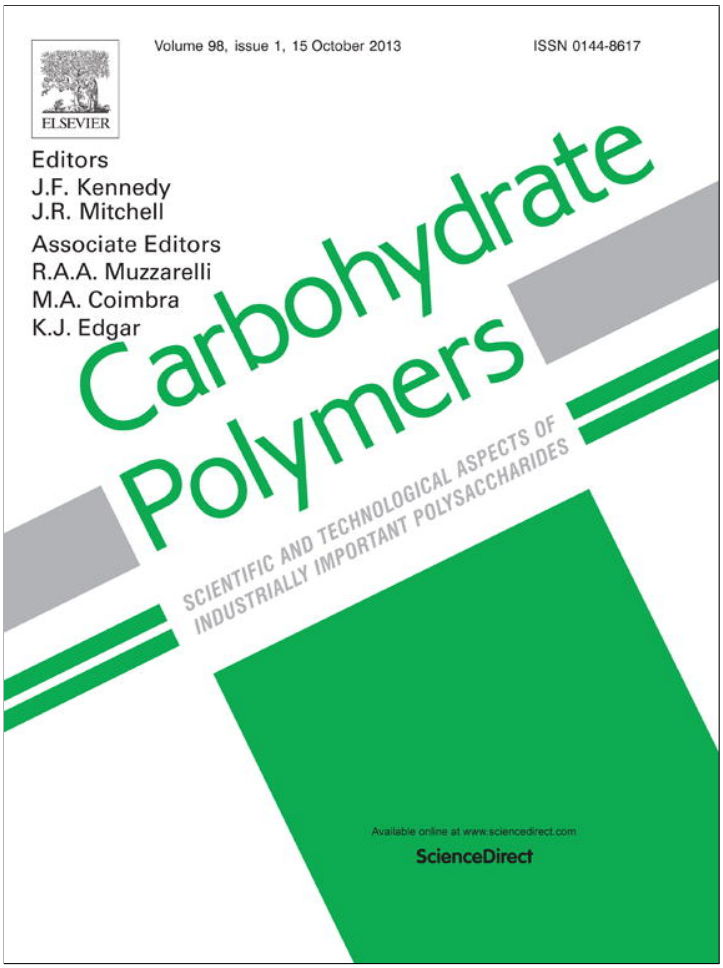

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Isolated starches from yams (*Dioscorea* sp) grown at the Venezuelan Amazons: Structure and functional properties

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ABSTRACT

This work aimed to characterize the molecular structure and functional properties of starches isolated from wild *Dioscorea* yams grown at the Amazons, using conventional and up-to-date methodologies. Among the high purity starches isolated (≥99%), the chain lengths were similar, whereas variations in gelatinization profile were observed. Starches have shown varied-shaped granules with monomodal distribution, and B-type crystallinity. Variations in amylose contents found by three analyses were hypothesized being related to intermediate material. Linear chain lengths were similar, and their amylopectins showed a dense, spherical conformation and similar molecular characteristics. The average molar mass and the radius of gyration of the chromatograms of the yam amylopectin, $\bar{M}_W$ and $\bar{R}_G$ were ranging between 174×10^6 g mol^{-1} and 237×10^6 g mol^{-1}, and 201 nm and 233 nm, respectively. The white yams starches were more sensible to enzymes than the other two. All starches have shown a wide range of functional and nutritional properties.

1. Introduction

Sources of starch commonly used in food technology and commercial applications of the food industry are limited. Moreover, starches have specifically functional properties defined by theirs botanical source. Additionally, processing of starch-based foods may bring about modifications of some specific functional properties that are required for adequate performance of starches in the containing food matrix and final products. The use of non-conventional starches such as those isolated from roots and tubers could provide options for extending the spectrum of desired functional properties, needed for added-value food product development. Due to high staple food demand, it has been postulated (Scott, Rosegrant, & Ringle, 2000) that the total use of roots and tubers in developing countries is expected to increase by 58% (from 232 mT to 635 mT), between 1993 and 2020. Isolation and purification of starches could be an excellent solution to cope with the competitive global market. Thus, there exists a high potential for the profitable commercial use of neglected tropical starches.

Indigenous starchy crops from the tropics are true wonders of nature since only with sun, tropical rain cycles, and without artificial inputs are able to grow in great abundance as staple food for the native local population (Satin, 1999). In the Venezuelan Amazons there are wild varieties of yams (*Dioscorea* genus) with a great diversity in shape and color which are also very rich in carbohydrates – specifically starch – and often contain important phytochemical compounds. Those crops and their starches have not been yet well characterized. Before suggesting any industrial application, their composition and functional properties must be investigated. It may help to drive their food and non-food uses. Further investigations are also needed toward molecular and supramolecular characterization to elucidate their functional behavior.

A preliminary study has been carried out in the Amazons and highlighted an amylose-free resource belonging to the neglected "Mapuey" yams (*Dioscorea trifida*) (Pérez et al., 2011). The authors reported quite high onset gelatinization temperatures and

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enthalpies (69.1–73.4 °C and 22.4–25.3 Jg^{-1}, respectively), as well as relatively high pasting temperatures at 5% starch suspension in the 1430–2250 cP range. Despite the report that the commercial starch from *Dioscorea* spp. displayed a C-type X-ray diffraction pattern (McPherson & Jane, 1999), it has been later established that yam starches from different origins displayed a B-type (Gunaratne & Hoover, 2002; Huang, Lin, & Wang, 2006; Nwokocha & Williams, 2011) with about 37.3% of crystallinity (Nwokocha & Williams, 2011). Recently, Pérez et al. (2011), also reported B-type pattern with a 24% of crystallinity for the wild genotypes exhibiting the highest amylose content. Without starch digestibility profiles, the authors also reported an average granule size in the 24.5–35.5 μm range, whereas amylopectin weight average molar mass ($\bar{M}_W$) by high-performance size exclusion chromatography coupled with multi-angle laser light scattering and differential refractometric index detection HPSEC-MALLS-DRI was surprising 3–5 times lower than those of common yam species (Rolland-Sabaté, Amani N'Guessan, Dufour, Guilois, & Colonna, 2003).

Therefore, this study aims at isolating, purifying and at characterizing the composition, molecular and supramolecular structure, physicochemical and functional properties of unknown starches of five wild yam genotypes belonging to the *Dioscorea* genus and harvested in the Venezuelan Amazons.

