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EXTRACTION AND CHARACTERIZATION OF STARCHES FROM PIGMENTED RICES

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ABSTRACT

The aim of this work was to extract and characterize starch from three varieties of pigmented rice (named white, red and black), preserving the bioactive compounds. The extraction yield was 44.0%, 47.0% and 35.7%, respectively. The scanning electron microscopy showed that the granules of the three varieties presented polygonal and angular format and absence of impurities. The chemical analyzes, showed more than 83.0% of carbohydrates in the three compositions. There was retention of the phenolic compounds from the raw material in the starches. With regard to pasting properties of the starches, differences were observed in initial temperature, which was of 80.6ºC, 79.1ºC and 88.8ºC for the starches of white, red and black rice, respectively. Black rice starch also showed slightly higher crystallinity and thermal stability than white and red rice starches. Gels of red rice starch have higher syneresis in five freeze-thaw cycles, when compared to the others.

Key-words: *Oryza sativa*, Polymer from renewable source, Bioactive compounds, Thermal analyzes.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most popular cereals in the world, especially in Asian countries [1]. The popularity of this cereal is due to its many characteristics, such as nutritional quality, practicability and applicability. In addition, its grain is hypoallergenic and non-toxic for people with celiac disease, so it can be a wheat substitute for products without gluten [2].

Red rice is considered a type of weed in many regions. Since this variety of rice contributes to a loss in white rice production, and due to the difficulties in being controlled, its use in the development of new food products has become an advantageous way for using the plant and its nutrients. Moreover, red rice has important compounds to be included in human diet [3,4].

Another variety of rice, which has a singular nutritional composition, is black rice. It is known for its anthocyanin and bioactive compounds content, which exhibit a high antioxidant activity and are considered as preventive agents for many diseases, like cancer [5,6].

Pigmented varieties of rice, as red or black, for instance, have higher phenolic content when compared with white rice [6]. Hence, the antioxidant capacity and functional properties associated with those varieties may be higher. Therefore, it could be used as a bioactive ingredient for food industries to improve the nutritional quality of their products.

Carbohydrates are the main constituents of rice, representing more than 80% of the product, which is starch. The concentration of starch in the grain is influenced by many factors, such as genetic, environmental, agronomic and others [7]. Industrial application of starch is related to its technological capacity and the properties that it provides to products, such as texture, viscosity, emulsion stability, among others.

The consumption of pigmented rice improve the intake of bioactive compounds, which can be associated to the inhibition of the activity of intestinal α-glucosidase and pancreatic α-amylase and a reduction of risk in type 2 diabetes, obesity, hypertension, hyperglycemia or dyslipidemia [8,9]. Moreover, those compounds present a great contribution as antioxidant agents in human health.

The use of rice starch in the food industry is expanding due to its characteristics, such as low ability to cause allergic reactions, mild taste, gel stability in freeze-thaw cycles, digestibility, acid resistance, and amylose and amylopectin content [10,11]. Thereby, the aim of this work was to extract and characterize starches from three pigmented varieties of rice, in order to study their bioactive compounds.

2. EXPERIMENTAL SECTION

2.1. Material

Three different varieties of rice were used: white, red and black (Armazém Santa Filomena, São Paulo, Brazil). Commercial rice starch (Dayelet, Spain) was used for comparison in thermal analyzes.

2.2. Methods

2.2.1. Starch extraction

The varieties of rice were submitted to wet extraction in order to obtain the starches, according to Ribeiro [12] with modifications. Firstly, the raw material was washed and weighed. The rice were submitted to fragmentation in water (1:2 rice:water) in an industrial blender model SPL-049 (SPOLU, Itajobi, Brazil) for 5min.

The fragmented material was filtered in order to separate the residues, which were again disintegrated and filtered.

. The filtered liquid was purified with a strainer (mesh 100 / 0.149mm) and placed on a bench for 24h to decant the starch. The starch was dried in an oven with air circulation (NG Científica, Campo Grande, Brazil) at 45ºC for 10h, and stored in plastic bags, in the dark. To extract starch from red rice, the filter was centrifuged at 1100rpm (G force = 318 x g, rotor radius 122.5mm) for 15min using a Simplex II centrifuge (ITR, Esteio, Brazil).

Extraction yield was calculated by initial mass of the raw material and the mass of dried starch (Equation 1).

$$Extraction\,yield\,(\%) = \frac{dry\,starch\,mass\,(g)}{raw\,material\,mass\,(g)} * 100 \qquad (1)$$

2.2.2. Starch microstructure

Starch microstructure was examined by optical microscopy and scanning electron microscopy. In optical microscopy, samples were placed in glass slides and diluted with distilled water. Images were captured with magnification of 40 and 100 times in an optical microscope (Nikon, Tokyo, Japan), by Micrometrics SE Premium 4 software.

The starch granules were analyzed in a low vacuum bench top scanning electron microscope TM 3000 (Hitachi, Tokyo, Japan) at 15kV. No gold coating was necessary to cover the starch granules.

2.2.3. Chemical characterization of starch

Moisture content was determined using an oven with air circulation at 105°C until constant weight [13]. Ash, lipid, protein and fiber content were determined according to methods n. 923.03, 945.38, 46.13 and 985.29, respectively [13]. The carbohydrate content was calculated by difference. Furthermore, the total caloric value was calculated with the results of lipids, proteins and carbohydrates, and expressed as kcal/100 g. The chemical characterization was performed in triplicate.

2.2.4. Amylose content

Amylose content was determined by the colorimetric method, as described by Martínez and Cuevas [14] and adapted by Zavareze et al. [15]. Amylose PA (Sigma®) was used as a standard. The amylose determination was performed in triplicate.

2.2.5. Phenolic compounds content

Extracts with different solvents (water, 70% acetone, 50% methanol and 50% ethanol) were prepared (based on previous tests). An aliquot (10g) of each sample was submitted to extraction with 100mL of solvent and placed in the dark for 5h (25ºC). Then, samples were centrifuged and the supernatant was used as extract. The total determination of phenolic compounds follows Folin and Ciocalteu [16] method, as developed by Singleton and Rossi [17], in triplicate. Gallic acid was used as a standard and results were expressed in mg GAE/100g. The phenolic compounds determination was performed in triplicate.

2.2.6. Colorimetry

Instrumental color of starches was determined by direct reading, assessed through a digital colorimeter CR 400 (Konica Minolta, New Jersey, USA) that shows L* (luminosity), a* (parameter from green to red) and b* (parameter from blue to yellow) values. The samples (10g) were weighed and placed in a Petri plate and read in five different places. Also, the chromaticity (C*) was calculated using values of a* and b* (Equation 2).

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

2.2.7. Pasting properties

The viscosity was carried out on a Rapid Visco Analyzer 4 RVA (Newport Scientific Pty Ltd, Warriewood, Australia) [18]. Starch suspension (3g of starch in 25mL water) adjusted to 14% moisture (wet basis). The time-temperature profile included mixing with the scoops, rotating at 960rpm in the first 10s and 160rpm until the end. Samples were heated between 50 to 90°C at a constant rate of 6°C/min, and then cooled at 50°C. Readings from the pasting curve were maximum peak viscosity, pasting temperature, breakdown viscosity, final viscosity and setback viscosity or retrogradation.

2.2.8. X-ray diffraction

The X-ray analyzes was performed on starch sample in powder form using a X-ray diffractometer X'Pert model (Philips, Almelo, Netherlands). The X-ray source used was a CuKα type radiation with a wavelength of λ=1.54056 Å, under the following conditions: Voltage and current of 40kV and 40mA, respectively; scanning range: diffraction angle 2ϴ from 5 to 30°; pitch: 0.1°; speed: 1°/min, equipped with a secondary graphite beam monochromator.

