



Starch Recovery Process from the Tiger Nut *Horchata* Processing Waste

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Abstract

Tiger nut horchata solid residue (HSR) has high starch content (about 23%, dry basis) that can be recovered to valorise this waste, together with other valuable components, such as oil, fibres, or phenolic compounds. In this study, a chemical-free, sustainable process was proposed to obtain starch with good yield and purity. This consists of a high-speed homogenisation step with distilled water, followed by filtration and phase separation by density in the slurry to obtain a starch-rich sediment that can be isolated by decantation. Five minutes of homogenisation at 10,200 rpm allows for obtaining 14.5 g of starch powder per 100 g of dried HSR, with a purity of about 85% and WI of 94.97. Applying 1 washing cycle to the filtering residue increased the starch powder yield up to 17 g/100 dried HSR, without notable losses in starch purity, whereas centrifugation at 15,000 g for 1 h in the phase separation step highly reduced separation time, increasing starch purity up to 92%. Thus, it was possible to recover up to 70% of the starch present in the HSR. The obtained starch had similar morphological characteristics, crystallinity degree, and gelatinisation behaviour to that present in the tiger nut tubers, exhibiting similar thermal stability to that reported for other native starches. Therefore, HSR can be used as a starch source for different uses.

Keywords Tiger nut horchata residue · Starch · Recovery process · Stability

Introduction

Tiger nut (*Cyperus Esculentus* L.) is a perennial and grass-like plant vastly grown worldwide. It is an underutilised crop that has been cultivated since early times (mainly in South Europe and West Africa) for its small, tuberous rhizomes which are eaten raw, roasted, used as hog feed, pressed for its juice to make beverages, such as tiger nut milk, or used to extract oil (Adejuyitan, 2017). Many authors have reported the nutritional value of tiger nut. It produces high-quality oil rich in unsaturated fatty acids. Likewise, the tubers are valued for their nutritious starch content, dietary fibre, and carbohydrates, being also rich in minerals (Gambo et al., 2014), and in phenolic compounds such as ferulic acid, p-hydroxybenzoic acid, sinapinic acid, and flavonoids (Olaosebikan et al., 2017). Therefore, there is a need for increased utilisation and awareness about its health benefits.

Horchata (a tiger nut milk) is a traditional Spanish beverage made from tiger nuts, mostly named chufa at local area, which generates a processing residue with very few uses and interesting composition to be valorised. The composition of tiger nuts cultivated in Spain (expressed on a dry basis) is 35.21% fat, 8.45% protein, 9.41% fibre, 1.97% ash, and 45.05% non-nitrogenous components (Codina—Torrella et al., 2015). The latter represents the extractable fraction where starch is the most abundant carbohydrate; it can reach 31%, whilst other carbohydrates range from 15.2 to 20% (Morell & Barber, 1983).

Before the horchata elaboration process, the harvested tubers are air-dried to reach the optimum moisture content (10–11%) for storage. At the *horchata* factories, called “*horchaterías*,” firstly, the tubers are thoroughly washed to eliminate all dirt and soil. Then, they are placed in salty water to remove the defective ones; afterwards, they are properly rehydrated; then disinfected in chlorine solution and rewashed to remove any chlorine traces. Once cleaned, they are wet milled with water and filtered. The filtrate is mixed with sugar and quickly cooled for storage. The resulting beverage is a colloidal system in which the aqueous phase is composed of water, sugars, soluble proteins like albumins, and minerals, whilst the dispersed

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phase is formed by suspended starch granules, oil droplets, water-insoluble proteins, and other small solid particles. The starch content in horchata ranges between 2 and 3.4% (Codina-Torrella et al., 2015; Martín Esparza et al., 2016), and the solid residue resulting from the filtration step of the horchata elaboration process represents 60% of the tubers used in the process. It is generally disposed of by combustion, composted, or used as animal feed (Sánchez-Zapata et al., 2009). Nevertheless, it is highly rich in starch that could be recovered. Other components, such as oil, fibres, or antioxidants present in the tuber residue, could also be obtained as added value compounds, contributing to the integral use and valorisation of the product, with zero residues, in the framework of the circular economy.

Previously reported starch extraction from tiger nut tubers has been performed by sodium metabisulfite extraction, alkaline extraction with NaOH, and water extraction. The yields previously reported were around 21% (Umerie et al., 1997). Sodium metabisulfite acts as a reducing agent; it denatures proteins and enzymes that contribute to starch degradation during the extraction process. It preserves colour as enzymatic activity can also lead to unwanted browning, and it also has antimicrobial properties (Deepa & Pongodi Vijayakumar, 2013). The alkaline medium helps in the partial solubilisation of starch, which is water insoluble, promoting swelling of granules, which aids the extraction process. As described for sodium metabisulfite, sodium hydroxide can also contribute to neutralising the enzymes and proteins that could break down the starch molecules during the extraction process.

Common to all described extraction processes, the first step is an initial washing of the tubers and their preparation for the process. In sodium metabisulfite extraction, tubers are first boiled in ethanol to deactivate enzymes. Defatting is a common step in all extractions reported as tiger nut tubers have a high lipid content. The extraction step is accomplished by wet milling the tubers, using only water or aqueous solutions of sodium hydroxide or sodium metabisulfite. The obtained slurry is filtered, and the suspension is left to stand to allow the settling of the starch granules. The recovered starch is washed several times to remove impurities and reactive traces. Finally, it is dried in an air oven not exceeding 50 °C for 18–24 h, pulverised, and correctly stored for further analysis (Akonor et al., 2019; Bolder et al., 2014; Jing et al., 2012; Manek et al., 2012).

People consume starch either directly from food or from products developed using commercial starch. It is commercialised in its original granular form, which is easily isolated by wet milling and often modified to alter its properties to meet the market needs. This market continues to expand year after year as industrial uses for starch are very diverse. It is used in the agrochemical, cosmetic, detergent, food, medical, paper, textile, or plastic industries (Ellis et al., 1998).

Because of its versatility, starch is a very significant global commodity that had a market estimated at 134.5 million Metric Tons in 2022 and is expected to reach 199.8 million Metric Tons by the year 2030 (Market Research, 2023). Starch is in various parts of plants such as the leaves, stems, roots, seeds, fruits, and storage organs (tubers); this makes the source for starch obtention directly the plant material. Nowadays, the most important crops used for starch production are cereals (maize, wheat, barley, rice), roots or tubers (potato, cassava, sweet potato), and legumes (peas, bean, chickpeas), whereas tiger nut could also be an interesting source of starch, especially if it is obtained from the tiger nut milk processing waste that is now underutilised.

Nowadays, the production, preparation, and consumption of food give rise to significant amounts of solid and liquid residues. This waste is becoming progressively difficult to manage, raising concerns about potential pollution. Furthermore, it signifies the loss of valuable biomass and essential nutrients. Horchata solid residue (HSR) has a non-nitrogenous content of 28.7 g/100 dry residue (Sánchez-Zapata et al., 2009), so it could be an interesting source of starch for different uses and applications, giving added value to this waste. Other components, such as oil, fibres or antioxidants could also be obtained in the framework of a refinery concept.

The aim of this study was to evaluate the feasibility of obtaining starch from the horchata solid residue using a high-shear homogenisation process followed by filtration and starch separation from the resulting slurry. The effect of different variables (homogenisation time, kind of aqueous solvent, washing cycles of the filtered residue and phase separation in the slurry) on the starch yield and purity was analysed. Obtained starch was characterised as to different relevant properties: amylose/amylopectin ratio, granule morphology and size, crystallinity degree, water holding capacity and solubility, gelatinization behaviour and thermal stability.