2. Materials and methods

2.1. Material

Five *Dioscorea* spp wild genotypes yams, gathered from different plants were collected including two white tubers: one with large size (YWL) and the other one irregularly shaped (YWI), two purple tubers: one with large size (YPL) and the other one irregularly shaped (YPI), and a yellow colored tuber irregularly shaped (YYI). All tubers known as: "ñame blanco", "ñame morado" and "ñame amarillo", were harvested within the "*Piaroa*" community of Puerto Ayacucho, in the Amazonas State, Venezuela. Yams tubers were cleaned and rinsed with a large amount of tap water and hand wiped.

2.2. Methods

2.2.1. Starch isolation and purification

The starch isolation was performed on independent batches of approximately 1–2 kg of each one of five *Dioscorea* spp wild genotypes yams. The starches were isolated and purified using the modified method C described in literature (Pérez, Bahnassey, & Breene, 1993), while extracting starch by blending (with Waring blender with twice the volume of water to edible tuber portion), sieving (200 mesh sieve) and centrifuging (1500 rpm for 15 min) in triplicate, prior to suspension in distilled water and sediment air drying at 45 °C.

2.2.2. Starch purity and physicochemical characteristics

Starches were analyzed for moisture, crude protein ($N \times 6.25\%$), ash, fatty material and damage starch following methods described in AACC (2003) and Smith (1967). Degree of purity and color profile (Pérez et al., 2011), titratable acidity and pH (AACC, 2003; Smith, 1967) were also determined.

2.2.3. Amylose content determination

The amylose content was determined in duplicate using Differential Scanning Calorimetry (DSC) and triplicate using Iodine Binding Capacity (IBC) method.

Calorimetric determination was performed using a DSC-7 (Perkin Elmer, Norwalk, USA) using 8 mg db starch sample with 40 μl of 2% (w/v) L-α-lysophosphatidylcholine solution (L4129, Sigma Chemical Co., St. Louis, USA), directly added into a micropan and hermetically sealed and held for 30 min as reported earlier (Pérez et al., 2011). The sample pan was heated against an empty reference one from 25 to 160 °C at 10 °C/min, held at 160 °C during 2 min prior to being cooled to 60 °C at a 10 °C/min rate. Amylose content was estimated from the energy of amylose-lysophospholipid complex formation against a pure potato amylose standard (Avebe, Veendam, Netherlands).

IBC was measured by the amperometry (Larson, 1953) after starch defatting (using a 5:95 water/dimethyl sulfoxide mixture (DMSO), precipitation in alcohol 90% and drying) and later solubilization in 1N potassium hydroxide for 3 days at 4 °C with 5 g/L of starch as per (Pérez et al., 2011). Amylose content of the starches was measured at 25 °C, using pure amylose as a reference for iodine binding capacity. A 1 ml of the starch solution was stirred at 25 °C with water (15 ml), hydrochloric acid (2 ml 1N) and potassium iodine solution (1 ml 0.4N) prior to titration with potassium iodate solution (0.005N). The iodine binding capacity represents the amount of iodine bound per 100 mg of polymer as described by Rolland-Sabaté et al. (2003). Finally, the amylose percentage is calculated by the ratio of the starch IBC to the reference IBC amylose.

2.2.4. Macromolecular analysis

Samples were DMSO-defatted as reported above and solubilized in water by microwave heating under pressure (Rolland-Sabaté et al., 2003; Rolland-Sabaté, Colonna, Mendez-Montealvo, & Planchot, 2007). The resulting solutions were filtered through 5 μm Durapore™ membranes and carbohydrate concentration was determined by the orcinol sulphuric method (Rolland-Sabaté et al., 2007). Solubilization recoveries were calculated from the ratio of the initial concentration to the final concentration in solution. The macromolecular characteristics of starch polysaccharides were determined as previously described (Rolland-Sabaté et al., 2007). A HPSEC KW 802.5 Shodex column (8 mm i.d. × 30 cm, Showa Denko K.K, Tokyo, Japan) coupled with a Shodex KW guard column (6 mm i.d. × 5 cm) was maintained at 30 °C using a Crococil temperature control system (Cluzeau, Bordeaux, France). A Dawn Heleos MALLS system fitted with a K5 flow cell, a GaAs laser at λ = 658 nm (Wyatt Technology Corporation, Santa Barbara, USA) and an ERC-7515A refractometer (Erma, Tokyo, Japan) were used for the dual detection of solutes. The water used to prepare the samples was produced by a RiOs™ and Synergy purification system (Millipore). Prior to elution at a flow rate of 0.3 ml/min, the mobile phase (Millipore water containing 0.2 gL^{-1} sodium azide) was carefully degassed and filtered through a Durapore GV (0.2 μm) membrane. Sample recovery rates were calculated from the ratio of the mass eluted from the column (DRI signal integration) and the mass injected. Injected masses were also determined using the sulfuric acid-orcinol colorimetric method (Rolland-Sabaté et al., 2003, 2007; Tetchi, Rolland-Sabaté, Amani, & Colonna, 2007).

The molar mass and the radius of gyration (M_i and R_{Gi}) were established, as previously described (Rolland-Sabaté et al., 2007). The average values $\bar{M}_w$, and $\bar{R}_G$ and the dispersity $\bar{M}_W/\bar{M}_n$ were calculated using summations taken over the whole amylopectin peak.

2.2.5. X-ray diffraction

The type and the degree of crystallinity were determined using X-ray diffraction performed on native starches as previously described (Pérez et al., 2011). After water content adjustment (stabilization with saturated barium chloride for 20 days under partial vacuum), the samples (20 mg each) were sealed between two tape foils prior to recording the diffraction diagrams using a D8 Discover spectrometer (Bruker, Karlsrühe, Germany) with a Cu KR1 radiation (λCu KR1 = 1.5405 Å) produced in a sealed tube at 40 kV and 40 mA with a double Gobël mirror parallel optics system (500 μm

beam diameter). Diffraction diagrams were collected with a two-dimensional GADDS detector with a 600 s recording time. The distance from the sample to the detector was set to 100 mm and at a 25° (2θ) angle. All recorded diagrams at the same integrated scattering (between 2θ values of 3° and 30°) were normalized prior to relative crystalline determination. A- and B-type recrystallized amyloses were used as crystalline standards with scaled subtraction of an experimental amorphous standard curve (potato dry extruded starch) to obtain null intensity in the regions without diffraction peaks. The degree of crystallinity of the structures was determined using the cellulose method as per Wakelin, Virgin, and Crystal (1959).