Samples were stored at 25ºC and 50% relative humidity in order to equilibrate the water within the sample prior analysis.

2.2.9. Differential scanning calorimetry

Differential Scanning Calorimetry analyzes was conducted on a TA Instruments Q2000 equipment, under nitrogen flow of 50 mL/min. During the test, the samples were heated at 10°C/min from 20ºC to 210°C and hold at this temperature for 3min. Then, they were cooled at 10ºC/min to -40°C, hold at -40°C for 3min, and then heated again at 3°C/min to 210°C. The peak temperature (T_{peak}) and the total heat of the endothermal transition (ΔH_{end}), related to the starch water loss, was determined for the first heating step. Eventual transitions during the cooling and second heating were analyzed. In order to compare, the same analyzes was conducted with commercial rice starch.

2.10. Thermal gravimetric analyzes

The study of the thermal stability of the rice starch was done using a TGA/DSC 1 (Mettler Toledo, Columbus, USA) STAR System analyzer with samples of around 5.0 mg, applying a heating ramp from 30 to 1000ºC at 10ºC/min under a nitrogen atmosphere (constant flow of 30mL/min). The weight loss evolution with temperature was analyzed using the STAR Evolution Software (Mettler Toledo, Columbus, USA). In order to compare, the same analyzes was conducted with commercial rice starch.

2.11. Syneresis by repeated freeze-thawings

Gels (10% s/v) of each studied starch were produced. They were obtained at 90ºC until complete gelatinization. The obtained gels (90g) were disposed in plates of 10cm of diameter and cooled at room temperature for 1h. Then, they were frozen for 20h and thawed at room temperature for 4h, before syneresis measurements. Gels were weighed prior freezing and removal of water, according to Takeiti [19]. The syneresis was determined according to Equation (3), expressed in percentage:

$$\% \, syneresis = \frac{Mi - Mf}{Mi} \, x \, 100 \quad (3)$$

Mi is the gel before freezing and *Mf* is the gel weight after excess of water removal. The measurements were performed in triplicate, during five cycles, for white, red and black starches.

2.12. Statistical analyzes

Data were submitted to analyze of variance (ANOVA) and compared by the Tukey test, at 5% significance level, with STATISTICA 8.0 software (Statsoft, Tulsa, USA).

3. RESULTS AND DISCUSSION

3.1. Starch extraction

The starch extraction method was efficient to obtain starches from different varieties of rice and they could be distinguished, with the naked eye, by the color.

All varieties of rice studied showed statistically different extraction yield; red rice presented the highest percentage (47.05%), followed by white rice (44.05%) and black rice with the lowest yield (35.74%). Yield variation is related to the raw material composition, since the quantity of each component changes for different raw materials. Ashogbon and Akintayo's [20] study on the extraction of rice starch obtained extraction yield ranging from 45 to 65% in varieties of rice cultivated in Nigeria (Igbemo, Efon Alayne, Illaje and Nerica II rice). Likewise, Zavareze et al. [15] extracted starch from rice with different amylose content (6.90-31.62% of amylose) and found that the yield increased in rice starches with higher amylose content. These authors associated such results with higher water absorption of starch granules with low amylose content, which blocks the protein separation causing loss of starch aggregated with the proteins [15]. Therefore, the data obtained in this work corroborate with the literature.

3.2. Starch microstructure

The size, format and structure of starch granules change as a function of the botanical species that is extracted. Nevertheless, there is no difference between starches studied in this work (Fig. 1 and 2). This is because the starches all come from rice, even from different varieties of the plant. Starches presented polygonal and angular format. Moreover, it was possible to observe, by looking at SEM images, the absence of impurities (Fig. 2), demonstrating the efficiency of the extraction of rice starch.

Ito et al. [21], when studying the physical-chemical, thermic, crystallographic and morphologic properties of black rice, concluded, by micrographs of SEM, that those granules present polyhedral structure with irregular forms, and acute angle and edged. Souza et al. [22] verified the same structure for white rice starch, as shown below (Figure 2). This starch is suggested to be used in pharmaceutical and food products because these may be a suitable way of using this raw material and could help to reduce negative environmental impacts [21].

40x 100x

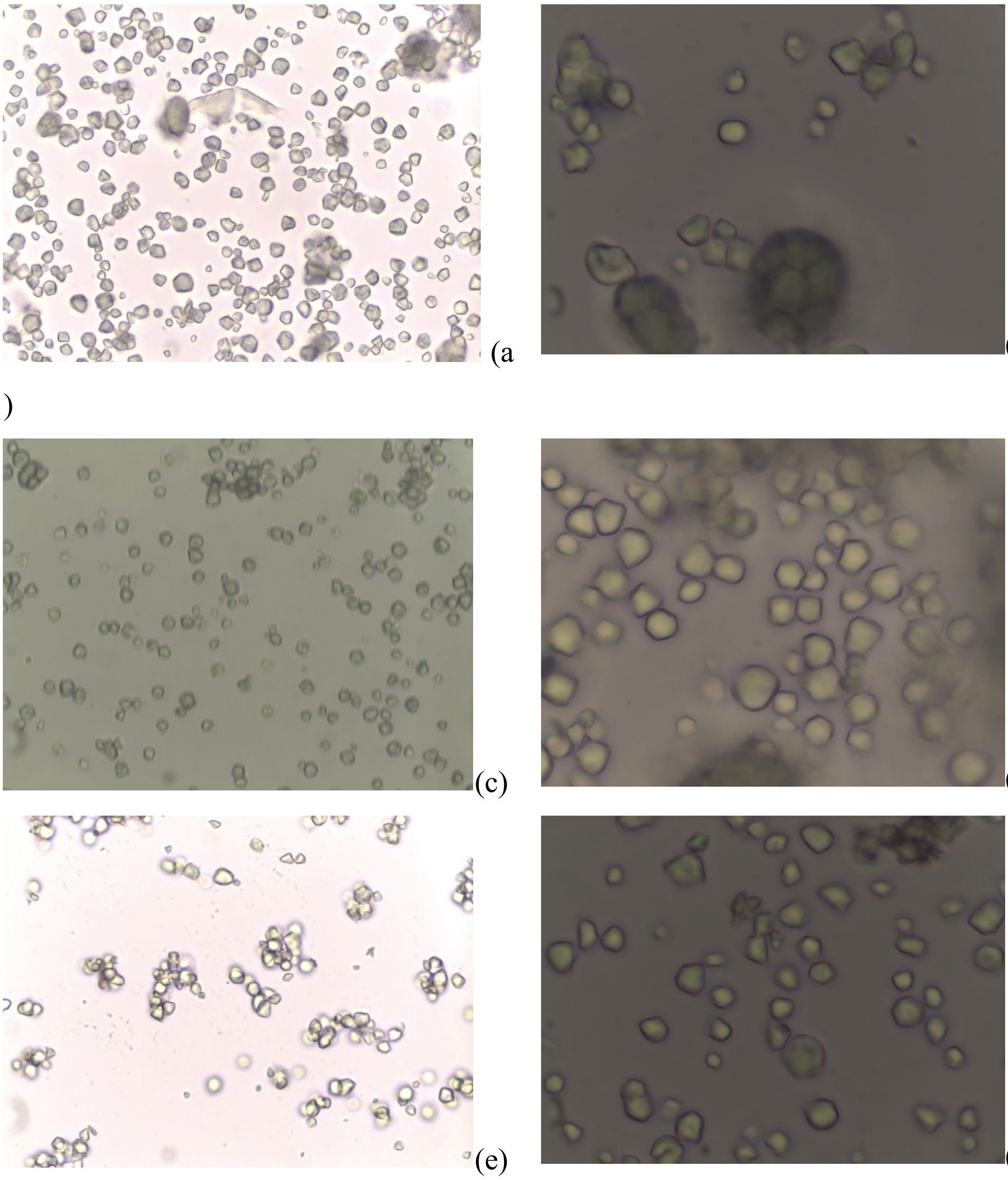


Figure 1. Optical microscopy of starch from white (a, b), red (c, d) and black (e, f) rice.

