Morphological and physicochemical characterization of porous starches obtained from different botanical sources and amylolytic enzymes

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Abstract

Porous starches might offer an attractive alternative as bio-adsorbents of a variety of compounds. However, morphology and physicochemical properties of starches must be understood before exploring their applications. Objective was to study the action of different amylolytic enzymes for producing porous starches. Wheat, rice, potato and cassava starches were treated with Amyloglucosidase (AMG), α-amylase (AM) and cyclodextrin-glycosyltransferase (CGTase). Morphological characteristics, chemical composition, adsorptive capacity and pasting/thermal properties were assessed. Scanning Electron Microscopy (SEM) showed porous structures with diverse pore size distribution, which was dependent on the enzyme type and starch source, but no differences were observed in the total granule surface occupied by pores. The adsorptive capacity analysis revealed that modified starches had high water absorptive capacity and showed different oil adsorptive capacity depending on the enzyme type. Amylose content analysis revealed different hydrolysis pattern of the amylases, suggesting that AMG mainly affected crystalline region meanwhile AM and CGTase attacked amorphous area. A heatmap illustrated the diverse pasting properties of the different porous starches, which also showed significant different thermal properties, with

25 different behavior between cereal and tuber starches. Therefore, it is possible to
26 modulate the properties of starches through the use of different enzymes.

27 **Keywords:** Porous starch; enzymes; amyloglucosidase; α-amylase; CGTase;
28 microstructure.

1. Introduction

Porous starch granules are becoming of great interest such as non-toxic absorbents, owing to their great absorption capacity derived from the major specific surface area [1]. Pores can protect sensitive elements as oils, minerals, vitamins, bioactive lipids, food pigments such as β-carotene and lycopene that are sensitive to light, oxidation or high temperature [2-4]. In fact, porous starches have been proposed as carriers or vehicles of colorants, spices, flavorings or sweeteners and pharmaceuticals [5]. Nevertheless, very scarce information exists regarding the characteristics of the pores and how to modulate them to extend the application of the porous starches [6].

Up to now, several enzymes, such as α-amylase (AM), β-amylase, amyloglucosidase (AMG), pullulanase, isoamylase and cyclodextrin-glycosyltransferase (CGTase) have been used for producing porous starches [7-10]. Pin-holes, sponge-like erosion, numerous medium-sized holes, distinct loci leading to single holes in individual granules and surface erosion are being observed after enzymatic action [11], but there is no clear understanding about the role of either the botanical origin of starch or the enzyme used. Aggarwal and Dollimore [12] observed an increase in the size of the pores on corn starch granules, when augmented the AMG concentrations, till the breakdown was so pronounced that walls around pinholes were broken, leading to large irregular holes and a disrupted structure. Recently, Benavent-Gil and Rosell [13] compared the effect of AMG, AM, CGTase and branching enzyme on corn starch properties, taking also into account the impact of enzyme level. Authors concluded that corn starches with varying number and size of pores could be obtained by controlling either the type of amylolytic enzyme or the level of enzyme.

In addition, it must be considered the intrinsic structural features of starches from different botanical origin, which might affect the amylolytic action. In fact, when corn,

54 mung bean or sago starches were treated with a mixture of AM and AMG at 35 ºC for
55 24 h, porous granules were obtained, whereas only enzymatic erosion occurred on the
56 surface of cassava starch granule [14]. According to Rocha, Carneiro and Franco [15],
57 AM degraded the external part of the granule surface of cassava, sweet potato, and
58 potato starches after hydrolysis at 37 °C for 48 hours; but Peruvian carrot starch showed
59 only some granules with internal degradation.

60 In previous literature, substantial variation was found in terms of hydrolysis time and
61 temperature, and enzyme type, which somewhat impedes the exploitation of porous
62 starches; meanwhile there is no clear knowledge about the role of those factors on the
63 pore development. Likewise, taking into account the variety of compounds to be
64 adsorbed from foodstuffs, pharmaceutical, cosmetic and chemical products, the
65 characterization of those starches would be needed from an industrial point of view.