2.2.6. Granule size distribution

The granule size distribution of starch particles was performed at room temperature by laser diffraction using a Malvern Mastersizer 3000. A spatula tip of each starch sample was suspended in tap water and the suspension was directly fed into the granule size measuring cell at room temperature. The suspension at a standardized 2% laser obscuration was submitted to a 10 s sonication prior to laser diffraction measurement. Based on triplicate measurements, the granule size distribution was estimated using the Fraunhofer approximation considering opaque particles.

2.2.7. Optical microscopy

Granular shape and Maltese crosses were observed by optical microscopy (Leica DM 2500, Leica Microsystems GmbH, Wetzlar, Germany) using a polarized light filter (Pérez et al., 2011). The microscope slices with a starch sprinkle and one or two drops of distilled water were covered with a slip cover glass, hold for 2 min, and then examined and photographed using an Optikam 3 digital camera (Optika Microscopes, Ponteranica, Italy).

2.2.8. Differential scanning calorimetry

Starch gelatinization and retrogradation measurements were done in duplicate using the same DSC7 apparatus as per Mestres, Matencio, Pons, Yajid, and Fliedel (1996), Pérez, Breene, and Bahanasey (1998); and Perdon, Siebenmorgen, Buescher, and Gbur (1999), respectively. The sample pan (8 mg of starch db with 40 μL of distilled water) and the empty reference pan were heated from 5 to 145 °C at a 10 °C/min rate. The gelatinization enthalpy variation (ΔH) and the onset gelatinization temperature (GT) of each sample were determined, as well as the end gelatinization temperature for the gelatinization range estimation. Retrogradation enthalpies (ΔHR) of the gelatinized samples were carried out at the same heating rate as for the gelatinization process after storage at 4 °C during a week. The retrogradation rate (%) was estimated as the ratio of the variation of the enthalpy of retrograded samples (ΔHR) to their gelatinization enthalpy variations (ΔH).

2.2.9. Rapid visco-analysis

Hot starch dispersion viscosity profiles were obtained with a Rapid Visco Analyzer (RVA, model RVA-4, Newport Scientific, Australia) (Pérez et al., 1998). Starch (1.25 g db) was dispersed in distilled water to get a 5% suspension. Viscosity was recorded using the temperature profile: holding at 50 °C for 1 min, heating from 50 to 90 °C at 6 °C/min, holding at 90 °C for 5 min, and then cooling down to 50 at 6 °C/min. The gel was then maintained for 2 min at 50 °C with continuous stirring at 160 rpm. Four parameters were measured: pasting temperature (PT), peak viscosity (PV), hot paste viscosity at the end of the plateau at 90 °C (HPV) and the cool paste viscosity (CPV) at 50 °C. Three additional parameters were calculated: breakdown (BD) estimated as PV – HPV, setback (SB) estimated as CPV – PV, consistency (CS), estimated as CPV – HPV as per Dufour et al. (2009).

2.2.10. In vitro starch availability

In vitro starch availability for gelatinized (water bath boiling during 1 min) and non-gelatinized starch suspension was analyzed (Holm, Björck, Asp, Sjoberg, & Lundquist, 1985). A α-amylase (1200 UI/mg and 27 mg of proteins/ml) from porcine pancreas preparation was used (A3176, Sigma Chemical Co., St. Louis, USA). About 700 mg of dry starch was suspended into 50 ml of a sodium and potassium phosphate buffer (0.5 M pH 6.9) and homogenized. The starch suspension was gelatinized during 1 min in boiling water during continuous stirring. The suspension was then cooled to room temperature. About 4 mg/ml of the α-amylase solution diluted in the phosphate buffer was mixed to both gelatinized and non-gelatinized starch suspensions and incubated at 37 °C for an hour. Sampling were carried out in triplicate at various time (5, 15, 30 and 60 min) in addition to the initial condition were no enzyme was used. A standard curve was prepared using pure dry maltose in the 0–2 mg/ml range in addition to the use of a control made of pure potato starch. The 3,5-dinitrosalicylic acid method (DNS) was used, while adding 0.2 ml sample, 0.8 ml of distilled water to 1 ml DNS solution in boiling water for 10 min, prior to cooling at room temperature and reading at 540 nm against a DNS blank. The extent of the hydrolysis was computed as the percentage of dry starch hydrolyzed (mg of maltose/100 mg of pure starch).

2.2.11. Statistical analysis

Analysis of variance (ANOVA) was performed on the raw data, using Statgraphics software 6.0 1992, Manugistics, Inc. & Statistical Graphics Corp., USA). Mean separations were performed by Duncan's Multiple Range Test at $P \leq 0.05$ using a Statgraphics software package.

3. Results and discussion

3.1. Starches purity and physicochemical properties

The moisture content, ash content, pH and titratable acidity of the yam starches were in the range accepted for dry products and commercial starches (Sriroth, Piyachomkwan, Wanlapatit, & Oastes, 2000; Swinkels, 1985; Thomas & Atwell, 1999). The crude protein and fatty material contents non-detected were considered being present, as traces (Table 1). Damaged starch contents were below 0.09% for all the starches, demonstrating the effectiveness of the procedure used to obtain starches. The starch color is related to purity and starch quality. The white indexes of the yams starches studied were close to 100. The White Index (WI) observed for the purple starches, were slightly lower than those of the white and yellow starches. The non-zero results for DE value are probably due to the traces of pigments that were already present in the edible yam pulps. In spite of the results discussed above in relation to pigmentation of the starches, the high purity of these starches can be corroborated (Table 1).

3.2. Macromolecular characteristics of starches

3.2.1. Amylose content

The amylose contents measured by IBC were higher than those obtained by DSC. It was also observed that amylose contents measured by IBC on the purple and yellow yams starches had highest values; as compared with its white counterparts, while those measured by DSC were quite similar. This discrepancy between measurements may have, among others, two origins: (i) the presence of lipids in the samples, (ii) the presence of intermediate material (IM), i.e., branched molecule having intermediate molar mass and chain length between amylose and amylopectin. The IBC measurement was made on defatted samples, whereas DSC measurement was made on the non-defatted samples. Since no fatty

Table 1
Purity (%db except moisture), IBC and DSC amylose contents, amylose:amylopectin relations (A:Ap), physical parameters of the five varieties of *Dioscorea* spp yams starches.