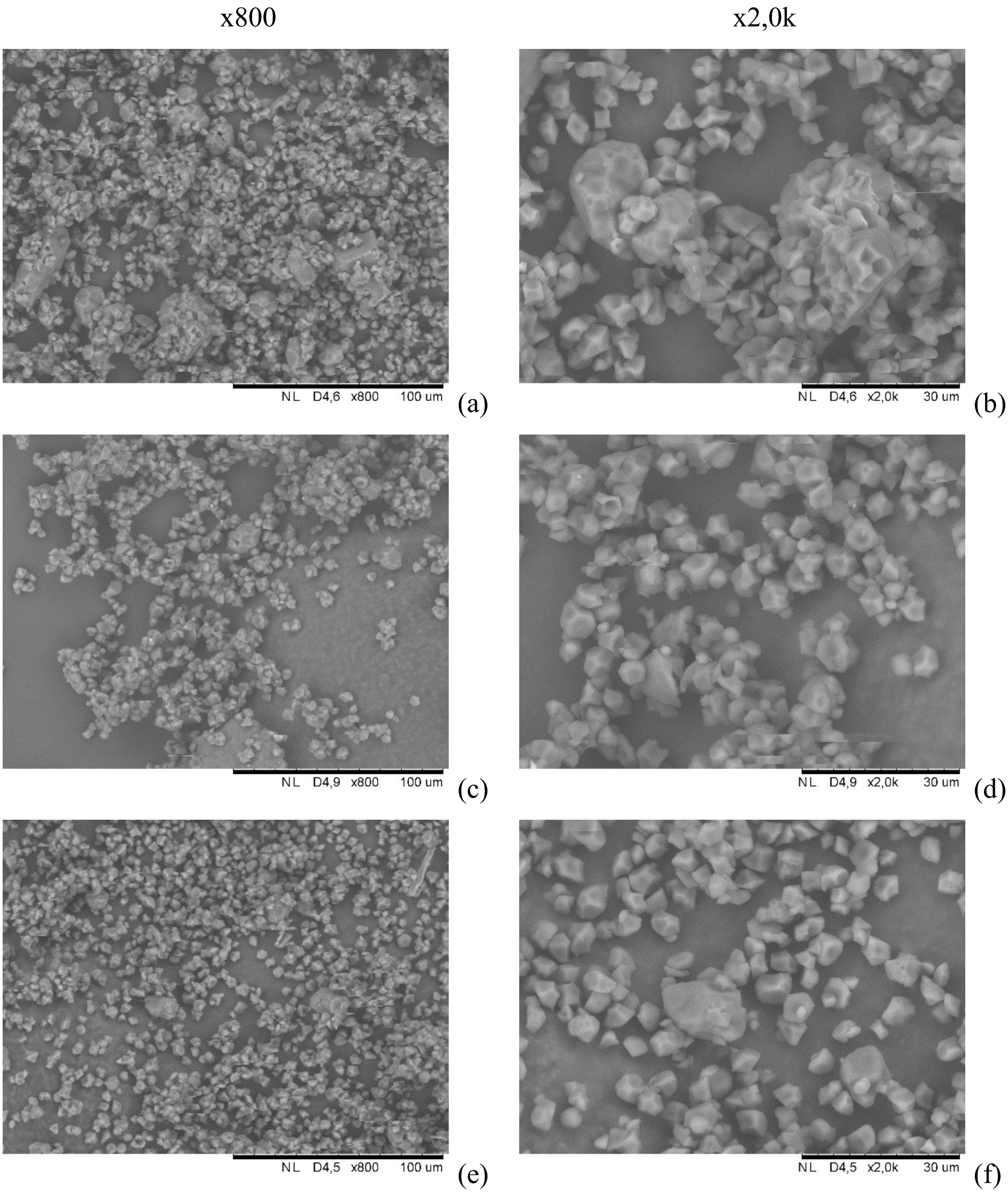


Figure 2. Scanning electron microscopy of starch from white (a, b), red (c, d) and black (e, f) rice.

3.3. Chemical characterization of starch

Black rice starch presented higher moisture content in comparison with others (Table 1). However, based on this parameter, all samples are in accordance with Brazil's legislation, which allows a maximum of 14% of moisture in this type of product [23].

Table 1. Chemical characterization of starch from different varieties of rice.

Sample	Constituents (%)					
	Moisture	Ash	Lipids	Proteins	Carbohydrates	Amylose
White	4.76±0.59^{c}	0.21±0.01^{b}	0.39±0.03^{b}	3.89±0.09^{a}	90.75±0.58^{a}	18.61±0.11^{c}
Red	6.89±0.21^{b}	0.80±0.05^{a}	0.51±0.05^{a}	3.73±0.27^{a}	88.07±0.26^{b}	25.75±0.26^{a}
Black	13.29±0.58^{a}	0.68±0.01^{a}	0.28±0.02^{c}	2.60±0.35^{b}	83.15±0.71^{c}	20.02±0.04^{b}

Means followed by the same letter in the columns do not differ statistically by Tukey test, at 5% of probability.

The ash content did not differ statistically for red and black rice starches, but showed a difference for white rice starch, which presented less minerals (Table 1). Along with the starch, white rice grains also presented low amounts of minerals (0.30%) [24]. However, essential micronutrients, such as iron, zinc and calcium, can be found in some varieties of rice [25].

Studied starches showed different lipid content depending on the type of rice (Table 1). It is in agreement with the quantity of this constituent in the raw material, as the lipid content in rice grains are influenced by the genotypic characteristics of the plant [24].

Rice is a cereal of low protein content, containing on average 7% of protein in its composition [24]. Therefore, rice starches have a low content of this nutrient, and they presented 3.89, 3.73 and 2.60% of proteins for white, red and black rice starch, respectively (Table 1). Only traces of fiber were found in the starch samples. The low amount of these components indicates the purity of the material studied, as observed in the images of microstructure. (Fig. 1 and 2).

Carbohydrates represent more than 80% of rice grain composition, even though it varies according to genetic, environmental and agronomic factors [26]. This is reflected on the starches obtained in this study, which present carbohydrate content ranging from 83.15 to 90.75% in black and white rice starch, respectively. Since the objective was to obtain starch, high carbohydrate values were extremely important in this study.

Starches presented statistical difference in total caloric value. The difference between the samples, regarding the content of lipids, proteins and carbohydrates, resulted in a different total calorific value. White rice starch presented the highest value (380.42 kcal/100 g) followed by red rice (371.46 kcal/100 g) and black rice with the lowest value (344.53 kcal/100 g).

3.4. Amylose content

The studied starches presented statistically different amylose content among them, being of 18.61, 25.75 and 20.02% for white, red and black rice starches, respectively. Zavareze et al. [15] classified amylose content of rice starches in high amylose (31.62%), average amylose (23.40%) and low amylose (6.90%). Thus, the starches analyzed in this work are in the range of average amylose. The results (Table 1) corroborate with the amylose content of different rice cultivars grown in Nigeria, ranging from 21.88 to 26.04% of amylose [21] and it is higher than starches from different rice cultivars, which ranged from 2.77 to 12.09% [27].