Materials and Methods

Materials

The solid residue from the horchata elaboration process was provided by Horchatería Rin (Alboraia, Valencia), with 62% moisture content, and stored at −18 °C until further use. Total starch (K-TSTA) and amylose/amylopectin (K-AMYL) determination kits were purchased at Megazyme Ltd. (Wicklow, Ireland). Sodium hydroxide, sodium metabisulfite, anhydrous sodium acetate, sodium chloride, calcium chloride dihydrate, magnesium chloride hexahydrate, manganese chloride tetrahydrate, and glacial acetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Sodium carbonate, phosphorus pentoxide, and magnesium nitrate-6-hydrate were supplied by Panreac Química, S.A. (Castellar del Vallès, Barcelona, Spain).

Composition of the Horchata Solid Residue (HSR)

Moisture (AOAC 934.06), protein (AOAC 920.152 using a conversion factor of 6.25), ash (AOAC 923.03), fibre (AOAC 991.43), and fat (AOAC 983.23) contents were determined in triplicate. Total starch was determined using a Megazyme K-TSTA kit following the AOAC 996.11 method (Horwitz, 2000).

Starch Extraction Process

Starch extraction from the horchata solid residue was designed based on the water extraction methodology previously described by other authors (Akonor et al., 2019; Builders et al., 2014; Jing et al., 2012; Li et al., 2017; Manek et al., 2012) for tiger nut tubers. This process, described in Fig. 1, consisted of aqueous high shear homogenisation of the residue, to favour the release of the starch granules from the plant matrix into the aqueous phase, and component separation through

different steps: (1) filtration to separate the non-water dispersible coarse particles (low starch residue) and the slurry that mainly contain starch granules and emulsified fat droplets, (2) gravitational phase separation in the slurry (sedimentation of starch granules and creaming of emulsified fat) and isolation of each phase. Before the extraction step, the horchata solid residue (HSR) was thawed for 1 h at 5–7 °C and then homogenised with the aqueous solvent (1:10 ratio) at room temperature by using a Thermomix TM-5 (Vorwerk Spain M. 1.1, S.C. Madrid, Spain). A high homogenisation rate (10,200 rpm) was selected in order to favour the plant cell disruption and the starch release to the aqueous medium, whilst two levels of homogenisation time were considered (2 and 5 min) on the basis of preliminary trials. Filtration was carried out in two steps: first, draining in a muslin cloth and subsequent filtration of the starch slurry through a nylon filter of 74 µm. Gravitational phase separation in the slurry was carried out by density difference of dispersed components, with starch remaining at the bottom due to its higher density. Natural sedimentation for 18 h at 5–7 °C and centrifugation at 15,000 g for 1 h were used in the phase separation step. After phase separation, the aqueous phase was decanted to obtain the sedimented starch. The sediment was washed in triplicate by re-dispersing the solid particles by manual stirring with copious amounts of water till obtaining a transparent liquid phase, and washed starch recovered by centrifugation at 15,000 g for 3 min. The washed wet starch was dried at 50 °C in a convection oven for 24 h to obtain the starch powder which was grounded with a glass mortar and properly stored for further analysis. A washing step of the extraction residue to recover the retained starch was also considered in the second experimental series. This step consisted of re-dispersion of solid particles in water (ratio 1:10), by manual stirring and subsequent filtration, incorporating the filtrate to the initially separated slurry. Zero, one, or two washing cycles were applied to analyse their impact on the process yield. A lower ratio of solids-water was used in the washing cycles: 1:4 and 1:3 for the first and second cycles, respectively.

Three experimental series were conducted to select the best values of the process step variables that provide good starch yield and purity. Each experimental series was performed in duplicate and starch yield was determined in each case as the ratio of the dried starch mass with respect to the corresponding dried mass of the HSR and expressed as a percentage. The *first experimental series* aimed to analyse the influence of the extraction solvent (pure distilled water, NaOH 13 mM or Na₂S₂O₅ 4 mM) using 2- and 5-min homogenisation time and natural sedimentation as the phase separation method. To promote the effect of reactants (NaOH or Na₂S₂O₅),

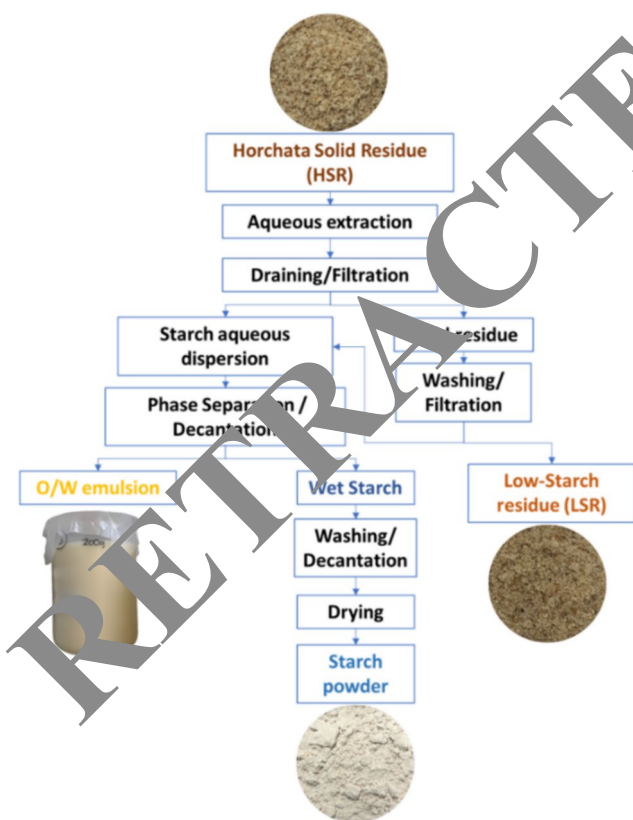


Fig. 1 Flow chart of the starch recovery process from the horchata solid residue (HSR)

a steeping step of HSR in the aqueous solutions was performed for 4 h at room temperature with a constant stirring of 800 rpm, before the homogenising step. A comparison of the obtained starch yield, purity, and whiteness index (WI) values of the different samples was carried out, as well as a comparison with other reported values, using different methods for starch extraction, such as the alkaline method with NaOH (Li et al., 2017) or the sodium metabisulfite method (Builders et al., 2014; Manek et al., 2012). The *second experimental series* analysed the impact of applying washing cycles to the extraction residue on the process yield and starch purity. One or two washing cycles were applied, and the starch yield, purity, and whiteness index were compared to those obtained without washing cycles. The *third experimental series* aimed to select the phase separation method. To this end, two homogenisation times (2 and 5 min) were tested, using natural sedimentation for 18 h and centrifugation at 15,000 g for 1 h in the phase separation step. In this series, no washing step of the extracted solid residue was applied. A comparison of obtained starch yield, purity, and white index was also performed.

Characterisation of the Obtained Starch

Starch Composition and Purity

Moisture (AOAC 934.06), protein (AOAC 920.152 using a conversion factor of 6.25), ash (AOAC 923.03), fibre (AOAC 991.43), and fat (AOAC 983.23) were determined in triplicate. Purity was measured with the Megazyme total starch assay kit (K-TSTA, Megazyme Int. Ireland Ltd., Co. Wicklow, Ireland), based on the AOAC 996.11 method (Horwitz, 2000).