Parameters	White		Purple		Yellow
	Large (YWL)	Irregular (YWI)	Large (YPL)	Irregular (YPI)	Irregular (YYI)
Moisture (%)	12.4 ± 0.4^{a}	11.8 ± 0.1^{a}	12.7 ± 0.2^{a}	12.6 ± 0.2^{a}	12.4 ± 0.4^{a}
Crude protein (%)	ND	ND	ND	ND	ND
Fatty material (%)	ND	ND	ND	ND	ND
Ash (%)	0.3 ± 0.0^{c}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.3 ± 0.0^{c}	0.1 ± 0.0^{a}
Purity[a] (%)	99.8 ± 0.0^{a}	99.8 ± 0.0^{a}	99.8 ± 0.0^{a}	99.7 ± 0.0^{a}	99.9 ± 0.0^{a}
Damaged starch (%)	0.05 ± 0.00^{a}	0.07 ± 0.02^{b}	0.05 ± 0.11^{a}	0.06 ± 0.03^{ab}	0.08 ± 0.03^{bc}
Amylose content (IBC) (%)	26.7 ± 0.3	26.7 ± 0.2	35.0 ± 0.2	29.3 ± 0.8	32.7 ± 0.2
A:Ap relation (IBC)	0.36	0.37	0.54	0.41	0.49
λ_{max} (nm)	587 ± 1.4	584 ± 2.8	582 ± 0.0	581 ± 1.4	587 ± 1.4
Amylose content (DSC) (%)	23.6 ± 0.49	24.5 ± 0.15	25.7 ± 0.18	25.3 ± 0.01	24.8 ± 0.01
Difference IBC-DSC[b]	3.1	2.2	9.3	4.4	7.9
A:Ap relation (DSC)	0.31	0.32	0.35	0.34	0.33
pH	6.1 ± 0.0^{b}	6.1 ± 0.0^{b}	5.5 ± 0.2^{a}	5.7 ± 0.0^{a}	5.4 ± 0.1^{a}
Titratable acidity (meq g^{-1})	$2 \times 10^{-4} \pm 0.00^{b}$	$2 \times 10^{-4} \pm 0.00^{b}$	$2 \times 10^{-4} \pm 0.00^{b}$	$2 \times 10^{-4} \pm 0.00^{b}$	$1 \times 10^{-4} \pm 0.00^{a}$
White index (WI)	94.07	92.79	91.80	90.45	94.88
Delta E (ΔE)	1.36	2.65	3.22	4.61	1.17

Results are means of three determinations. Means with different letters in the same column within the same varieties differs significantly ($p < 0.05$).
ND: not detected.
[a] 100 – (%Crude protein + %Fatty material + %Ash).
[b] Difference calculated from the amylose content determined by IBC and the amylose content determined by DSC.

material was detected here (Table 1), and an intermediate response was shown between amylose and amylopectin using the iodine-bonding procedure (Baba & Arai, 1984; Colonna & Mercier, 1984) it is most probably that those starches exhibiting the highest amylose content with IBC measurements (YPI, YYI and YPL) may contain a large proportion of IM. The long chains of IM could probably contribute to the increase of the values obtained by IBC, and to a lesser extent to the values obtained by DSC, since this latter technique is more sensitive to the presence of α-(1,6) linkages (Gérard, Barron, Colonna, & Planchot, 2001), leading then to an overestimation of calculated amylose:amylopectin ratio by IBC.

In spite of the gap between results by DSC and IBC, the amylose contents determined were in agreement with those reported by several other authors for *Dioscorea* spp yam starches (Amani N'Guessan et al., 2005; Gallant et al., 1982).

For the five yam starches, λ_{max} values fluctuated in the 581–587 nm range (Table 1). If λ_{max} values depend on the amylose content and more generally on the degree of polymerization between two α-(1,6) links (Buléon, Colonna, Planchot, & Ball, 1998; John, Schmidt, & Kneifel, 1983), the higher the λ_{max} is, the higher the amylose content is expected, and/or the higher the molecule chain length should be. The five yam starches exhibited similar λ_{max} values which suggest that the starch samples average chain lengths are most probably quite similar. However, λ_{max} values obtained were lower than those reported in literature for yam and cassava starches at equivalent amylose contents (Amani N'Guessan et al., 2005; Pérez et al., 2011; Rolland-Sabaté et al., 2012). The five *Dioscorea* spp wild genotypes yams starches studied here may be expected to present shorter linear chains, than those the other yam and cassava starches being already reported in the literature.

3.2.2. Macromolecular features

The five yam starches (Table 2) exhibited solubilization recoveries of 91% for YYI and higher than 96% for YWL, YWI, YPL and YPI. Elution recoveries (the percentage of macromolecules percolated through the HPSEC system) ranged from 85% for YWL and YPI to 100% for YYI. The total mass recovery was then of 88% on average. This high mass recovery denotes a quantitative analysis for all starch samples.

For the five yam samples, chromatograms of starches revealed two Differential Refractive Index (DRI) peaks and one light scattering (LS) peak, corresponding to the biggest fraction (i.e. amylopectin fraction) (Fig. 1a). The first DRI peak at an elution volume of 5.95 mL was attributed to the amylopectin fraction and the second at an elution volume of 6.65 mL to the amylose fraction (Rolland-Sabaté et al., 2003). The amylose:amylopectin ratio, calculated from the

Table 2
Macromolecular characteristics of the five varieties of *Dioscorea* spp yams starches determined by HPSEC-MALLS. Solubilization and elution recoveries, amylose:amylopectin ratio (A:Ap relation), weight average molar mass ($\bar{M}_W$), z-average radius of gyration ($\bar{R}_{Gz}$), hydrodynamic coefficient (ν_G), apparent molecular density (d_{Gappw}) and dispersity index ($\bar{M}_W/\bar{M}_n$) for the amylopectin population.