By comparing the obtained results with the amylose content found in native corn starch (25.16%) [28] or native cassava starch (19.41%) [29], which are conventional and widely used in industry, we can say that rice starch is similar with regard to amylose content.

3.5. Phenolic content

The influence of solvents on the extraction of phenolic content from rice starch samples was confirmed by the solvents used (water, 70% acetone, 50% methanol and 50% ethanol) (Table 2). The best solvent for the extraction of phenolic content from rice starch is water, which showed the highest results: 288.95, 478.46 and 661.60 mg GAE/100g for starch of white, red and black rice, respectively. Starch obtained from different rice varieties had a higher phenolic content than babassu starch, which resulted in 8.2 and 63.7 mg GAE/100g of sample extracted by alkaline and wet method, respectively [30].

Table 2. Phenolic content (mg GAE/100g) of starches from different varieties of rice.

Samples	Solvent			
	Water	70% Acetone	50% Methanol	50% Ethanol
White	288.95±0.53^{Ac}	201.36±0.33^{Cc}	246.05±0.09^{Bc}	147.42±0.42^{Dc}
Red	478.46±0.55^{Ab}	344.63±0.45^{Ba}	336.24±0.18^{Cb}	318.05±0.25^{Db}
Black	661.60±0.37^{Aa}	340.86±0.21^{Db}	461.46±0.19^{Ca}	524.92±0.14^{Ba}

Means followed by the same lower letter (in the columns) and followed by the same capital letter (in the lines) do not differ statistically by Tukey test, at 5% of probability.

The starch samples presented statistical differences with regard to the phenolic content (Table 2). This agrees with the literature, since the chemical composition of the plants is influenced by several factors, such as genotypic factors [31], environmental factors, management, processing, storage or production location [5].

3.6. Colorimetry

The three samples studied presented a statistical difference in the evaluated parameters (Table 3). The highest value of L* was observed for white rice starch, indicating a greater clarity compared to the others. The red rice starch presented positive values in the parameters a* and b*, demonstrating a tendency to a reddish coloration. The values of the parameter a* in the black rice starch were positive and higher than those obtained in the red starch, however the parameter b* was lower. Thus, results indicate a purple coloration for black rice starch.

Table 3. Color parameters of starches from different varieties of rice.

Samples	Color parameters			
	L*	a*	b*	C*
White	98.92 ± 1.41^{a}	0.04 ± 0.02^{c}	4.89 ± 0.27^{b}	11.99 ± 1.28^{c}
Red	82.13 ± 2.04^{b}	6.02 ± 0.17^{b}	8.57 ± 0.09^{a}	54.90 ± 1.68^{a}
Black	63.82 ± 0.59^{c}	9.29 ± 0.04^{a}	0.45 ± 0.04^{c}	43.26 ± 0.42^{b}

Means followed by the same letter in the columns do not differ statistically by Tukey test, at 5% of probability.

The maintenance of the color from the raw material was also verified in the babassu starch, which showed a yellowish coloration in all treatments [30]. On the other hand, Soison et al. [32], when studying the starches of colored varieties of sweet potato, found that the extracted starches did not maintain the raw material color, and all of them were white. The authors have attributed the color dispersion to the wash performed during the starch purification process.

3.7. Pasting properties

During the gelatinization of starch, promoted by heating under excess of water, the crystalline regions breakdown, this phenomenon characterized by an increase of viscosity between 3 and 5min of initial heating [33]. In this work, there was no change in viscosity in the first 3min , but there was difference in the initial paste temperature: 80.6ºC, 79.1ºC and 88.8ºC for white, red and black rice starch, respectively. It demonstrated that starch from white and red rice showed similar gelatinization process, whereas the starch from black rice needed a higher temperature for this phenomenon (Fig. 3).

According to Franco et al. [34], starches that their amylopectin content presents high percentages of branched chains need higher temperatures of gelatinization. The value of the pasting temperature of white rice in the present study was similar a reported by Gonzáles et al. [35] for the native rice starch (79.1ºC).

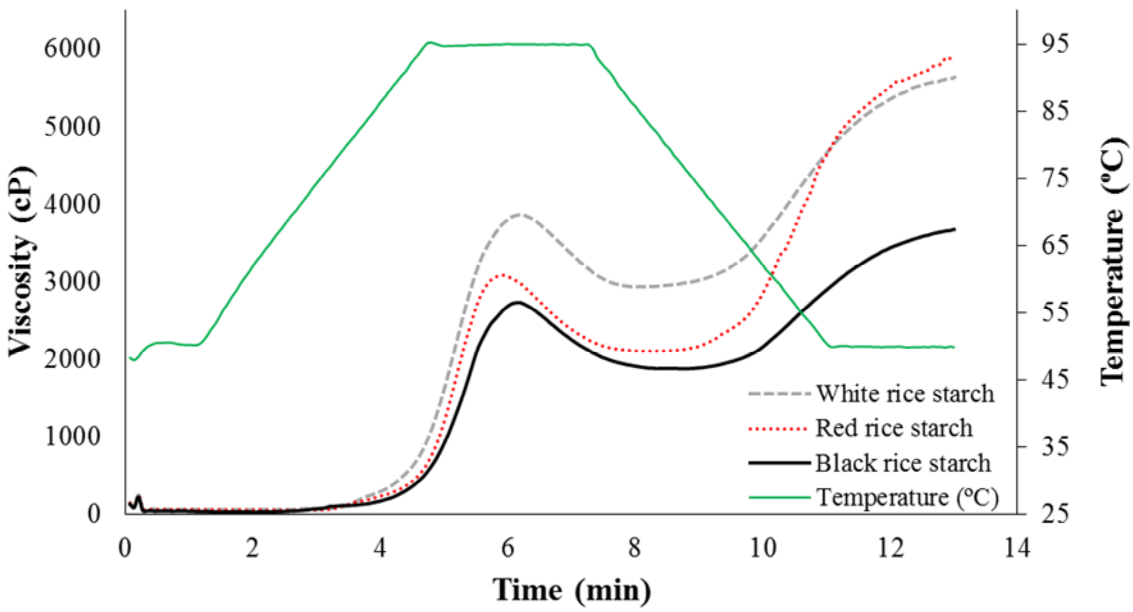


Figure 3. Viscoamylographic properties (RVA) of starches from white, red and black rice.

Wani et al. [36], in their analyzes, verified values from the literature between 63.80 (Indian Rice Starch) and 95.10ºC (commercial rice starch from Thailand) for paste temperature in starches of different varieties of rice. The determination of this parameter is essential for the industrial application of starches, such as in the development of biodegradable packaging.

Starches showed maximum cold viscosity reading ranging from 51.0cP to 68.5cP for black and white rice, respectively and the final viscosity (maximum values) values were 3872.0cP, 3082.5cP and 2731.5cP for white, red and black rice starches, respectively.

In the cooling stage, the starches minimum viscosity of 2937.0, 2103.5 and 1879.0cP and a maximum of 5618.5, 5892.5 and 3655.0cP for the white, red and black rice starches, respectively.

The starches presented 935.0, 979.0 and 852.5 of breakdown viscosity values and 2681.5, 3789.0 and 1776.0cP of setback values for the white, red and black rice starches, respectively. Breakdown values can be used to predict resistance of starch paste to processing conditions in the industry. Usually, this parameter is related to the degree of swelling of the starch granules during heating [37].