Whiteness Index

The colour determination to obtain the CIE $L^*a^*b^*$ colour coordinates (L^* , a^* , b^*) was conducted using a Spectrocolorimeter (CM-360d, Minolta Co. Tokyo, Japan) with 8 mm target mask. Whiteness index (WI) was calculated using Eq.

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

Morphology of Starch Granules

Microscopical observations of extracted starch were carried out using an optical microscope (Optika Microscope B-350, Italy) equipped with a camera (Optikam B2). The diameter of

at least 200 granules was measured using the Optika Vision Lite programme. Additionally, the microstructure of different samples was characterised by High-Resolution Field Emission Scanning Electron Microscope (GeminiSEM 500, Zeiss, Oxford Instruments, Oxford, UK). For this analysis, samples were gold-coated before the microscopic observations, carried out at an accelerating voltage of 3 kV.

Particle size distribution of the starch granules was obtained with a laser diffraction particle size analyser (Mastersizer® 2000, Malvern Instruments Ltd., Malvern, UK) equipped with a wet dispersion unit (Hydro 2000 MU). The laser diffraction method and Mie theory, following the ISO 13320 normative (AENOR, 2009), were used. Particles were dispersed in water at 25 °C using ultrasound and constant stirring at 1900 rpm. Particle refraction and absorption indexes were, respectively, 1.52 and 0.1. The mean value of ten replicates was reported.

Thermal Analysis

Differential scanning calorimetry (DSC) was used to determine the gelatinisation temperature and enthalpy of the extracted starch. A DSC 1 STARe System analyser (Mettler Toledo, Switzerland), operating under nitrogen flow (30 mL min⁻¹) was used. Samples (1:4, 2:3, and 3:2 starch:water ratios) were prepared as a paste and then placed into medium pressure 7 mm diameter aluminium crucibles with o-ring (ME 2692) to be hermetically sealed. Samples were conditioned at 25 °C for 1 h and submitted to heating from 25 to 150 °C at 5 °C min⁻¹ to obtain the gelatinisation endotherm.

The thermal stability of dried starch was determined using a thermogravimetric analyser (TGA/SDTA 851e, Mettler Toledo, Schwarzenbach, Switzerland) by heating the samples (about 4 mg) in an alumina crucible from 25 to 900 °C, at 10 °C min⁻¹ under nitrogen flow (30 mL min⁻¹).

X-Ray Diffraction Analysis (XRD)

The X-R diffraction spectra of starch samples was obtained, using an X-ray diffractometer (Bruker AXS D8 Advance, Karlsruhe, Germany) with K α -Cu radiation (λ : 1.542 Å), operating at 40 kV and 40 mA. Scanning between diffraction angles of 5° and 40°, with a step size of 0.05 and a dwell time of 1 s was considered. The data was obtained using XRD Commander software (Bruker AXS GmbH, Karlsruhe, Germany) and processed with DIFFRAC.EVA (Bruker AXS GmbH, Karlsruhe, Germany) and DRXWin software. The degree of crystallinity was expressed as a percentage and calculated with Eq. 2.

$$X_c = A_c / (A_a + A_c) \quad (2)$$

where X_c is the degree of crystallinity, A_c is the crystalline area, and A_a is the amorphous area of the spectra.

Solubility and Swelling Power

Solubility was determined following the method described by other authors (Akonor et al., 2019; Liu et al., 2021) with slight modifications. Approximately 150 mg of starch sample was weighed (W_1) into centrifuge tubes, and 10 mL of distilled water was added. The suspension was vortexed at 2500 rpm and incubated at 85 °C in a water bath at constant stirring for 30 min. The samples were left to cool to room temperature and centrifuged at $3000 \times g$ for 35 min. The supernatant was carefully decanted and dried in a convection oven until constant weight was reached at 105 °C (W_2). The swollen starch remaining in the centrifuged tubes was also weighed (W_3). Solubility and swelling power were calculated using the equations:

$$\text{Solubility (\%)} = \frac{W_2}{W_1} \times 100 \quad (3)$$

$$\text{Swelling power} = \frac{W_3}{W_1 \left(1 - \frac{\text{solubility}}{100}\right)} \quad (4)$$

Amylose/Amylopectin Ratio

Starch samples were previously treated with DMSO and ethanol to remove lipids, and then, a modification of the Concavalin-A method (Yun & Matheson, 1995) was employed using a Megazyme K-AMYL amylose/amylopectin determination kit.

Statistical Analysis

The statistical analysis of the data was carried out by one-factor (ANOVA) or multifactorial (MANOVA) analysis of variance, using Statgraphics Centurion XVIII software (Statgraphics Technologies, Inc. The Plains, Virginia). Fisher's least significant difference at a 95% confidence level was used. Significant differences between treatments are indicated in the tables with different letters as superscripts (abc...).

Results and Discussion

Starch Separation Process: Yield and Purity

The horchata solid residue used in the study had $62.6 \pm 0.7\%$ moisture content, whilst the dried solids contained $61 \pm 2\%$ of total fibre, $7.2 \pm 0.2\%$ of fat, $3.7 \pm 0.2\%$ of protein, $2 \pm 0.5\%$ of ashes and $22.7 \pm 1.0\%$ of starch. These components are arranged in the ground cellular structure of tiger nut that has been previously water extracted during the processing to obtain horchata (tiger nut milk). Figure 2 shows the microstructure of the residue at different magnifications where the different cell-constituted particles can be observed highly washed during the milk processing. At a higher magnification, the remaining cell walls and cavities containing different ratios of adhered starch granules on the particle surface. A higher content of starch granules and other cell components is expected to be present in the internal cells of the particles. In fact, the usual content of tiger nuts ranges from about 30%, only slightly higher than that determined in

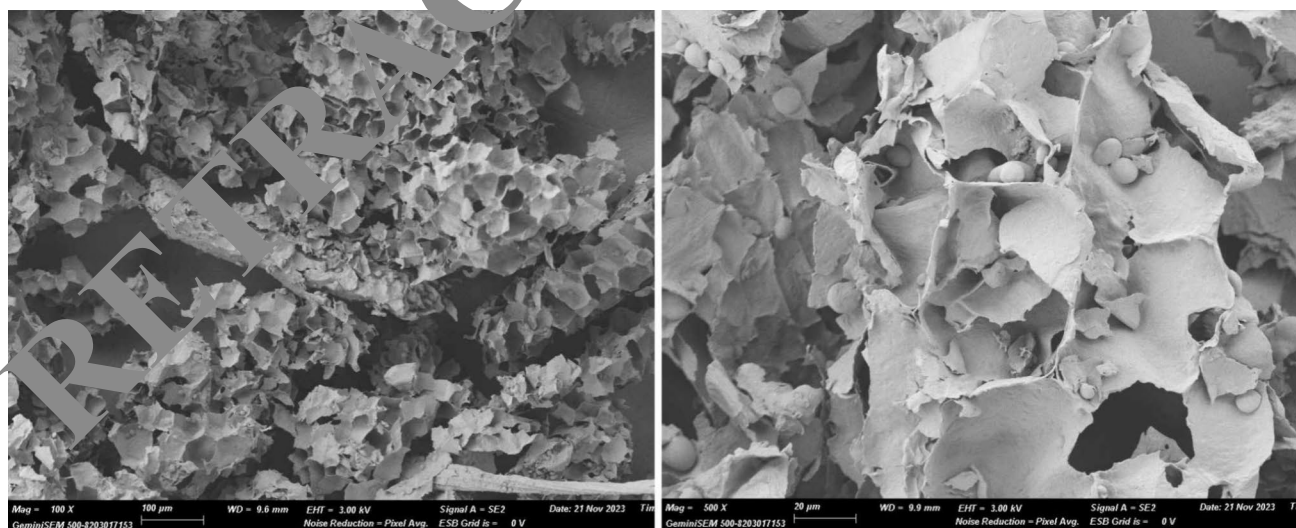


Fig. 2 FESEM micrographs of the horchata solid residue (HSR) at different magnifications show the cellular nature of the HSR particles and the residual starch granules adhered to the open, external cells of the particles

the HSR. This suggests that a strong homogenisation process would be required to release the remaining internal starch of the particles.