Parameters	White		Purple		Yellow
	Large (YWL)	Irregular (YWI)	Large (YPL)	Irregular (YPI)	Irregular (YYI)
Solubilization recovery (%)	99	96	97	100	91
Elution recovery (%)	85	88	98	85	100
A:Ap relation[a]	0.39	0.61	0.52	0.39	0.69
$\bar{M}_W \times 10^{-6}$ 10^{-6} (g mol^{-1})	237 ± 15	201 ± 1.4	193 ± 3.7	174 ± 4.3	196 ± 0.8
$\bar{R}_{Gz}$ (nm)	233 ± 9.1	217 ± 0.3	206 ± 1.4	201 ± 2.5	212 ± 3.0
$\bar{M}_W/\bar{M}_n$	2.2 ± 0.2	2.2 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.2 ± 0.1
ν_G	0.29 ± 0.02	0.30 ± 0.02	0.30 ± 0.02	0.28 ± 0.02	0.29 ± 0.02
d_{Gappw} (g mol^{-1} nm^{-3})	8.6 ± 0.4	9.3 ± 0.4	9.9 ± 0.4	8.8 ± 0.2	9.4 ± 0.3

$\bar{M}_W$, $\bar{R}_{Gz}$, ν_G, d_{Gappw} and $\bar{M}_W/\bar{M}_n$ values were taken over the whole amylopectin peak.
ν_G: slope of the log–log plot of the radius of gyration *versus* the molar mass.
A $d_{Gappw} = \bar{M}_W/(4\pi/3)\bar{R}^3_{Gw}$.
[a] Determined from the ratio of the chains amount at the apex of the amylose and of the amylopectin peaks.

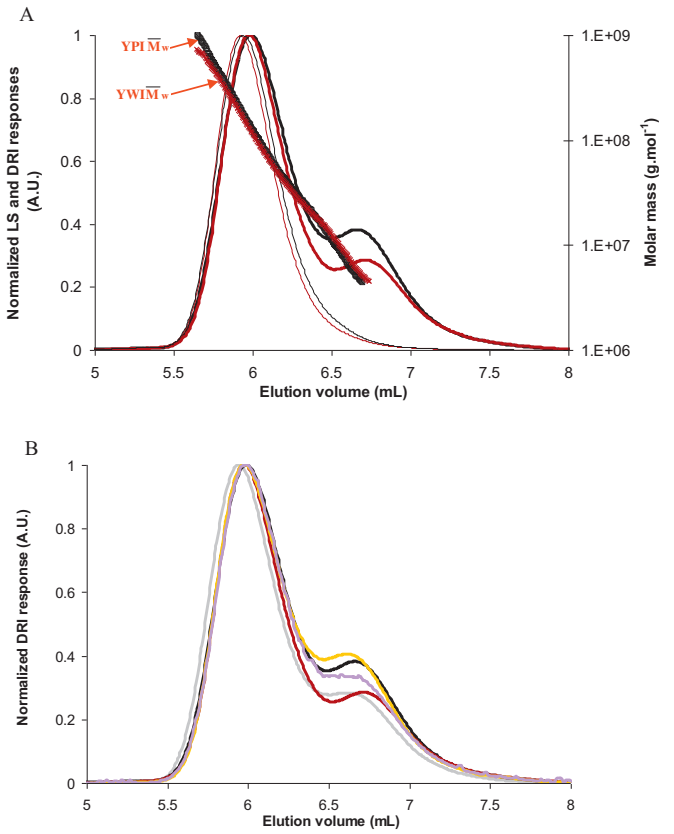

Fig. 1. Chromatograms and molar masses distributions of the five yams obtained by HPSEC. (A) The thick lines represent the normalized DRI responses in dark red and black for YPI and YWI, respectively. The thin lines represent the normalized light scattering responses at 90° in dark red and black for YPI and YWI, respectively. The dark red crosses and the brown triangles represent the molar masses of YPI and YWI, respectively. (B) The thick lines represent the normalized DRI responses in dark red, gray, purple, black and yellow for YPI, YWL, YPL, YWI and YYI, respectively. With, YPI: irregular purple tubers; YWL: large white tubers; YPL: large purple tubers; YWI: irregular white tubers; and YYI: irregular yellow tubers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

ratio of the chains amount at the apex of the amylose and of the amylopectin peaks, should then increase when amylose content increase. It is not the case for the samples studied here (Table 2). As HPSEC separates by size, linear and branched molecules of the same size elute at the same elution volume. Moreover, the separation between amylose and amylopectin is not complete, then, the peak attributed to amylose (Fig. 1) can be regarded as the sum of the amylose peak, the amylopectin peak tail with the lowest hydrodynamic radii, and possibly a part of the intermediate material peak (which spread between the amylopectin and the amylose peak). This discrepancy between HPSEC and IBC and DSC data may thus be explained by the presence of an intermediate material (presenting intermediate size and a molecular structure between amylose and amylopectin) in significant proportions in particular for YYI, YWI and YPL. Even if the calculation of amylose:amylopectin ratio by means of HPSEC chromatograms leads to an overestimation of its value (Table 2) due to the absence of separation between the peaks, HPSEC analysis gave additional evidence for the presence of an intermediate material in important proportions in YYI and YPL.

Amylopectin $\bar{M}_W$, $\bar{R}_G$, and the $\bar{M}_W/\bar{M}_n$ values ranged from 174×10^6 g mol^{-1} to 237×10^6 g mol^{-1}; 201–233 nm and 2.0–2.2, respectively (Table 2). The $\bar{M}_W$ and $\bar{R}_G$ values were of the same order of magnitude as those reported previously for cassava and yam starches (Pérez et al., 2011; Rolland-Sabaté et al., 2007; Tetchi et al., 2007), even if they were of significantly lower magnitude compared with Rolland-Sabaté et al. (2003) data, due probably to biological variations. YWL amylopectin exhibited the highest $\bar{M}_W$ and $\bar{R}_G$ values and YPI the smallest whereas YWI, YPL and YYI showed intermediate $\bar{M}_W$ and $\bar{R}_G$ values (Table 2).

Structural data could be determined from the exponent ν_G using the equation $R_G = K_G M_i^{\nu G}$, where K_G is a constant. ν_G values depend on polymer conformation and are theoretically 0.33 for a sphere, 0.50–0.60 for a random coil, and 1.0 for a rod. The experimental ν_G values decrease when molecular density increase and for branched polymers, a ν_G decrease was believed to correspond to an increase of branching. Experimental ν_G values obtained for the five yam amylopectins by plotting the radius of gyration and the molar mass of the same fraction were all around 0.30 in line with other data reported in the literature (Pérez et al., 2011; Rolland-Sabaté et al., 2003; Tetchi et al., 2007). Accordingly, YWL, YWI, YPL, YPI and YYI amylopectins showed a very dense and spherical conformation and they seemed to have similar densities; i.e., probably similar branching degrees.