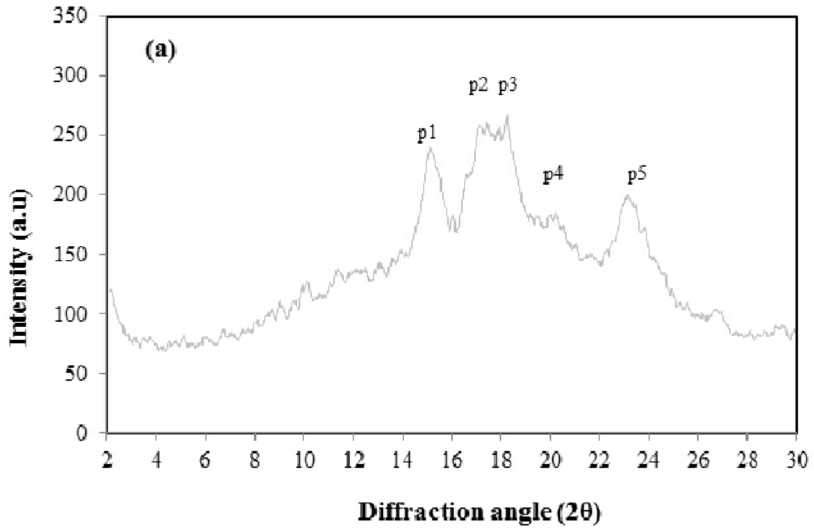
3.8. Crystalline structure

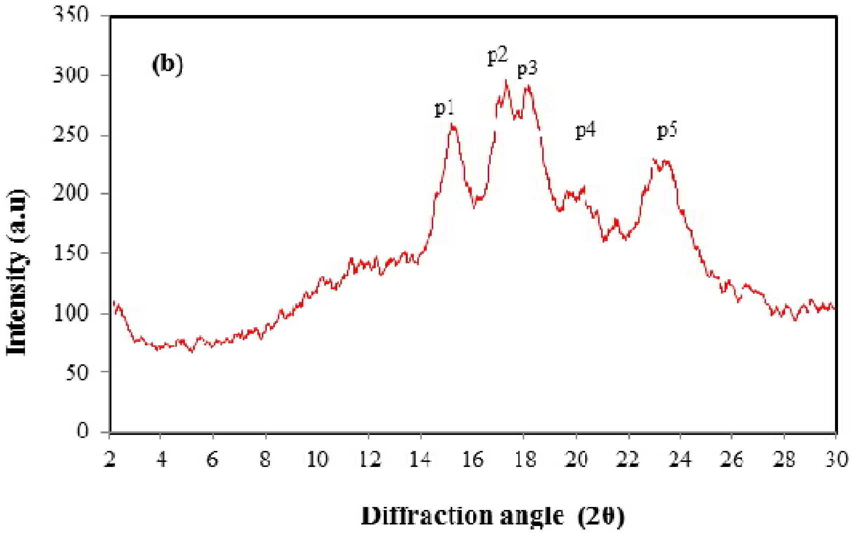
According to Zobel [38], in a study on X-ray diffraction in starch granules, type A starches showed strong signals at 2θ equal to 15.3, 17.1, 18.2 and 23.5º, whereas for type B starches, strong bands appeared at 5.6, 14.4, 17.2, 22.2 and 24.0º, and for those of type C, the signals were stronger in 2θ equal to 5.6, 15.3, 17.3 and 23.5º. In this study, the three types of starch studied did not show peaks at angle 2θ equal to 5 degrees, being all classified as type A starches (Table 4 and Fig. 4).

Table 4. Crystalline characteristics of the three rice starches.

Rice	Peak diffraction angle (deg)					Normalized peak area				Cristallinity
starch	p1	p2	p3	p4	p5	p1	p2+p3	p4	p5	(%)

White	15.10	17.10	18.30	20.20	23.20	3.53	11.35	4.71	6.14	25.70
Red	15.20	17.30	18.20	20.30	23.00	4.25	11.24	2.05	7.99	25.50
Black	15.20	17.10	18.00	20.20	23.10	6.01	13.53	2.44	4.69	26.70





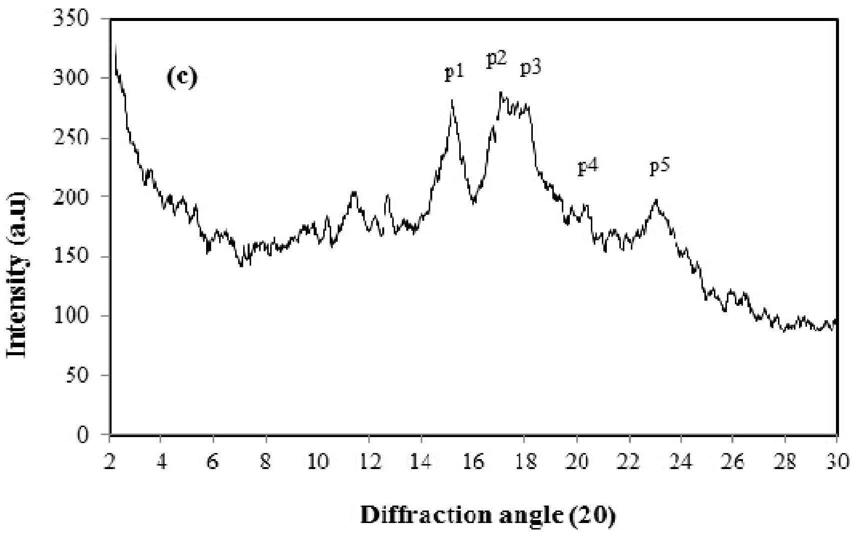


Figure 4. X-ray diffraction of starch of white (a), red (b) and black (c) rice.

The different starches have presented peaks at angle 2θ at 20.2º, 20.3º and 20.2º for starch from white, red and black rice, respectively. Those peaks are mainly found in starches from cereals, with is attributed to V-amylose-phospholipid complexes [39].

The black rice starch (Fig. 4c) presented the highest crystallinity and a spectrum when compared with white and red rice starches (Fig. 4a and 4b, respectively). According to Singh et al. [40], the differences in starch crystallinity could be related to differences in amylose and amylopectin ratio. In the present study, the highest ratio between amylose and amylopectin, as well as the highest crystallinity, is observed for the red and black rice starch. Additionally the presence of compounds, other than polysaccharides, could also be affecting the crystallinity degree of the starch, by hindering the regular spatial arrangement of the macromolecules. As indicated before, joint to slightly higher amylose content, the black rice starch contains much more phenolic compounds than the white rice starch (and more than the red one).

3.9. Thermal stability

The heating curves obtained by DSC for the three studied starches, displaying the endothermal transition related to the starch water loss (Fig. 5).

Values of the peak temperature (T_{peak}) were found to be 117.8ºC, 119.6ºC and 111.6ºC for white, red and black rice starch respectively, and the total heat absorbed during the first heating (ΔH_{end}), related to the starch water loss, was found to be 302.6J/g, 288.5J/g and 328.8J/g for white, red and black rice starch respectively. No thermal transitions were observed during the cooling and the second heating of the starch samples. For comparison proposals, the thermal analyzes was also carried out on a commercial rice starch, that displayed T_{peak} = 98.4ºC and ΔH_{end} = 335.8J/g. As can be observed, the starches of the three pigmented varieties of rice were found to be more stable in terms of the endothermal transition, which resulted less intense and displaced to higher temperatures. Among the pigmented varieties, the black rice starch displayed the endothermal transition located at the lowest temperature with the highest value of ΔH_{end}, being related with its highest water content.