In this sense, the process shown in Fig. 1 was applied in the first experimental series considering two homogenisation times (2 and 5 min) and three different solvent types to analyse their effect on the starch release and its final yield and purity, as well as the whiteness index (WI) of the obtained starch powder after phase separation of the homogenised slurry. The increase in the homogenisation time may contribute to the plant tissue disruption, affecting the release degree of starch granules from the most internal cells. Starch granules with higher density (about 1500 kg/m³) were sedimented at the bottom of the container, but other particles of similar density, such as cellulosic cell walls, can contaminate this fraction. Decantation and water washing allow for the starch-rich powder to be obtained after the subsequent drying.

Table 1 shows the mean values and standard deviation of the three parameters measured in the starch powder for the treatments applying 10,200 rpm for 2 and 5 min, using pure water and alkaline and reducing water solutions. A multifactor ANOVA was applied to the data to better reflect the influence of factors (homogenisation time and solvent) on the analysed parameters. As concerns starch yield, both factors, time ($F=80.1$) and solvent ($F=7.8$) significantly affect the starch yield, as well as their interactions ($F=14.2$). As expected, starch yield rose as the homogenisation time increased since more starch granules were delivered from the internal cells of the initial particles, becoming more disrupted during the homogenisation step. The solvent effect was less significant (lower F values), but also affected to a different extent, depending on the time. Specifically, at short homogenisation time, a similar starch yield was obtained in alkaline and neutral water that was higher than in sulphite solution. In contrast, with longer homogenisation time, the yields were very similar for the three solvents (about 14%).

When the starch yield is expressed with respect to the total starch content of dried HSR, the values ranged between 56.9–63.0% (water extraction), 47.4–63.9% (Na₂S₂O₅ extraction), and 50.5–59.6% (alkali extraction). These yields did not reflect a notable improvement of the starch recovery by using metabisulfite or alkali extraction. Nevertheless, in all

cases, these were lower than that obtained in other studies with different starch sources. Tessema and Admassu (2021) reported 68, 71, and 74% yields for starch obtained from anchote tubers, using water, metabisulfite, and sodium hydroxide as extraction solvents, respectively. Likewise, alkali extraction also led to a higher starch yield from chestnuts than water extraction (81.7 against 68.4%) (Correia & Beirão-da-Costa, 2010). Usually, methods including alkaline or sodium metabisulfite extraction yielded more starch due to the breakdown of structural components or the protein-starch complexes, favoring the starch delivery. However, this effect can vary depending on the starch source. The HSR used obtained after the horchata production, could be even affected by the action of natural enzymes which could make the starch more accessible regardless the solvent used at high homogenisation speed.

In terms of starch purity, no significant effect of homogenisation time nor of extraction solvent was observed. Nevertheless, starch purity slightly tended to decrease when homogenisation time rose due to the promotion of higher release of small cellular fragments with similar density to starch, such as cell wall fragments. Likewise, the highest purity values were obtained in pure water at both homogenisation times, where starch purity tended to decrease in the metabisulfite and alkali extractions, mainly when the homogenisation time rose. This could be attributed to the greater cellular rupture provoked by these solvents, generating particles with similar density to starch that contaminate the sediment. The purity of starch greatly affects the quality and applicability of the starch, leading to different functional properties of starch (Ghoshal & Kaur, 2023; Liu et al., 2024; Nie et al., 2023; Rashwan et al., 2024). Greater purity implies better separation of starch from proteins, lipids, and fibres, this affecting the final starch concentration and influencing the water solubility, the viscous properties or the colour and clarity of the starch solutions (Cornejo-Ramírez et al., 2018).

Figure 3 shows the FESEM micrographs of the starch powders obtained in the three solvents, where no qualitative differences between samples were observed. Separated granules with a smooth surface, spherical and oval-shaped appear, together with some granule aggregates adhered to the cell wall rests. This indicates that starch interactions with the polysaccharidic cell wall components (with

Table 1 Yield, starch purity, and whiteness index of the obtained starch at 10,200 rpm for 2 and 5 min, using water, NaOH 13 mM, or Na₂S₂O₅ 4 mM as an extraction solvent, and natural sedimentation as phase separation method (first experimental series)

Extraction solvent	Time (min)	Starch yield (g/100 g)	Starch purity (g/100 g)	Whiteness index
Water	2	13.1 ± 0.3 ^a	87 ± 2 ^a	95.17 ± 0.03 ^a
	5	14.5 ± 0.5 ^a	85 ± 2 ^a	94.97 ± 0.04 ^a
Na ₂ S ₂ O ₅	2	10.9 ± 0.2 ^b	86 ± 5 ^a	95.32 ± 0.01 ^a
	5	14.7 ± 0.6 ^a	79 ± 4 ^a	95.19 ± 0.06 ^a
NaOH	2	13.0 ± 0.8 ^a	81 ± 7 ^a	95.48 ± 0.02 ^a
	5	13.7 ± 0.1 ^a	79 ± 2 ^a	95.19 ± 0.05 ^a