Apparent particle density (d_{Gappw}) determination is another means to approach the conformation of the molecule which could give additional indication on branching. The values of d_{Gappw} reported in Table 2 were calculated on the basis of a smeared uniform density in the particle and based on the following equation for equivalent homogeneous spheres: $d_{Gappw} = \bar{M}_W/(4\pi/3)\bar{R}^3_{Gw}$. For the five yam amylopectins studied, d_{Gappw} values ranged from 8.6 to 9.9 g mol^{-1} nm^{-3} for YWL and YPL amylopectins respectively (Table 2). d_{Gappw} values must be compared for the same molar mass, as it decreases when molar mass increase for single molecules. The density values reported for the five yams amylopectins are very similar, confirming that these samples have very similar molecular amylopectin structures. Nevertheless, the low value (8.8 g mol^{-1} nm^{-3}) obtained for YPI, which exhibited also the lower molar mass, indicate a significantly less dense structure compared to the others. The densities were generally in agreement with the values calculated from literature data for yam and cassava amylopectins (Pérez et al., 2011; Rolland-Sabaté et al., 2003, 2012; Tetchi et al., 2007). However, d_{Gappw} values of these five yams amylopectins appeared to be slightly higher compared to those reported previously for cassava amylopectins (Rolland-Sabaté et al., 2012), meaning that yam amylopectins probably exhibited a more dense branching structure than cassava ones. This conclusion is in line with the particularly low λ_{max} values observed for yam starches which account for an important amount of short chains in the samples.

3.3. Starch macrostructure

All starches investigated exhibited B-type crystallinity. Crystallinity degrees were 35% for YWL and 40% for the other starches. In this study the crystallinity degree seem not to be related to the amylose content. The B-type crystallinity agreed well with those reported by many other authors (Amani N'Guessan et al., 2005; Hoover, 2001; Jayakody, Hoover, Liu, & Donner 2009; Moorthy, 2002; Tetchi et al., 2007).

Granules sizes ranged from 24.5 to up 60.0 μm with an average value of 45.3 μm with monomodal distributions (Fig. 2), but the granules sizes were inversely distributed for the white yam starches as compared with the purple counterparts. YWL and YWI starches exhibited highest fraction of larger granules (higher than 40 μm), followed by a population of granules between 20 and 40 μm, and a minor fraction corresponding to starch granules sizes smaller than 20 μm). Contrary, the YPI and YPL starches exhibited inverse trend, whereas the YYI starch presented half of the population of granules between 20 and 40 μm, and half higher than 40 μm size. These results were in the same order of magnitude to those reported by several authors for yam starches of different

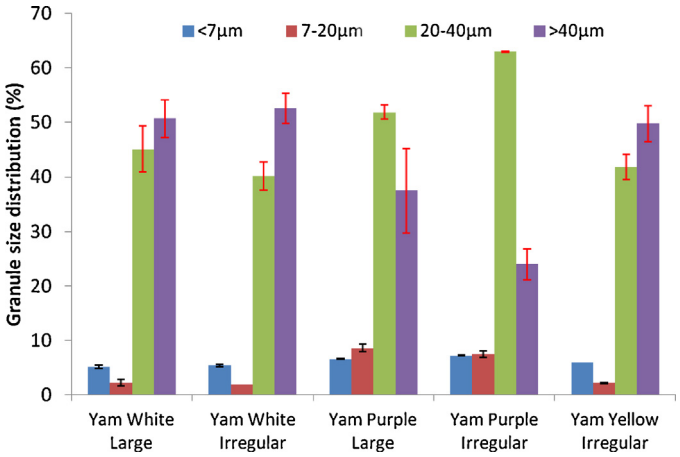

Fig. 2. Five yam starch granule distributions with standard deviations.

origins (Amani N'Guessan et al., 2005; Hoover, 2001; Jayakody et al., 2009; Moorthy, 2002; Tetchi et al., 2007).

Optical light polarized micrographs allowed pointing out quite similar irregular shape for the granules of each of the five starches. The starches granules appeared to be large, oval, shell-shaped or diamond-shaped, with smooth surfaces, with no evidence of fissure, and forming figures aggregated such as: snake, pyramid, or stylish figures as illustrated by the arrows in Fig. 3. These aggregated forming shapes have not been so far reported in the literature. The typical Maltese cross by birefringence, mostly due to molecular chains in the amorphous regions of granules (Zobel, 1988) was in an eccentric position on the native starches granules.

3.4. Functional properties

3.4.1. Thermal properties

The onset gelatinization temperature varied from 69.7 to 72.4 °C and the highest gelatinization temperature (82.5 °C) was exhibited by YPI (Table 3). The gelatinization enthalpy variation (ΔH) was in the 20.0–21.4 J g^{-1} range, while the highest enthalpy change was due to YWL, without any significant statistical differences highlighted among starches. These values were slightly higher than those reported in the literature for *Dioscorea* yam tubers collected in Ivory Coast, where a wide ΔH variation was reported, in the 13.7–20.3 J g^{-1} range (Amani N'Guessan et al., 2005), whereas the change in the retrogradation enthalpy ΔHR was in the 4.7–6 J g^{-1} range. If some similar retrogradation rates (between 23.5 and 29%) were observed among landraces when computing the ratio of gelatinization ΔH to retrogradation ΔHR, corresponding yam percentages extracted from the literature are often reported between 37.5% and 55% (McPherson, & Jane, 1999; Zobel, 1988) and even in the 56–84% range for some *Dioscorea alata* and *esculenta* landraces (Jayakody et al., 2009; Lawal, Lechner, & Kulicke, 2008). As reported earlier, the low retrogradation rates observed here might be due to a high proportion of short chain branches (Lu, Chen, & Lii, 1997). Except for waxy and amylomaize starches, the retrogradation rate is reported as being species-dependent among several starchy resources, with a variation reported in the 25–59% range from cassava to mung bean, respectively (Jane et al., 2003). The retrogradation rate is also probably landraces-dependent within the *Dioscorea* genus (including wild varieties). Moreover, if the value of enthalpy provides a quantitative measurement of the energy during the melting process of recrystallized amylopectin (Karim, Norziah, & Seow, 2000), each apparatus provides specific values which cannot quantitatively and objectively being compared. In addition, the promotion of retrogradation by the refrigerated storage favoring nucleation combined or not with a heating stage reported to favor the propagation process of starch recrystallization, may also contribute to some of the differences in retrogradation rates observed for the wild varieties with some of the literature.