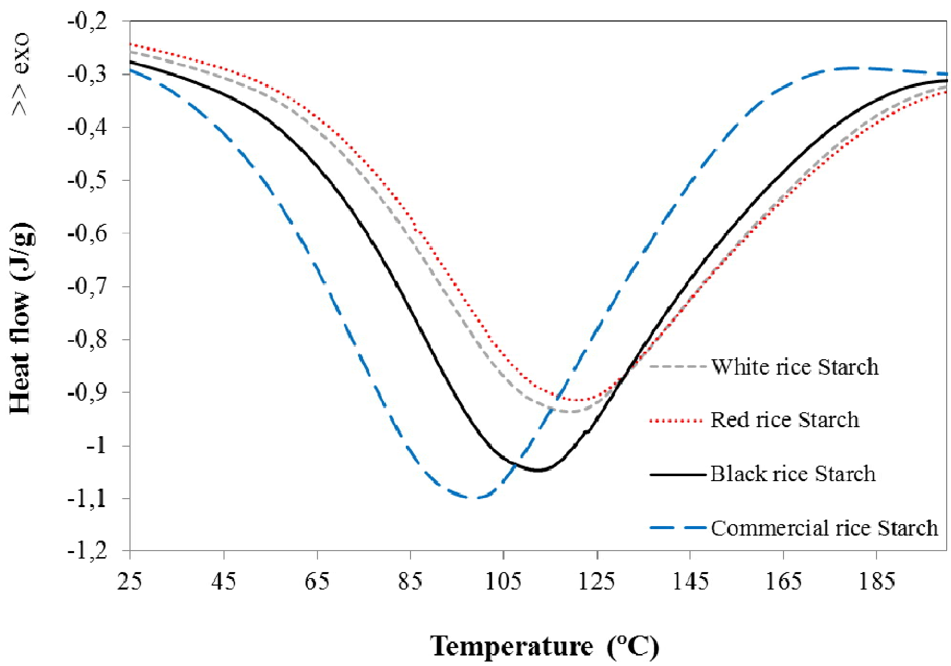
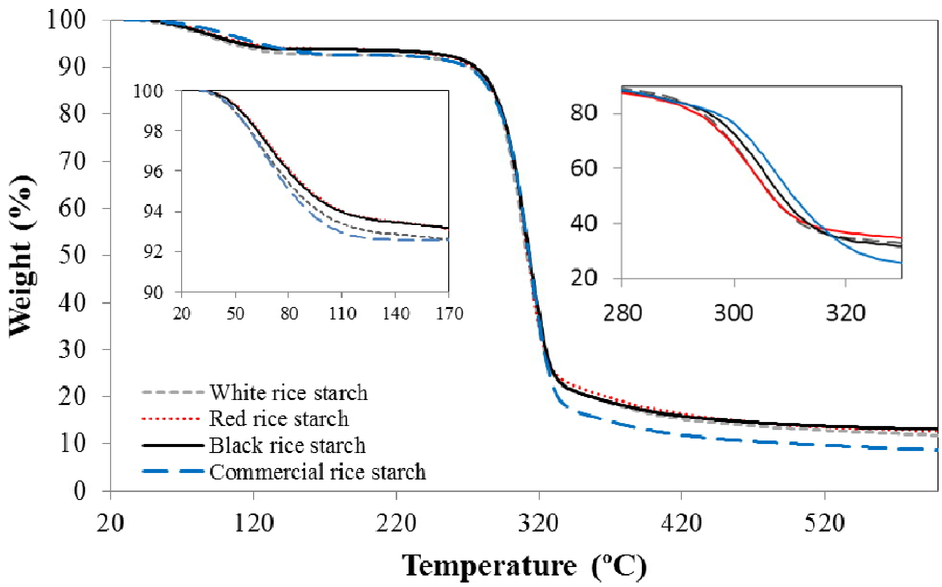
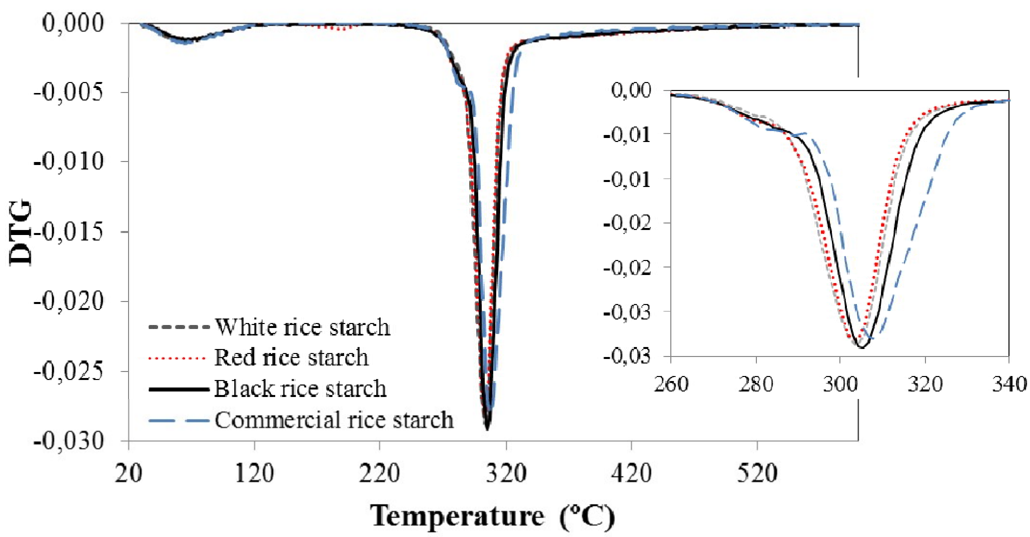


Figure 5. DSC heating curves of the three studied starches and that of the reference.

As can be seen in Fig. 6a, the thermogravimetric analyzes (TGA) revealed two stages of weight lost in all the starches. Firstly, the water lost step extended up to 120ºC approximately for all starches, with commercial and black rice starches displaying slightly higher water loss than white and red ones (first inset in Fig. 6a). The second weight loss step accounts for the thermal decomposition of the polymer, with lower weight loss for the three pigmented starches with respect to the commercial one (see detail in the second inset in Fig. 6a). As can be seen in Fig. 6b, the derivative DTG curves demonstrate that the thermal breakdown of the rice starches is produced in a single step. In addition, the pigmented varieties displayed lower stability than the commercial rice starch, since this variety presents its DTG curve moved to higher temperatures. Among the pigmented varieties, the black rice starch showed a slightly higher thermal stability than the white and red starches.



(a)



(b)

Figure 6. TGA curves (a), and derivative curve of the thermal decomposition (b) of three studied starches and that of the reference.

3.10. Syneresis by repeated freeze-thawings

The syneresis (%) of rice starch gels during freeze-thaw cycles are shown in Fig. 6. Under the experimental conditions, red rice starch gel lost higher percentage of water, when compared to the others. Also, it was observed an increase water loss with the increase of freeze-thaw cycles. After five freeze-thaw cycles, red rice starch gel presented 66.46% of synerisis, while black and white rice starch gels presented 51.39% and 31.18%, respectively. The syneresis of these starches is associated to the amylose content of each sample, also with the retrogradation tendency observed by viscoamilografic properties. Gels of the studied starches have presented lower syneresis than waxy maize (7.81%) and sweet potato (16.74%) starches at the 5th cycle of freeze-thaw [19]. Its difference based on amylose content can be seen in other studies, as described by Ashwar [41]. Considering rice starch gels with amylose content from 8.56 to 17.85%, he ones with high amylose content showed similar results to the present study, in particular white rice starch gel in the 3th (14.66%) and 5th (27.09%) freeze-thaw cycles.