66 Therefore, the main objective of this study was to identify the potential of starches from
67 different botanical sources to obtain porous starches with different type of hydrolases.
68 Particularly, to characterize and compare the effect of amyloglucosidase, fungal α-
69 amylase and cyclodextrin-glycosyltransferase on the morphological and
70 physicochemical properties of selected starches from cereals and tubers. In this study,
71 morphological, chemical, thermal and pasting properties of different enzymatically
72 modified starches were studied. Thereby, the granule characteristics as well as the
73 enzyme attack on starch granules were visualized by scanning electron microscopy
74 (SEM) and analyzed by a micrograph processing tool. In order to establish a possible
75 correlation, these values were combined with chemical, pasting and thermal properties.

76 ## 2. Materials and methods

77 ### *2.1. Materials*

Potato starch (Tereos Syral, Marckolsheim, France), wheat starch (NATILOR Chamtor company, Pomacle, France), intermediate amylose rice starch (Sigma-Aldrich, Spain) and cassava starch (local market) were used as substrates for enzymatic modification. Amyloglucosidase (EC 3.2.1.3), fungal α-amylase (EC 3.2.1.1) and cyclodextrin-glycosyltransferase (EC 2.4.1.19) activities were provided by commercial food grade preparations (Amyloglucosidase 1100 L declared activity 1100 AGU/g product, Fungamyl® 2500SG declared activity 2500 FAU/g product and Toruzyme® 3.0 L declared activity 3KNU/mL product) supplied by Novozymes (Bagsværd, Denmark). All other reagents were of analytical grade. The water used was deionized.

2.2. Preparation of porous starch

The preparation of porous starch was based on the method of Benavent-Gil and Rosell [13]. The selection of enzyme levels (16.5 AMG U/g, 11 AM U/g and 0.2 CGTase U/g) was based on preliminary experiments, which showed that under the experimental conditions used (50 ºC, 2 hours), maximum number of pores were obtained without distorting the granule. Native starches were included for comparison, and starches subjected to treatment conditions in the absence of enzymes were used as controls.

2.3. Scanning electron microscopy (SEM)

The granule morphology of native and modified starches was observed using a JSM 5200 scanning electron microscope (SEM) (JEOL, Tokyo, Japan). Samples were coated with gold in a vacuum evaporator (JEE 400, JEOL, Tokyo, Japan) prior to observation. The obtained samples were examined at an accelerating voltage of 10 kV and magnified 3,500x times.

The microstructure analysis was carried out using the image analysis program (ImageJ, UTHSCSA Image Tool software). The SEM images were saved as 8-bit tiff format.

Scale was initially set using the relationship between pixels and known distance. Threshold was assessed applying the default algorithm and then particle analysis was carried out. The following parameters were measured: granule and pore area . The area occupied by pores in a starch granule was calculated as the sum of the areas of all the pores of a starch granule divided by granule area. Values were the average of 20 independent measurements.

2.4. High performance anion exchange chromatography (HPAEC)

The hydrolysis compounds (oligosaccharides and cyclodextrins) lixiviated during enzymatic treatment were quantified using HPAEC (Dionex ICS3000, Thermo Fisher Scientific, Waltham, MA, USA) according to the methodology described by Dura and Rosell [16].

2.5. Analysis of chemical and physicochemical properties of modified starches

The amount of amylose/amylopectin in the starches was analyzed using a commercially available kit (Amylose/Amylopectin Assay Kit, Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland) following supplier instructions. This enzymatic method is based on the concanavalin A method [17]. Water and sunflower oil adsorptive capacities of starches were determined following the method described by Yousif, Gadallah and Sorour [18], with slight modifications. Samples (0.100 g ± 0.005 g) were mixed with distilled water or oil (1 ml) and centrifuged at 3,000 x *g* for 10 min. Adsorptive capacities were expressed as percent weight of solvent retained by the sample. Each measurement was performed in duplicate.