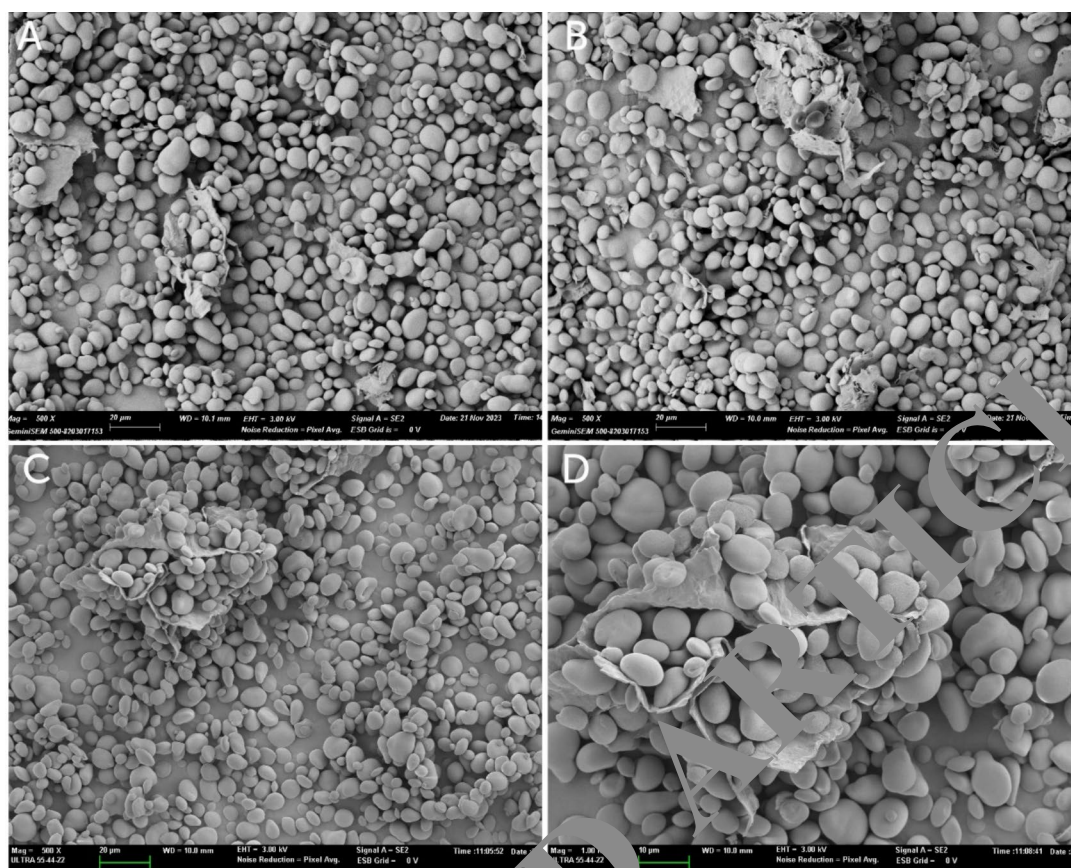


Fig. 3 FESEM micrographs of HSR starch powder obtained by 5 min homogenisation time with **A** sodium metabisulfite ($\times 500$), **B** sodium hydroxide ($\times 500$), and **C** distilled water ($\times 500$). **D**: water extracted starch powder at higher magnification ($\times 1000$)

similar density) limited the granule isolation to a certain degree, regardless of the aqueous solvent used, affecting the starch purity degree. The structure of cell wall-starch clusters can be better observed in Fig. 3D at higher magnification, where the tightly packed starch granules appear into de cell wall cavity.

Similar behaviour was observed for the WI, where the longer homogenisation time provoked a significant decrease of WI ($F=133.8$) whilst solvent also affected ($F=81.6$) as well as their interactions ($F=6.85$). The lowest values of WI were obtained in water, whereas the highest values were reached in the alkaline medium. Nevertheless, at 5 min homogenisation, WI ranged between 94.7 (in water) and 95.19 (alkaline medium), which suppose a hardly appreciable difference. The loss of WI was mainly related to the delayed sedimentation on the top of a starch layer of small particles coming from the disrupted peel cells of tiger nuts with brown colour. Figure 4 shows the appearance of the starch sediment (top layer) with the brown top layer and the microstructure of this top layer observed by FESEM. The thin coating of starch

granules with other lamellar particles can be observed in the micrograph, as well as the presence of other cellular fragments embedded in the starch bed. These fragments, polysaccharidic in nature, with similar density to starch, will contribute to reducing the separated starch purity.

Based on the obtained results with the different solvents, water would be more convenient than solutions with chemicals in order to ensure a cleaner process. In this sense, 5 min homogenisation in water could be proposed, which allows for obtaining 14.5 g of starch powder per 100 g of dried HSR, with a purity of about 85% and WI of 94.97.

Another critical point of the process was the recovery of starch retained in the homogenisation residue after draining and filtering. In this sense, 1 and 2 washing cycles (WC) were applied, using pure water-residue in a ratio of 4:1 and 3:1, respectively, and the yield, purity and WI of starch powder were compared with respect to that obtained without washing cycles. Table 2 shows the mean values and standard deviation obtained for each treatment applying 2- and 5-min homogenisation times. WC ($F=36.3$) and time ($F=17.1$) significantly affect starch yield without

significant interactions between these factors. As previously observed, the highest time increased the yield as well as the WC, mainly the first cycle. As expected, an opposite, less significant effect was observed in starch purity for WC ($F=14.8$) and time ($F=3.1$). In agreement with this, WI significantly decreased when time ($F=55.5$) or WC ($F=125.1$) rose. Based on these results, 5 min homogenisation and one WC would be recommendable to increase starch yield up to 16.6%, without notable loss of purity (up to 82%) or WI (up

to 94.55). In this case, 60% of the starch content of the HSR was recovered, similar to those recovered with 2 WC, but with higher starch purity.

To better analyse the effect of WC on the starch partition between the slurry and low starch residue (LSR) obtained after draining and filtration, the yield and final starch content were also analysed for the LSR in the two dried fractions obtained in the muslin draining and filtering. These values are shown in Table 3, where the reduction of starch content

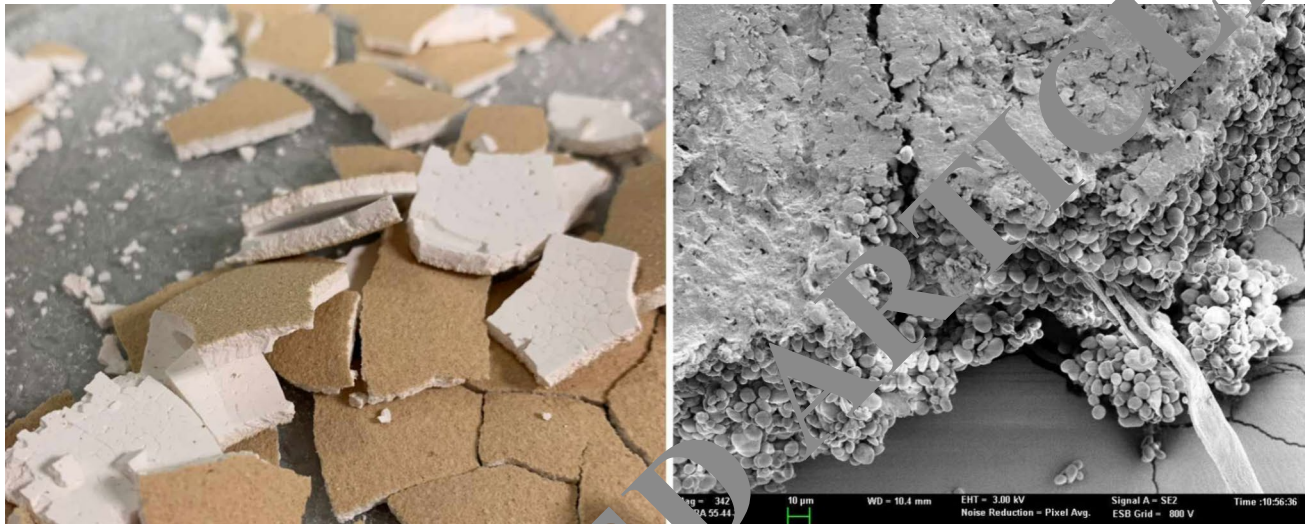


Fig. 4 Appearance of the brown top layer in the starch sediment after the phase separation step and FESEM micrograph of this layer

Table 2 Yield, starch purity, and whiteness index of the obtained starch using different washing cycles of the extraction residue (second experimental series)