3.4.2. Rheological properties

The overall gelatinization profiles are different from each other with the presence of a peak viscosity for all starches, except for the YYI (Fig. 4). The maximum viscosities developed at 7% suspension were in the range of 3426–4798 cP. Starches YWL, YWI and YPI exhibited the highest peak viscosities and the lowest viscosities during the holding and cooling time, bringing as a result, lower consistencies and higher setback and breakdown than starches YPL and YYI (Table 3). The initial pasting temperature varied from 75.7 to 77.3 °C and the highest pasting temperature (90 °C) was reached by YWI and YPL, however, those of the other starches was rather near to this temperature. These values were close but slightly lower than those reported for 4% yam starch suspensions from Ivory Coast (Amani N'Guessan et al., 2005). The large granule size of YWL, YWI

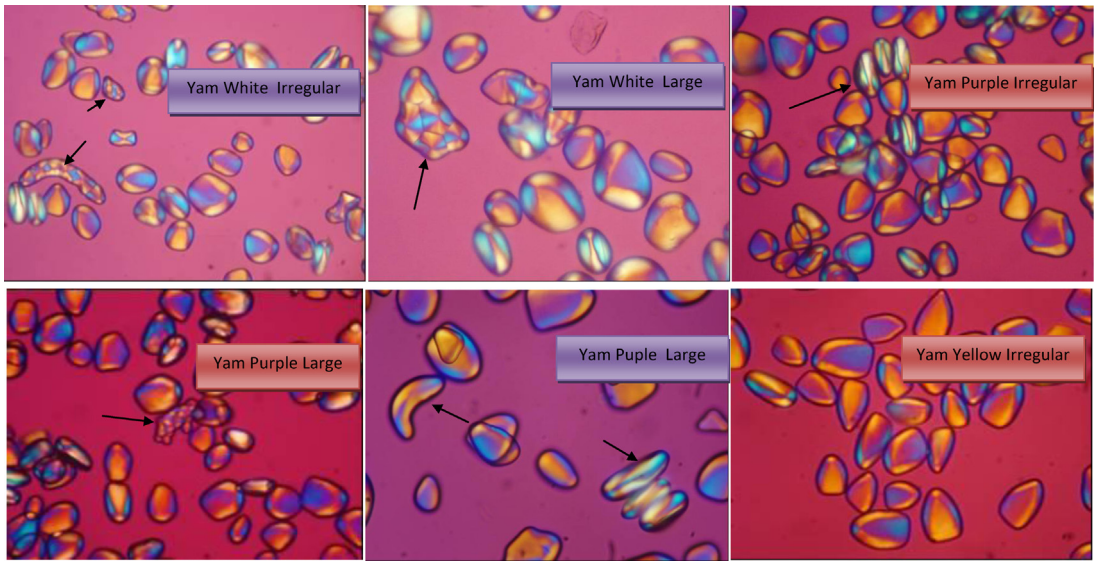

Fig. 3. Optical light polarized microphotography of the starches isolated from the five yams.

Table 3
Thermal and pasting properties of the five varieties of *Dioscorea* spp yams starches.

Parameters	White		Purple		Yellow
	Large (YWL)	Irregular (YWI)	Large (YPL)	Irregular (YPI)	Irregular (YYI)
Gelatinization temp. range (°C)	71.6–78.3	71.7–78.6	72.4–79.7	72.0–82.2	69.7–78.0
Gelatinization (Tend-Tonset) (°C)	6.7 ± 0.2a	6.9 ± 0.2a	7.3 ± 0.2a	10.2 ± 0.1b	8.3 ± 0.2a
ΔH (J g^{-1}) Gelatinization	21.4 ± 1.0a	20.0 ± 1.0a	21.2 ± 0.7a	20.0 ± 1.1a	21.0 ± 1.2a
Retrogradation temp. range (°C)	51.2–72.2	50.8–74.9	51.2–76.8	50.8–76.8	50.9–74.7
Retrogradation (Tend-Tonset) (°C)	21 ± 0.7a	24.1 ± 0.b	25.8 ± 0.2c	26 ± 0.2c	23.8 ± 0.6b
ΔH (J g^{-1}) Retrogradation	5.6 ± 0.1b	4.7 ± 1.1a	6.0 ± 0.1b	5.8 ± 0.3b	5.2 ± 0.2a
Retrogradation (%)	26.2	23.5	28.3	29.0	24.8
Pasting temp. range (°C)	75.7–89.8	76.5–90.0	77.3–90.0	77.1–88.6	75.7–88.6
Maximum peak (cP)	4798 ± 255	4251 ± 84	3665 ± 406	4656 ± 192	3426 ± 17
Breakdown (cP)	2395 ± 55	1468 ± 41	1047 ± 771	1822 ± 59	984 ± 61
Setback (cP)	−2279 ± 240	−1370 ± 115	−254 ± 356	−1470 ± 206	−342 ± 13
Consistency (cP)	99 ± 54	99 ± 18	353 ± 32	117 ± 26	544 ± 274

Results are means of three determinations. Means with different letters in the same column within the same varieties differs significantly ($P < 0.05$).

and YPI may explain their higher viscosity values obtained at the peak and the higher consistency of YYI and YPL is probably linked to their higher amylose content obtained by IBC, the longer chains contained in these samples having more ability to associate to each other. When comparing the gelatinization temperature measured using RVA with those obtained with DSC (Fig. 4 and Table 3), temperatures are always higher. Such well-know discrepancy is due to the principle of the method itself. Using DSC approach, the method highlights the temperature (onset temperature) at which the starch granule start to lose its crystallinity, whereas the RVA (pasting temperature) measures a later phenomenon as the temperature at which the swelling granules induce an increase of the viscosity of the media.