2.6. Viscosity measurement

The pasting properties of native and enzymatically modified starches were measured using a Rapid Visco Analyzer (RVA-4500, Perten Instruments, Hägersten, Sweden).

Starch (2.00 g ± 0.01 g based on 14% moisture content) was added to 20 mL of distilled water placed into the aluminum RVA canister. Slurries underwent a controlled heating and cooling cycle, from 50 to 95 ºC in 282 s, holding at 95 ºC for 150 s and then cooling to 50 ºC. The initial speed for mixing was 960 rpm for 10 s, followed by a 160 rpm paddle speed that was maintained for the rest of assay. Peak viscosity, final viscosity, breakdown (peak viscosity-through), setback (final viscosity-through) and onset temperature for pasting formation were determined from the viscosity plot and recorded using Thermocline software for Windows (Perten Instruments, Hägersten, Sweden). The level of hydrolysis at 95 ºC and 50 ºC was defined as the %-change in paste viscosity recorded in the RVA at 50 ºC and 95 ºC.

2.7. DSC thermal analysis

Gelatinization properties of modified starches were measured using a differential scanning calorimeter (DSC) from Perkin–Elmer (DSC 7, Perkin–Elmer Instruments, Norwalk,CT). The slurry of starch and water (1:3) was placed into stainless steel capsules. The sealed capsules were equilibrated at room temperature for one hour before analysis. The samples were then heated from 30 to 120 °C at a heating rate of 10 °C/min under nitrogen atmosphere, using an empty stainless steel capsule as reference. The onset (To), peak (Tp) and conclusion (Tc) temperatures were determined from the thermogram. The enthalpy of gelatinization (ΔH) was estimated based on the area of the main endothermic peak, expressed as joule per gram sample (J/g).

2.8. Statistical analysis

The data reported are the mean of replicates and expressed as a mean ± standard deviation. Statistical analyses were carried out with Fisher's least significant differences test with a significance level of 0.05. Pearson correlation coefficient (r) and P-value

were used to indicate correlations and their significance using Statgraphics Centurion XV software (Bitstream, Cambridge, N). The correlation coefficient was classified in different levels of correlation: perfect ($|r| = 1.0$), strong ($0.80 \leq |r| \leq 1.0$), moderate ($0.50 \leq |r| \leq 0.80$), weak ($0.10 \leq |r| \leq 0.50$), and very weak (almost none) correlation ($|r| \leq 0.10$).

3. Results

3.1. Microstructure of modified starches

Fig. 1 shows SEM micrographs of native, references and modified starches. As expected, SEM micrographs revealed the broad variation in shape and area among native starches from different sources (Fig. 1, A1, B1, C1 and D1). Granules from wheat were composed of two different populations. The large A-type granules exhibited lenticular or disk shapes, while the small B-type granules exhibited principally spherical or ellipsoidal shape. The granules average area was 242.90 μm^2 and 13.11 μm^2 for A-type and B-type granules, respectively (Fig. 1, A1). Rice starch granules displayed polygonal shapes and 17.53 μm^2 in granules average area (Fig. 1, B1). Potato starch was composed of large granules and similar to wheat, two different populations were observed (Fig. 1, C1). The largest granule fraction was ellipsoid in shape with a granules average area of 1098.04 μm^2, and the smallest fraction was basically spherical in shape and average area of 291.95 μm^2. Cassava starch granules showed many truncated granules and with several grooves on the surface and average area of granules was 117.99 μm^2 (Fig. 1, D1). The observed microscopic appearances are in agreement with literature [19]. The reference starch granules, subjected to treatment in the absence of enzymes at pH 4.5 (Fig. 1, A2, B2, C2 and D2) and pH 6.0 (Fig. 1, A3, B3, C3 and D3), kept their integrity, and there was not significant difference observed in rupture,

breakage or pores due to the incubation with buffer (Fig. 1 A2-3, B2-3, C2-3 and D2-3) as has been previously reported [7, 16].