Time (min)	Washing cycles	Starch yield (g/100 g)	Starch purity (g/100 g)	Whiteness index
2	0	13.1 ± 0.3 ^a	87 ± 2 ^a	95.17 ± 0.03 ^a
	1	15.5 ± 1.1 ^b	82 ± 0 ^{ab}	94.96 ± 0.04 ^b
	2	15.8 ± 1.7 ^b	80 ± 2 ^b	94.65 ± 0.06 ^c
5	0	14.5 ± 0.5 ^a	85 ± 2 ^a	94.97 ± 0.04 ^b
	1	16.6 ± 0.9 ^b	82 ± 4 ^{ab}	94.55 ± 0.02 ^c
	2	17.6 ± 1.0 ^b	75 ± 1 ^b	94.84 ± 0.02 ^b

Table 3 Values of yield (g/100 g dried HSR) and starch content of the low starch residue (LSR) in the fractions obtained by draining (D) and subsequent filtering (F). The total starch content of the LSR and total starch losses throughout the process with respect to the HSR starch content are also shown

Time (min)	Washing cycles	Yield (g/100 g HSR)		Starch content (g/100 g)			Starch loss (%)
		D Fraction	F Fraction	D Fraction	F Fraction	Total LSR	
2	0	64.6	8.1	8.0	15.3	8.8	22
	1	46.7	2.0	6.4	8.8	6.5	30
	2	57.0	0.8	5.0	14.0	5.1	31
5	0	51.0	12.1	6.7	16.3	8.6	22
	1	64.5	0.5	6.8	9.3	6.8	21
	2	53.6	0.6	4.3	10.2	4.3	32

*D, drained with muslin cloth; F, filtered with nylon 74 µm filter; HSR, horchata solid residue; and LSR, low starch residue

in the total LSR significantly decreased with WC from about 9 to 5% for 2 min homogenisation and from 9 to 4% for 5 min homogenisation, thus reflecting the positive effect of both the highest homogenisation time and WC on the starch recovery from the HSR. As expected, the starch richness of the fractions retained in the filter (the smallest particles) was higher than in the drained fraction, but the former represented a very small fraction of the total LSR.

Figure 5 shows the FESEM micrographs of the LSR after 0 and 2 washing cycles, where the lower presence of starch granules on the open cells can be qualitatively observed. The values of starch loss percentage (non-quantified in the sediment or LSR) with respect to the content of HSR in the whole process, throughout the phase separation and washing steps are also shown in Table 3. These were deduced from the mass balance considering starch powder and dried LSR and the total content in the dried HSR. These values ranged between 20 and 30% of the total starch content of the HSR.

A third experimental series was carried out to compare the efficiency of centrifugation and natural sedimentation in the phase separation step, applying 2- and 5-min homogenisation times. Table 4 shows the yield, purity and WI of the obtained

starch powders for the different treatments. MANOVA did not reflect a significant effect of the phase separation method ($F=3.47$) against the significant effect of homogenisation time ($F=53.8$) on the starch yield. Nevertheless, centrifugation significantly ($F=21.40$) increased the starch purity up to 92–96%, whereas the statistical significance of homogenisation time was reduced ($F=2.65$). WI also significantly increased in centrifuged samples ($F=21.2$), but it was significantly reduced ($F=67.3$) when homogenisation time rose. This suggests that centrifugation allowed for more selective sedimentation of cellular fragments but not enough to separate the brown fragments of tiger nut peel that are more present when the homogenisation time rose. Therefore, centrifugation instead of natural sedimentation allows for better phase separation and starch purity, although it did not improve the WI of the starch powder.

Hence, based on the obtained results, 5-min homogenisation time with distilled water, one WC and the slurry centrifugation would be the best conditions to enhance the starch yield and purity of the recovery process of starch from the HSR, giving rise to an adequate starch powder for different uses. The relevant properties of this product are discussed in the next section.

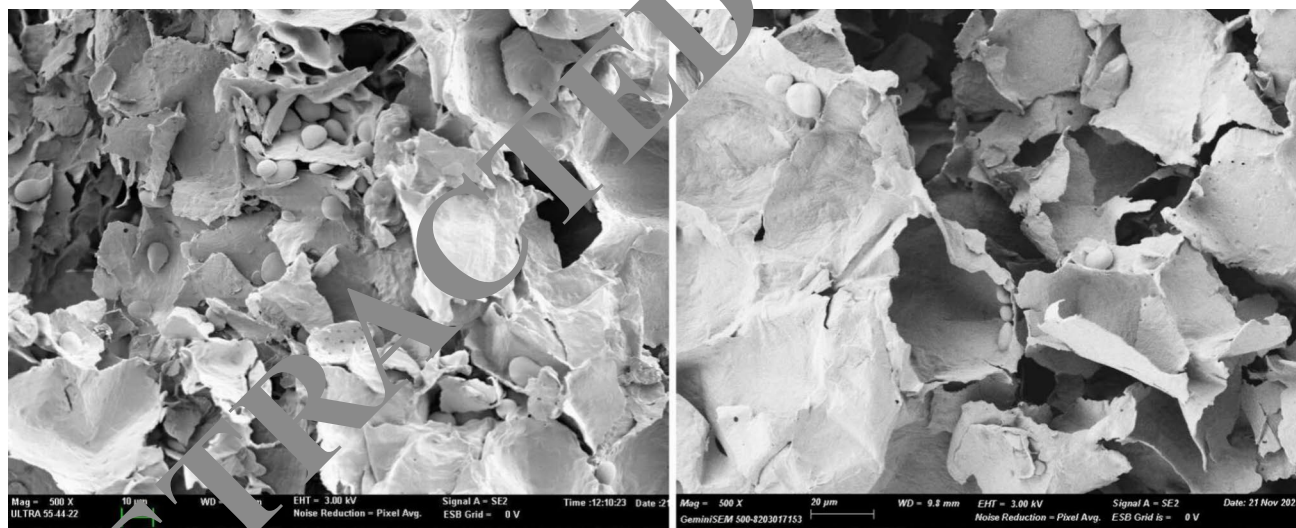


Fig. 5 FESEM images of the low starch residue (LSR) after 0 and 2 washing cycles ($\times 500$)

Table 4 Yield, starch purity, and whiteness index of the obtained starch in the different treatments as a function of the homogenisation time and phase separation method

Phase separation method	Time (min)	Starch yield (g/100 g)	Starch purity (g/100 g)	Whiteness index
Sedimentation	2	13.1 $\pm$ 0.3 ^b	87 $\pm$ 2 ^b	95.17 $\pm$ 0.03 ^a
	5	14.5 $\pm$ 0.5 ^a	85 $\pm$ 2 ^b	94.97 $\pm$ 0.04 ^b
Centrifugation	2	12.7 $\pm$ 0.4 ^b	96 $\pm$ 1 ^a	95.03 $\pm$ 0.03 ^a
	5	14.1 $\pm$ 0.8 ^a	92 $\pm$ 3 ^a	94.93 $\pm$ 0.01 ^b

Physicochemical Properties of Isolated Starch

The obtained dried starch contained 9.1% moisture content, whilst the dried solids contained low traces of total fibre, protein, lipids, and ash: 0.5%, 0.15%, 1%, and 0.4%, respectively. Other authors report similar results for tiger nut starch extracted directly from the tubers (Jing et al., 2012; Neto et al., 2018; Salwa et al., 2010). The amylose content of the obtained starch was 23%, similar to that previously reported for the tuber starch (Lv et al., 2022; Wang et al., 2022). Amylose and amylopectin are interspersed and intertwined with each other to preserve the integrity of the granule; amylose holds amylopectin molecules together (Jane, 2006), so the amylose content affects many fundamental properties of starch, such as solubility and swelling power. The solubility value at 90 °C was 6%, notably lower than previously reported values by other authors for tiger nut starch. Akonor et al., (2019) reported solubility of starch from whole tubers of 11.6% and 13.5% for black and yellow cultivars, respectively, whilst higher values (up to 18.6%) were obtained by Liu et al. (2019). Even though solubility was tested at higher temperature than gelatinization temperature to promote solubility, it resulted in a low value. The swelling power obtained in water was 10 g /g, similar to previously reported values (Akonor et al., 2019; Manek et al., 2012). It is a measure of the associative forces in the granules as well as their hydration capacity. The smaller granular size tends to confer more compact packing, hence a greater degree of association, which results in lower swelling power than other starches, such as maize or potato starch (Manek et al., 2012).