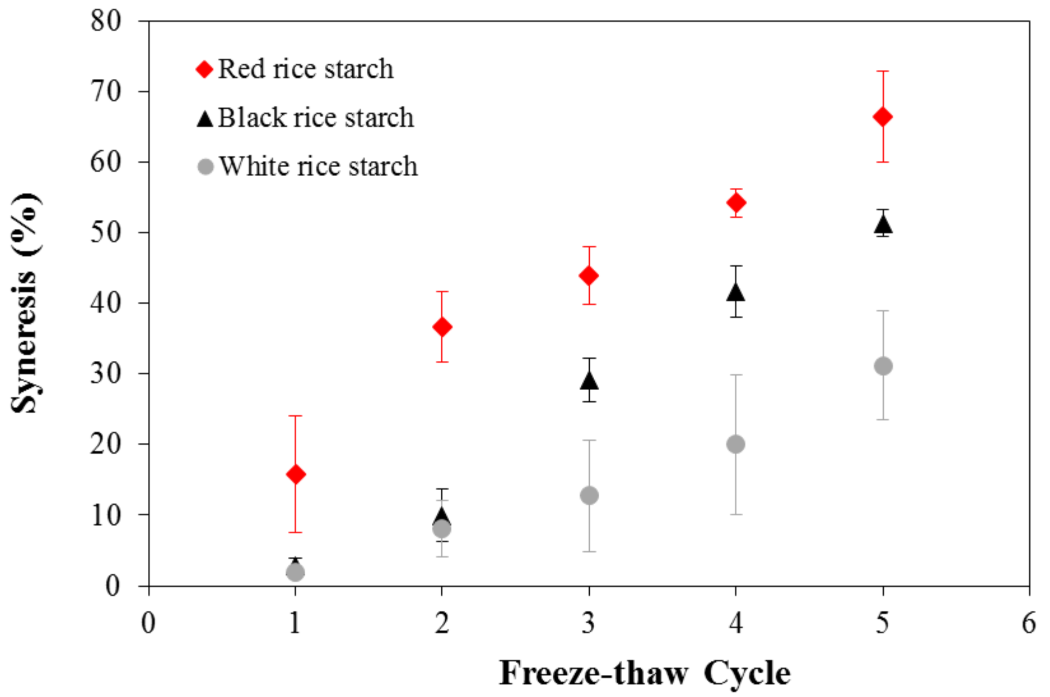


Figure 7. Syneresis of starch gels from white, red and black rice during freeze-thaw cycles.

4. CONCLUSION

Rice is a raw material suitable for the extraction of starch due to its high extraction yield. Even with the differences between rice varieties, the starches present granules of polygonal and angular formats, as visualized by optical microscopy and SEM.

Starches composition presented statistical differences for some components, according to the rice variety; however, the major components were the carbohydrates, which are represented mainly by the starch.

The starches presented a high phenolic content, even after the extraction and drying process, and this content was different according to the solvents used, among which water showed a better performance. Starch from red rice presented the highest amylose content (25.75%), retrogradation tendency and syneresis observed in freeze-thaw cycles.

Starches from white and red rice being all classified as type A starches. In addition, starch from black rice have presented the highest crystallinity area (26.7%), among the others, also this starch needs higher temperature of gelatinization (88.8ºC) than white and red rice starches. Besides that, the black rice starch also showed a slightly higher thermal stability than the white and red starches.

The studied starches can serve as viable functional food ingredients, and they are suitable to be used for industries, mainly at food industry, where they can be applied for the development of food products or edible films and coatings. In addition, starches from pigmented varieties of rice may be better choices as sources of phenolic compounds than from white rice.

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5. REFERENCES

[1] A. Folorunso, S. Omoniyi, A. Habeeb, Proximate composition and sensory acceptability of snacks produced from broken rice (*Oryza sativa*) flour, Am. J. Food. Nutr. 6 (2016) 39-43. https://doi.org/10.5251/ajfn.2016.6.2.39.43.

[2] A.B. Das, S. Bhattacharya, Characterization of the batter and gluten-free cake from extruded red rice flour, LWT – Food. Sci. Technol. 102 (2019) 197-204. https://doi.org/10.1016/j.lwt.2018.12.026.

[3] S. Hayashi, E. Yanase, A study on the color deepening in red rice during storage, Food. Chem. 199 (2016) 457-462. https://doi.org/10.1016/j.foodchem.2015.12.023.

[4] Z. Hu, X. Tang, J. Liu, Z. Zhu, Y. Shao, Effect of parboiling on phytochemical content, antioxidant activity and physicochemical properties of germinated red rice, Food. Chem. 214 (2017) 285-292. https://doi.org/10.1016/j.foodchem.2016.07.097.

[5] V.C. Ito, L.G. Lacerda, Black rice (*Oryza sativa* L.): A review of its historical aspects, chemical composition, nutritional and functional properties, and applications and processing technologies, Food. Chem. 301 (2019) 125304. https://doi.org/10.1016/j.foodchem.2019.125304.

[6] V.C. Ito, A.A.F. Zielinski, I.M. Demiate, M.H.F. Spoto, A. Nogueira, L.G. Lacerda, Effects of gamma radiation on the stability and degradation kinetics of phenolic compounds and antioxidant activity during storage of (*Oryza sativa* L.) black rice flour, Braz. Arch. Biol. Technol. 62 (2019) 01–14. https://doi.org/10.1590/1678-4324-2019180470.

[7] P. Van Hung, Phenolic compounds of cereals and their antioxidant capacity, Crit. Rev. Food. Sci. Nutr. 2016*, 56* [1], 25–35. https://doi.org/10.1080/10408398.2012.708909.

[8] C.J. Rebello, F.L. Greenway, J.W. Finley, Whole grains and pulses: A comparison of the nutritional and health benefits, J. Agric. Food. Chem. 62 (2014) 7029–7049. https://doi.org/10.1021/jf500932z.

[9] L.G. Ranilla, Y.I. Kwon, E. Apostolidis, K. Shetty, Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America, Bioresour. Technol. 101 (2010) 4676–4689. https://doi.org/10.1016/j.biortech.2010.01.093.

[10] S. Wang, P. Li, T. Zhang, J. Yu, S. Wang, L. Copeland, *In vitro* starch digestibility of rice flour is not affected by method of cooking, LWT – Food. Sci. Technol. 84 (2017) 536-543. https://doi.org/10.1016/j.lwt.2017.06.023.

[11] R. Wang, J. Wan, C. Liu, X. Xia, Y. Ding, Pasting, thermal, and rheological properties of rice starch partially replaced by inulin with different degrees of polymerization, Food. Hydrocoll. 92 (2019) 228-232. https://doi.org/10.1016/j.foodhyd.2019.02.008.

[12] A.E.C. Ribeiro, D.P.R. Ascheri, J.L.R. Ascheri, Amidos fosfatados da *Swartzia burchelli*: propriedades físicas e físico-químicas, Bol. Centr. Pesq. Process. Aliment. 35 (2017) 1-17. http://dx.doi.org/10.5380/cep.v35i1.55933.

[13] AOAC. Official methods of analysis of the Association of Official Analytical Chemists. 18 ed.; Association of Official Analytical Chemists: Mayland, 2005.

[14] C.Y. Martínez, F. Cuevas, Evaluación de la calidad culinaria y molinera del arroz: CIAT, 1989.