Fig. 1. Scanning electron micrograph (wheat: A; rice: B; potato: C; cassava: D) of native starches (1), starches treated enzymatically (AMG: 4; AM: 5; CGTase: 6) and their counterparts subjected to treatment conditions without the presence of enzymes (2-3). Magnification 2,000×.

Enzymatic treatments modified the surface of starches and the extent of the effect was highly dependent on the source of starch. Differences in the enzymatic action could be related to the starches susceptibility to be attacked (Fig. 1 A4-6, B4-6, C4-6 and D4-6). Generally, the enzymatic action on the starches provoked the formation of deep holes in cereal starches, while more superficial attacks were observed in the tuber starches [20]. In order to quantitatively stablish possible differences associated to enzyme type and starch botanical origin, the pore size and the ratio pore area to starch granule area (related to the abundance of pores per granule) were assessed (Fig. 2). Enzyme type and starch source significantly affected the pore size distribution (Fig. 2A). Nevertheless, the ratio pore area to granule area (Fig. 2B) was similar regardless starch source and enzyme type, with the exception of wheat starch treated with AMG that showed sponge-like erosion structures. Specifically, AMG treated wheat starch showed the formation of holes along the equatorial groove, suggesting main hydrolysis at these points, and in some cases leading to rupture of the granules. Rice, potato and cassava starches also showed pores on the surfaces of granules after AMG treatment, but in rice seems to be deeper than in cassava and potato starches. Aggarwal and Dollimore [21] observed that potato starch offers greater resistance to AMG attack than wheat, rice, and corn starches. Image analysis did not reveal significant changes in the pore size distribution

among starch types, but an increase in the abundance of pore per granule in treated wheat starch was observed.

The action of AM led to similar pore size distribution on the granules surface, independently on the starch source, with the exception of potato starch that displayed bigger holes and wider distribution. In the case of treatment by CGTase, cereal starches exhibited wider distribution of pore sizes than tuber starches (Fig. 2A). Overall, SEM suggested that enzymatic hydrolysis of cereal starches was initiated from granule surfaces and then spread toward the granule interior, producing deeper holes compared to tuber starches. Possibly the presence of pores and channels in cereal starches allowed enzymes to penetrate towards the granule interior, while the rigid and smooth surface of tuber starches acted as a barrier to enzymes [20, 22]. Moreover, tuber starches are more resistant to the enzymatic hydrolysis than cereal starches, due to a high number of branch points in non-crystalline regions, which lead to high density amorphous regions and stable crystallites [23], yielding less deep holes.

Fig. 2. Pore size (A) and pore surface area distribution (B) obtained for each enzymatic treatment of starches from different origins. Notations are referred to the starch botanical source (Wheat, Rice, Potato, Cassava) followed by the abbreviations of the enzyme used (AM, AMG, CGT).

3.2. Cyclodextrins and oligosaccharides released during enzymatic treatment of starches

Enzymes acted differently on the starch granules as indicated by the compounds released (glucose, oligosaccharides and cyclodextrins) during enzymatic treatment, and also depending on the botanical origin of starch (Table 1). In the absence of enzymes, no hydrolysis products were released (data not shown). In line with other reports, the

222 only product of hydrolysis after AMG treatment was glucose. AMG is a well-known
223 exo-amylase, releasing only glucose residues from amylose or amylopectin chains [24].
224 Regardless of starch source, the amount of glucose seemed constant, likely due to
225 glucoamylase level was enough to produce saturation of the non-reducing-ends of starch
226 chains [9]. AM and CGTase are known to cleave α-1–4 glycosidic bonds existing in the
227 internal part (endo-) of a polysaccharide chain. The main compounds produced during
228 AM treatment differed between cereal and tuber starches. The products of hydrolysis
229 were mainly maltose followed by glucose and oligosaccharides with a DP of 3–4.
230 Conversely, the glucose was predominantly released from potato, whereas maltotetraose
231 was the major hydrolysis product from cassava. CGTase treatment converted starch into
232 a mixture of α-, β- and γ-CD and smaller amounts of oligosaccharides with a DP of 1–4,
233 regardless starch source. Nevertheless, α-, β- and γ-CD contents varied between
234 starches depending on the enzyme specificity [25] but also on the substrate [26].