Morphology and Crystallinity

Figure 6 show the micrographs of starch granules obtained by optical microscopy with and without polarised light and FESEM micrographs. The well-preserved starch granules can be observed in the micrographs, with the typical spherical-oval shape and smooth surface. The crystalline nature of the granules could be confirmed from the images with polarised light (Fig. 6B). No notable differences in the granule morphology and size were observed with respect to that reported by other authors for tiger nut starch granules (Akhobor et al., 2018; Akonor et al., 2019; Jing et al., 2012; Manek et al., 2012).

Starch crystallinity was also quantified through the XRD spectrum (Fig. 6D) shows the typical X-ray diffraction pattern of the crystalline structure of amylose and amylopectin in starch granules with A-type structure, more typical of cereal starch (Zobel et al., 1995; Ai & Jane, 2015). This exhibited high-intensity peaks at 15°, 17°, 18°, and 23° Bragg diffraction angles (2 θ) whilst the rest of the diffractogram

is conformed of amorphous scattering. The diffraction pattern of *Cyperus esculentus* starch is very similar to maize starch, which can be attributed to similarities in amylose/ amylopectin contents, as well as to their particle size distribution. The degree of crystallinity obtained was 38.4%, similar to that previously reported for this tuber starch. It was higher than that reported for potato starch (21%), similar to maize starch (39%) (Manek et al., 2012), and lower than sweet potato starch (56%) (Liu et al., 2024).

Figure 7 shows the size distribution curves of starch granules obtained from image analysis in the optical micrographs and laser diffraction. The starch granules were within the small and medium size ranges. Granule diameters ranged between 3 and 23 μm (mean 8 μm), as deduced from image analysis. In contrast, the particle size distribution obtained by laser diffraction shows a bimodal distribution where the low-size particles had a similar size distribution to that obtained by image analysis, but high-sized particles were also detected. These may be attributed to aggregates of granules produced by orthokinetic flocculation during the measure with the sample agitation, as well as to the present clusters of granules adhered to the cell wall rest, previously described, although the granules were relatively smaller, the shapes and surface features of the HSR starch closely resembled starches from other tropical tubers such as sweet potato.

Gelatinisation Behaviour and Thermal Stability

Starch gelatinisation was analysed at three different starch–water ratios (1:4, 2:3, and 3:2). Table 5 shows the onset, peak, and end temperatures of the gelatinisation endotherms, as well as the enthalpy per mass unit of starch, obtained in each case. No significant changes were observed in the gelatinisation temperature range with the water ratio, but the expected enthalpy decrease was observed when the water availability decreased. Similar values were obtained in previous studies for starch extracted from tiger nut tubers (T_p :70.96 °C and ΔH_g : 16.57 J/g) using a water-starch ratio of 3:1 (Liu et al., 2021). Gelatinisation temperature was in the range reported for other starches, such as potato starch (T_p 62 °C), maize starch (T_p 64 °C), rice starch (T_p 80 °C), or wheat starch (T_p 59–64 °C) (Ellis et al., 1998).

Thermal stability of starch is a very important issue for developing a rational thermal processing technology, such as obtaining thermoplastic starch. Figure 8 shows the obtained TGA curve of the HSR starch where its thermal degradation behaviour can be observed, reflected in three mass-loss events as a function of temperature, as previously observed for other native starches (Liu et al., 2013). The first is associated with the evaporation of bounded and structural water, representing 14% of the total weight

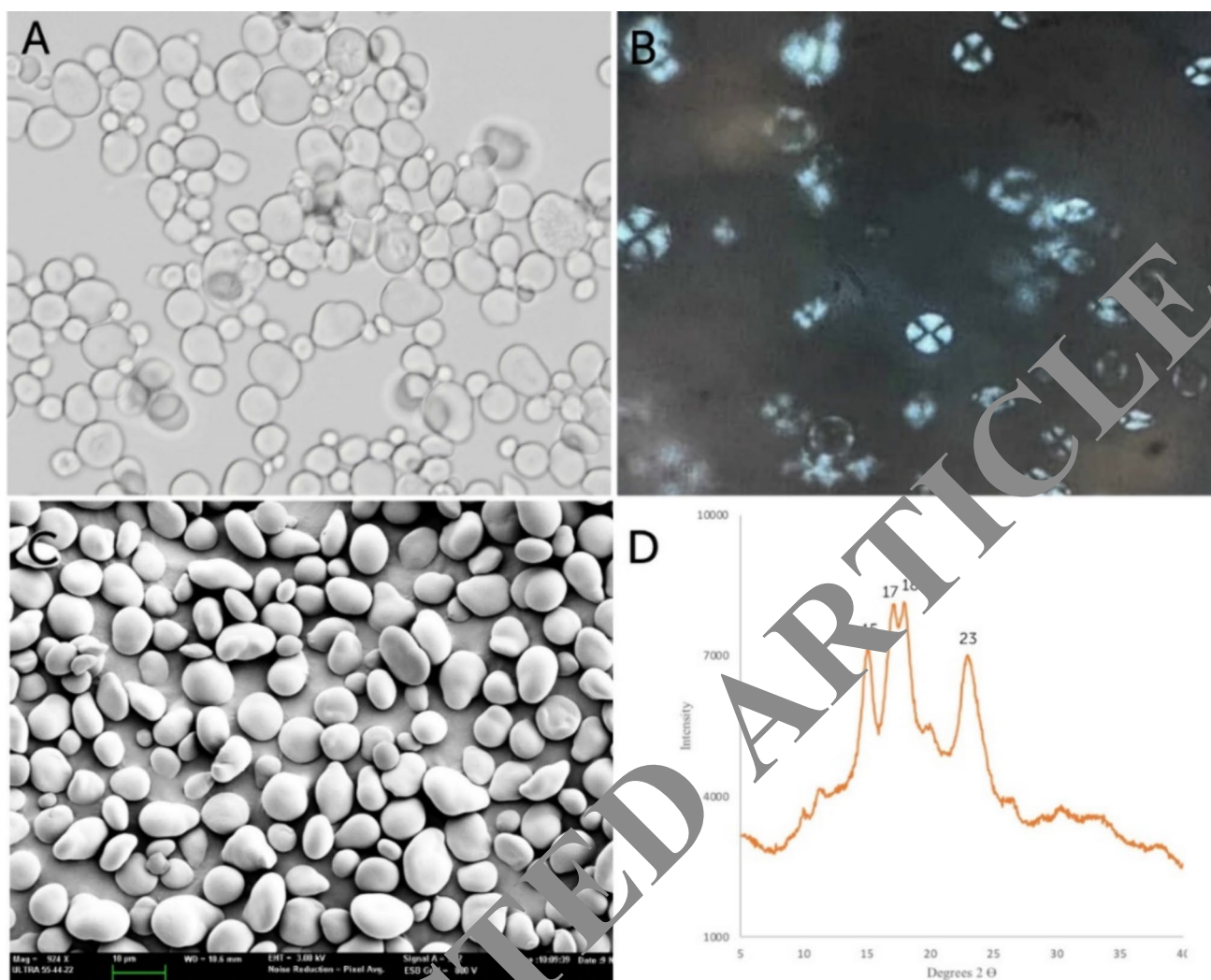


Fig. 6 Microscopic observation of the HSR starch by optical microscopy ($\times 1000$) (A), polarised light optical microscopy ($\times 1000$) (B), FESEM ($\times 1000$) (C), and XR diffraction spectra (D)