3.5. *In vitro* starch availability

The extent of the hydrolysis of the raw yam starches studied varied from 4.6 to 7.8% (Fig. 5). Ungelatinized starches from the white yams were more sensible to the enzymes (Fig. 5A) than the purple and yellow ones at 60 min hydrolysis, whereas no significant differences were observed at the same time between gelatinized starches of the different genotypes. This could be attributed to their lower amylose:amylopectin ratio, since a high amylose content is known to decrease the susceptibility of starch to hydrolysis (Srichuwong, Candra, Mishima, Isono, & Hisamatsu, 2005). During cooking process in boiling water (98 ± 1 °C) during 1 min, the amount of starch hydrolyzed increased dramatically from 4.6 to 75.8%, in agreement with previous works reporting that any treatment that destroys the starch granular structure increases the susceptibility of starch to enzymes action (Englyst, Kingman, & Cummings, 1992; Singh, Dartois, & Kaur, 2010; Snow & O'Dea, 1981). A fraction of approximately 25%, which has not suffered enzymatic degradation, remained as a potential indicative of the presence of steric effect and/or bond forces resistant to the enzymatic attack. A stronger resistance (before 40 min) is offered by both large (white and purple) compared to the other three yams starches (Fig. 5B). Indeed, for nutritional purposes, Englyst et al. (1992) postulate has to be kept in mind. These authors have proposed a classification of starch based on the rate and extent of the starch *in vivo* digestion: rapidly digestible starch (RDS), the amount of glucose release after 20 min; slowly digestible starch (SDS), the amount of glucose release between 20 and 120 min of *in vitro* digestion; and resistant starch (RS) total starch minus the

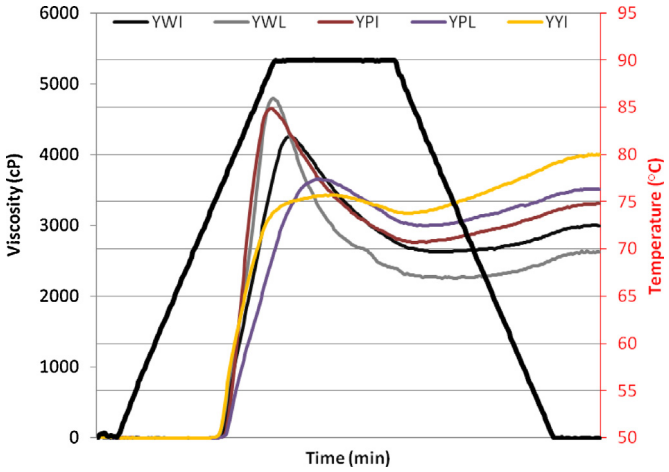

Fig. 4. RVA-pasting properties of the five varieties of *Dioscorea* spp yams. With, YWI: irregular white tubers; YYI; YWL: large white tubers; YPI: irregular purple tubers; YPL: large purple tubers; and YYI: irregular yellow tubers.

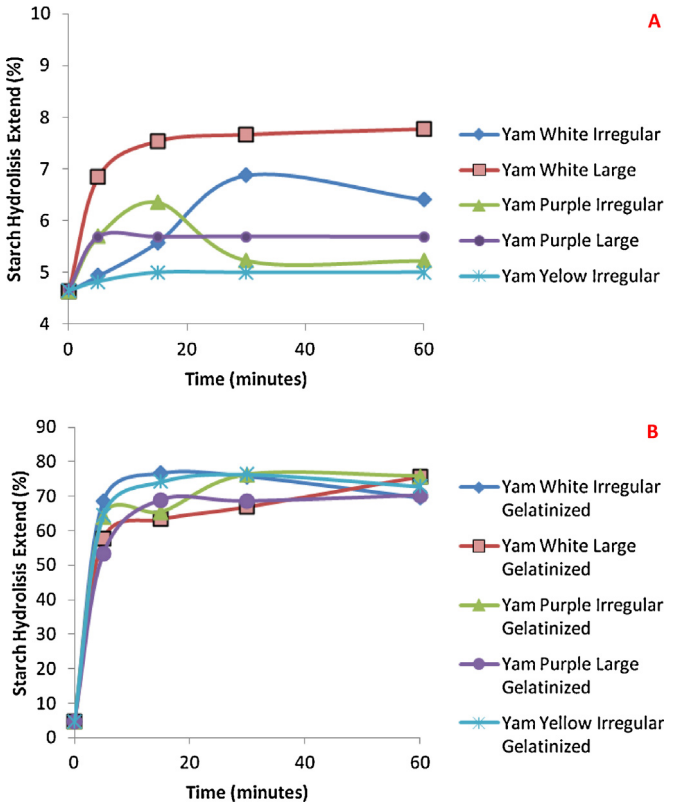

Fig. 5. Starch hydrolysis of the five yams starches samples: (A) native non-gelatinized and (B) gelatinized during 1 at 98 ± 1 °C.

amount of glucose release after 120 min of *in vitro* digestion. They concluded that the RDS has a large impact in the glycaemic response in humans, while the impact from SDS is limited. On the other hand, the RS escapes the digestion in the small intestine (may be digested in the large intestine), without causing a glycaemic response. The 25% non-hydrolyzed fraction remaining after 20 min of hydrolysis shall correspond to SDS and shall then be responsible for an uncompleted glycaemic response.

4. Conclusions

Starches with high purity and with similar physical and physicochemical properties were isolated from the edible portion of five kinds of yams investigated. The amylose contents were about 20–30% in agreement with those determined by several other authors for *Dioscorea* spp yams. Nevertheless, some discrepancies between amylose content determined by DSC, IBC and the HPSEC analysis were found, and considered as an index of the presence of an intermediate material exhibiting intermediate molar mass and chain length between amylose and amylopectin. The studied amylopectins exhibited similar molar mass, radii and densities and the numerical values were close to those generally reported in literature for yam and cassava starches. All yam starches studied exhibited a pure B-type crystallinity, and a crystallinity degree of 35–40%. The granules are large, oval and could form aggregated structures. They have a monomodal size distribution from 24.5 to up 60 μm. The YYI and YPL starches which exhibit the highest amylose content by IBC measurements have more retrogradation tendency, than their counterparts. Native starches from the white yams were more sensible to the enzymatic hydrolysis than the other two (purples and yellow), and this could be attributed to their lower amylose:amylopectin ratio. Whereas, a portion of 25% of the starches gelatinized during 1 min at 98 ± 1 °C, remained non-hydrolyzed, after 20 min of hydrolysis by α-amylase. An incomplete glycaemic response could then be produced after the ingestion of these starches, making them products with good potential nutritional/health properties.

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