235 *3.3. Amylose content and adsorptive capacity*

236 In agreement with data reported in the literature [27], significant differences in amylose
237 contents were detected among the starches from different sources (Table 2). The cereal
238 starches (wheat and rice) contained lower average amylose content compared to the
239 tuber starches.

240 Enzymatic treatment for obtaining porous starches affected amylose contents and the
241 effect was significantly dependent on the starch origin and the enzyme type (Table 2).
242 The ratio amylose/amylopectin remained unchanged in the case of wheat starch,
243 whereas in the other starches the enzymatic treatment led to a decrease in the amylose
244 content, with the exception of AMG modification. Specifically, AMG treatment
245 increased the amylose content of rice starch, and no significant effect was detected in

246 the other starches. Taking into account that a decrease in starch crystallinity has been
247 correlated with an increase in amylose content [28], it seems that amylopectin of rice
248 starch was preferentially hydrolyzed by AMG, since amylopectin has many more non-
249 reducing ends. AM and CGTase preferentially hydrolyzed the amylose chains in rice
250 and potato starches resulting in a decrease of this polymer, because the amylose is
251 located in the amorphous regions. This observations agrees with the inverse relationship
252 between the amylose content and the amount of hydrolyzed starch previously reported
253 [29]. However, no significant effect was observed in the amylose content when cassava
254 starch was treated with AM. It seems that AM and CGTase attacked amorphous
255 domains, where the majority of the amylose is located [30], leading an increase in the
256 amount of amylopectin. Therefore, results on amylose content revealed that depending
257 on enzymatic treatment amylose or amylopectin are primarily hydrolyzed.

258 The water adsorptive capacity (WAC) of starches was significantly dependent on the
259 starch source, being higher for cereal starches; whereas no trend was observed for
260 adsorptive oil capacity (OAC) (Table 2). Enzymatic treatment significantly affected the
261 WAC and OAC (Table 2). All enzymatic treatments increased the ability of starch to
262 bind water molecules, which suggested that hydrophilic tendency of starch increased
263 after enzymatic treatment. Among them, wheat starch treated with AMG showed the
264 greatest absorption. Likely, the pore surface area originated by AMG was responsible of
265 this behavior due to the increase of the surface area. It is generally assumed that the
266 holes created in the starch surface after enzymatic treatment increase the surface area,
267 having significant influence on starch water retention [9].

268 Nevertheless, no clear tendency was observed for the OAC. AMG treatment did not
269 significantly affect that property in rice and potato starch, while this enzyme enhanced
270 and reduced the OAC of wheat and cassava starches, respectively. The addition of AM

to rice and cassava starches resulted in lower values for their OAC, but no change was induced in wheat and potato starch. Conversely, the treatment of wheat and rice starches with CGTase led to porous starches with higher OAC, but this enzyme reduced this parameter in cassava starch and there is no change observed in potato starch. These observations suggested that rice after AM treatment and cassava porous starch, obtained with any of the tested enzymes, had more lipophilic surface. A significant negative and moderate correlation was identified between the OAC and the amylose content (r=-0.684, P<0.000). Therefore, the ratio amylose/amylopectin must play an essential role in the OAC, being responsible of the different trend observed with each enzymatic treatment.