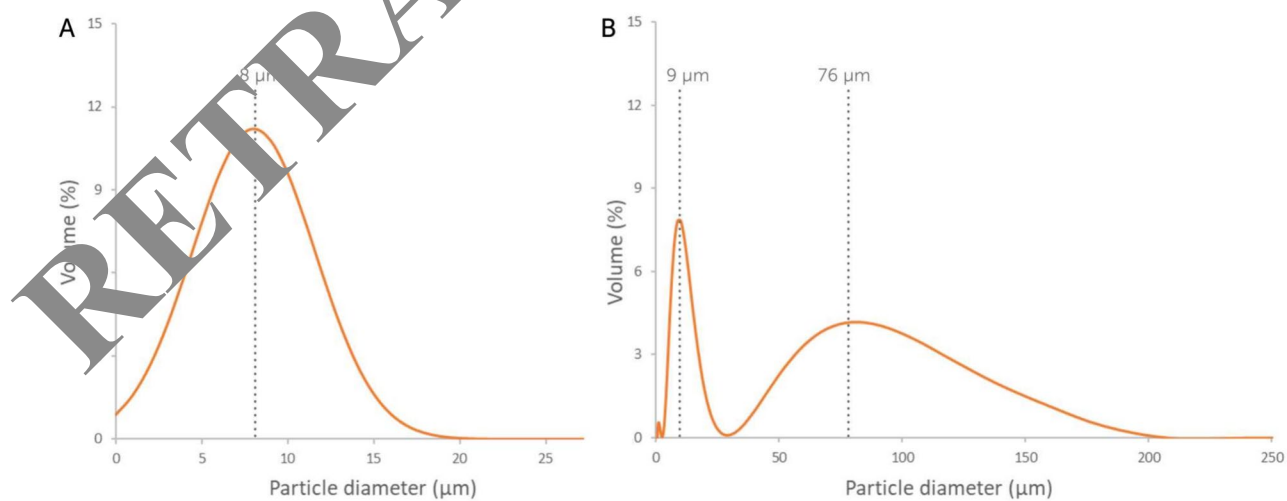
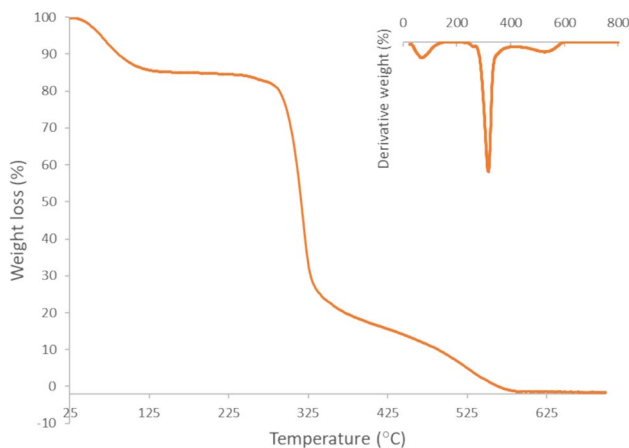


Fig. 7 Particle size distribution of starch granules obtained from microscopic analysis (A) and laser diffraction (B)

Table 5 Gelatinisation temperatures (onset: T_o , peak: T_p , and end: T_e) and enthalpy (ΔH_g , J/g starch) obtained from DSC analysis for HSR starch with different water ratios

Ratio starch:water (w/w)	T_o (°C)	T_p (°C)	T_e (°C)	ΔH_g (J/g starch)
1:4	67.9 ± 0.2	72.50 ± 0.05	77.7 ± 0.4	12.4 ± 0.2
2:3	68.6 ± 1.7	73.4 ± 1.6	80.6 ± 2.8	5.7 ± 0.9
3:2	66.7 ± 0.6	71.9 ± 0.5	76.57 ± 0.06	1.3 ± 0.0

**Fig. 8** TGA curve of starch from HSR and embedded derivative curve

loss, but it would depend on the starch moisture content. This finishes around 130 °C, with T_p (peak temperature: maximum degradation rate) at 73 °C. The second and main degradation event exhibited the highest degradation rate and the highest weight loss (61%); starting at about 320 °C, with T_p at 315 °C, and end temperature at 340 °C. In this event, the decomposition of starch occurred through the long-chain scission and subsequent glucose ring degradation (Liu et al., 2013). Thermal degradation temperatures of the obtained starch were similar to those of other native starches, such as maize starch (Awale & Lele, 2000). Liu et al. (2013) reported that the thermal stability of starch decreased with increasing amylose content, but no significant relationship between microstructure (crystallinity, granules size) and the thermal degradation process was observed. The last thermal event occurring after the main degradation step finished around 590 °C with no practical mass residue. This can be attributed to the final degradation of products formed during the main degradation step. Therefore, the thermal stability of the HSR starch was similar to that of other native starches widely used in the thermal processes, exhibiting high stability.

Conclusions

Starch recovery from tiger nut *horchata* solid residue was possible with good yield and purity, using a green process without chemicals. A high-speed homogenisation step with distilled water, followed by filtration and phase separation by density in the slurry, allows for obtaining starch-rich sediment that can be isolated by decantation and purified by water washing. Five minutes of homogenisation at 10,200 rpm allows for obtaining 14.5 g of starch powder per 100 g of dried HSR, with a purity of about 85% and WI of 94.97. Applying 1 washing cycle to the filtering residue increased the starch powder yield up to 27 g/100 dried HSR, without notable losses in starch purity, whereas centrifugation at 15,000 g for 1 h in the phase separation step highly reduced separation time, increasing starch purity up to 92%. In this way, it was possible to recover up to 70% of the starch present in the HSR (23% on a dry basis). The obtained starch had similar morphological characteristics, crystallinity degree and gelatinisation behaviour to that present in the tiger nut, without notable alterations due to the *horchata* processing. It had a relatively high amylose/amylopectin ratio (23% amylose), with moderate solubility and adequate swelling capacity in water and thermal stability. Therefore, HSR can be used as a source of starch for different uses, by applying the proposed process that could be scalable and adapted to recover other interesting compounds, such as fat (separated in the slurry) or insoluble fibres and phenolic compounds present in the filtering residue. In this way, a biorefinery approach could be used to valorise the HSR, obtaining starch and other valued components with near-zero residues in the global process.

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Author Contribution Anita Patrón: Investigation, Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. Amparo Chiralt: Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision, Project administration. Chelo González-Martínez: Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision. Eugenia Martín-Esparza: Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

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