[15] E. Zavareze, S. Halal, J. Pereira, A. Radunz, M. Elias, A. Dias, Caracterização química e rendimento de extração de amido de arroz com diferentes teores de amilose, Braz. J. Food. Technol. IISSA (2009) 24-30.

[16] O. Folin, V. Ciocalteu, On tyrosine and tryptophane determinations in proteins, J. Biol. Chem.73 (1927) 627-650.

[17] V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Viticult. 16 (1965) 144-158.

[18] D.M. Santos, D.P.R. Ascheri, A.L. Bukzem, C.C. Morais, C.W.P. Carvalho, J.L.R. Ascheri, Physicochemical properties of starch from avocado seed (*Persea americana* Mill), Bol. Centr. Pesq. Process. Aliment. 34 (2016) 1-12. http://dx.doi.org/10.5380/cep.v34i2.53138.

[19] C. Takeiti, F.M. Fakhouri, R. Ormenese, C.J. Steel, F. Collares, Freeze-thaw stability of gels prepared from starches of non-conventional sources. Starch/Starke. 59 (2007) 156-160. http://dx.doi.org/10.1002/star.200600544.

[20] A. Ashogbon, E. Akintayo, Morphological, functional and pasting properties of starches separated from rice cultivars grown in Nigeria, Int. Food. Res. J. 19 (2012) 665-671.

[21] V.C. Ito, E. Schnitzler, I.M. Demiate, M.E.S. Eusébio, L.G. Lacerda, R.A. Castro, Physicochemical, termal, crystallographic, and morphological properties of biodynamic black rice starch, and of residual fractions from aqueous extraction, Starch. 70 (2018) 01-07. https://doi.org/10.1002/star.201700348.

[22] D. Souza, A.F. Sbardelotto, D.R. Ziegler, L.D.F. Marczak, I.C. Tessaro, Characterization of rice starch and protein obtained by fast alkaline extraction method, Food. Chem. 191 (2016) 36-44. https://doi.org/10.1016/j.foodchem.2015.03.032.

[23] BRAZIL. Leis, decretos, etc. Decreto nº 12.486 de 20 de outubro de 1978. Normas técnicas especiais relativas a alimentos e bebidas. Diário Oficial do Estado de São Paulo, 21 out., 1978. 20 p.

[24] M. Walter, E. Marchezan, L.A. Avila, A. Arroz: composição e características nutricionais, Ciênc. Rural. 38 (2008) 1184-1192. https://doi.org/10.1590/S0103-84782008000400049.

[25] N. Renuka, S.V. Mathure, R.L. Zanan, R.J. Thengane, A.B. Nadaf, Determination of some minerals and β-carotene contents in aromatic indica rice (*Oryza sativa* L.)

germplasm, Food. Chem. 191 (2016) 02-06. https://doi.org/10.1016/j.foodchem.2015.05.045.

[26] V. Ziegler, C.D. Ferreira, J.T.S. Goebel, S.L.M. El-Halal, G.S. Santetti, L.C. Gutkoski, E.R. Zavareze, M.C. Elias, Changes in properties of starch isolated from whole rice grains with brown, black, and red pericarp after storage at different temperatures, Food. Chem. 216 (2017) 194–200. https://doi.org/10.1016/j.foodchem.2016.08.045.

[27] N. Tangsrianugul, R. Wongsagonsup, M. Suphantharika, Physicochemical and rheological properties of flour and starch from Thai pigmented rice cultivars, Int. J. Biol. Macromol. 137 (2019) 666-675. https://doi.org/10.1016/j.ijbiomac.2019.06.196.

[28] M. Lopez-Silva, L.A. Bello-Perez, E. Agama-Acevedo, J. Alvarez-Ramirez, Effect of amylose content on morphological, functional and emulsification properties of OSA modified corn starch, Food. Hydrocoll. 97 (2019) 105212. https://doi.org/10.1016/j.foodhyd.2019.105212.

[29] J. Figueroa-Flórez, E.M. Cadena-Chamorro, E. Rodríguez-Sandoval, J. Salcedo-Mendoza, H. Ciro-Velásquez, Cassava starches modified by enzymatic biocatalysis: effect of reaction time and drying method. DYNA. 86 (2019) 162-170. https://doi.org/10.15446/dyna.v86n208.72976.

[30] B.C. Maniglia, D.R. Tapia-Blácido, Isolation and characterization of starch from babassu mesocarp, Food. Hydrocoll. 55 (2016) 47-55. https://doi.org/10.1016/j.foodhyd.2015.11.001.

[31] S. Petropoulos, Â. Fernandes, L. Barros, I.C. Ferreira, Chemical composition, nutritional value and antioxidant properties of Mediterranean okra genotypes in relation to harvest stage, Food. Chem. 242 (2018) 466-474. https://doi.org/10.1016/j.foodchem.2017.09.082.

[32] B. Soison, K. Jangchud, A. Jangchud, T. Harnsilawat, K. Piyachomkwan, Characterization of starch in relation to flesh colors of sweet potato varieties, Int. Food. Res. J. 22 (2015) 2302-2308.

[33] M. Zeng, C.F. Morris, I.L. Batey, C.W. Wrigley, Sources of variation for starch gelatinization, pasting, and gelation properties in wheat, Cereal. Chem. 74 (1997) 63-71. https://doi.org/10.1094/CCHEM.1997.74.1.63.

[34] C.M.L. Franco, K.S. Wong, S.H. Yoo, J.L. Jane, Structural and functional characteristics of selected soft wheat starches, Cereal. Chem. 79 (2002) 243-248. https://doi.org/10.1094/CCHEM.2002.79.2.243.

[35] L.C. Gonzáles, M.A. Loubes, M. Tolaba, Incidence of milling energy on dry-milling attributes of rice starch modified by planetary ball milling, Food. Hydrocoll. 82 (2018) 155-163. https://doi.org/10.1016/j.foodhyd.2018.03.051.

[36] A.A. Wani, P. Singh, M.A. Shah, U. Schweiggert-Weisz, K. Gul, I.A. Wani, Rice starch diversity: Effects on structural, morphological, thermal, and physicochemical properties - A review, Compr. Rev. Food. Sci. F. 11 (2012) 417-436. https://doi.org/10.1111/j.1541-4337.2012.00193.x.

[37] Y.L. Chung, H.M. Lai, Molecular and granular characteristics of corn starch modified by HCl-methanol at different temperatures, Carbohydr. Polym. 63 (2006) 527-534. https://doi.org/10.1016/j.carbpol.2005.10.031.

[38] H.F. Zobel, Developments in Carbohydrate Chemistry, in: R.J. Alexander, H.F. Zobel (Eds.), The American Association of Cereal Chemists: St. Paul, MN, 1994.

[39] C. Gerárd, P. Colonnna, A. Buléon, V. Planchot, Order in maize mutant starches revealed by mild acid hydrolysis, Carbohydr. Polym. 48 (2002) 31-141. https://doi.org/10.1016/S0144-8617(01)00219-3.

[40] N. Singh, Y. Nakaura, N. Inouchi, K. Nishinari, Fine structure, thermal and viscoelastic properties of starches separated from *Indica* rice cultivars. Starch. 59 (2007) 10-20. https://doi.org/10.1002/star.200600527.

[41] B.A. Ashwar, A. Gani, I.A. Wani, A. Shah, F.A. Masoodi, D.C. Saxena, Production of resistant starch from rice by dual autoclaving-retrogradation treatment: *In vitro* digestibility, thermal and structural characterization. Food.Hydrocol. 56 (2016) 108-117. https://doi.org/10.1016/j.foodhyd.2015.12.004.