3.4. Pasting and thermal starch properties of porous starches

A heatmap was constructed (Fig. 3) to visualize differences between pasting characteristics of the porous starches from different sources. The heatmap of the hierarchical clustering of the RVA properties was analyzed on the basis of similarities and differences in starch pasting properties, including onset, peak viscosity, through, breakdown, final viscosity, setback, hydrolysis percentage at 95°C and 50°C. In line with previous studies [19], the tuber starches displayed different paste viscosity patterns compared to their cereal counterparts. Starches from cereals showed lower peak viscosity, breakdown and final viscosity compared to tuber starches. The resulting dendrogram differentiated three different clusters. First cluster only contained cereal starches, whereas second and third essentially comprised porous starches from cassava and potato, respectively. It was evident from the dendrogram that amylolysis changed the pasting performance of starch suspensions and the effect was highly dependent on the starch source. Nevertheless, there was more similarity among porous starches from same botanical source than among starches from different botanical origin treated with

296 the same enzyme. This effect was more pronounced in the case of tuber starches than in
297 cereal starches, which showed greater similarities between them. It has been reported
298 that the enzymatic susceptibilities of starches varied depending on factors such as
299 granule area, strength of association between starch components, ratio of amylose and
300 amylopectin, crystallinity, polymorphic type (A, B, C), amylose-lipid complex, type of
301 enzyme, and hydrolysis conditions [6, 7, 16]. Significant correlations were found
302 between pasting parameters and OAC of the starches. Particularly, OAC was
303 significantly positive correlated with pasting temperature and setback (r=0.827,
304 P<0.000; r=0.617, P<0.000), but showed a negative correlation with peak viscosity,
305 through and breakdown (r=-0.665, P<0.000; r=-0.463, P<0.008; r=-0.633, P<0.000).
306 The onset temperature or temperature where viscosity started to increase was
307 significantly ($P < 0.05$) augmented in cereal starches and decreased in tuber starches by
308 AMG. Wheat and potato starches treated with AM had higher and lower onset
309 temperature, respectively; while rice and cassava starches remained unchanged after
310 hydrolysis. The CGTase action induced an enhancement of this parameter in all starches
311 studied, except when added to cassava starch, which showed opposite behavior.

312 **Fig. 3.** Hierarchical clustering of RVA profiles. A heat map representing the
313 hierarchical clustering of the Z-scores of the enzyme activities related to viscoelastic
314 properties, when compared starches from different sources obtained from AMG, AM
315 and CGTase enzymatic treatment. The *Z*-scores represent the dispersion around the
316 overall mean of the viscoelastic properties and weighted by their standard errors. The
317 scale of the intensity is shown in the top corner. Row represents samples and column
318 represents viscoelastic properties. Notations are referred to the starch botanical source
319 (Wheat, Rice, Potato, Cassava) followed by the abbreviations of the enzyme used.

Porous starches obtained with AMG showed low peak viscosity compared to their native counterpart, particularly rice starch displayed the lowest peak viscosity, likely due to its large amylose contents after enzymatic hydrolysis as suggested Chung, Liu, Lee and Wei [31]. Porous starches from cereals also showed a reduction of through and final viscosity values, but only low setback was observed on rice starch after AMG action. Besides, it was observed the presence of an additional peak viscosity (Pv1) during heating, prior to the common peak viscosity at 95 ºC, in the case of wheat starch treated with AMG. A similar result was recently reported by Benavent-Gil and Rosell [13] when studying the addition of different AMG levels to corn starches, observing a progressive increase of that peak with the level of AMG added, and a simultaneous decrease of the maximum peak viscosity. Pertaining to enzymatic treatment, diverse effect was promoted. Specifically, tuber starches showed a significant ($P < 0.05$) decrease of the peak viscosity, breakdown and setback after AM action. Moreover, only cassava starch decreased through and final viscosity, while potato starch enhanced these parameters after AMG treatment. It seems that AM preferentially disrupted the amorphous growth rings of cereal starches, but the amorphous and crystalline regions in tuber starches [8]. CGTase attack produced a significant ($P < 0.05$) decrease in pasting parameters of potato starch. In the case of wheat and cassava starches, CGTase treatment resulted in a low peak viscosity, through, final viscosity and setback, but a high breakdown. Similar effects were reported when wheat starch was treated with CGTase [32]. Conversely, rice starch modified by CGTase showed an increase in breakdown and setback, but showed a decrease in through and final viscosity.

The gelatinization temperatures (To, Tp and Tc) as well as the enthalpy changes (ΔH) of native and modified starches are summarized in Table 3. A significant difference in gelatinization temperature was observed between cereal and tuber starches. The highest

To, Tp and Tc values were found for rice starch followed by cassava, potato and wheat starches. Enzymatic treatment only promoted significant ($P < 0.05$) differences on the Tc (Table 3). Taking into account the interaction between botanical source and enzymatic treatment, it was observed that main differences were detected on To and Tc. Porous starches from cereals (wheat and rice) showed higher To, with the exception of rice starch treated with AM; whereas porous starches from tubers exhibited lower Tp and Tc. Similar results were obtained after partial hydrolysis using glucoamylases of wheat, corn and rice starches, which showed a high To [21]. The different behavior of the AM treated rice starch might be related to degradation of amorphous areas, as suggested the amylose content analysis. Therefore, enzymatic treatment of cereal starches affected mainly the beginning of the gelatinization, in opposition to tuber starches where the last part of the gelatinization was more affected. Likely, factors such as granular pores and channels and length of amylopectin spacers and branches could be responsible of that behavior [8].

Regarding the gelatinization enthalpy, no relationship was found neither with the botanical origin of the starches or the enzyme type. The highest ΔH values were noted in the potato starches followed by the cassava, wheat and rice starch. After the enzymatic treatment, porous starches showed lower ΔH compared to their native starches, except porous starches from rice that showed higher ΔH. Again, this result suggested that the state of the crystalline and amorphous regions of porous rice starches differed from the others. ΔH has been related to the amount of ordered carbohydrate structure in the granule that is disrupted during gelatinization [33]. Therefore, the low ΔH values indicated that porous starches from wheat, potato and cassava required less energy to promote starch gelatinization, thus less energy was needed to uncoiling and melt the unstable double helices during gelatinization [34].

4. Conclusions

Starches from cereal and tuber sources could be used to obtain porous starches with different structural and functional features, which also depended on the enzyme used to produce the surface pores or cavities. Cereal starches were more susceptible to enzymatic hydrolysis than tuber starches, presenting deep holes with some degradation of its internal part. The size distribution of the pores was dependent on the type of enzyme and botanical source of starch, but the number of pores per granule was independent of the above. The right combination of type of starch and enzyme could provide porous starches with different degree of porosity, as well as varied pasting performance, thermal properties, WAC and OAC.

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480 **Table 1.** Oligosaccharides and cyclodextrins released after starch hydrolysis by AMG, AM and CGTase. Results are expressed in mg 100 g^{-1} of starch.

Starch source	Enzyme type	Glucose	Maltose	Maltotetriose	Maltotetraose	Maltopentaose	α-CD	β-CD	γ-CD
Wheat	AMG	23.94±0.71^{e}	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	AM	8.46±0.33^{c}	18.88±0.30^{e}	2.07±0.17^{e}	5.98±0.38^{f}	n.d	n.d	n.d	n.d
	CGTase	0.86±0.02^{a}	1.38±0.00^{b}	1.09±0.09^{c}	1.33±0.05^{c}	0.02±0.00^{b}	2.81±0.04^{b}	0.56±0.01^{a}	1.57±0.00^{a}
Rice	AMG	25.42±0.72^{f}	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	AM	7.04±0.29^{b}	20.04±0.34^{f}	1.59±0.15^{d}	4.99±0.38^{e}	0.04±0.00^{c}	n.d	n.d	n.d
	CGTase	0.21±0.02^{a}	0.38±0.00^{a}	0.34±0.04^{a}	0.28±0.01^{a}	n.d	3.00±0.05^{d}	1.11±0.01^{c}	3.32±0.00^{d}
Potato	AMG	24.03±1.18^{e}	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	AM	10.12±0.29^{d}	6.12±0.41^{c}	5.83±0.13^{g}	2.22±0.20^{d}	0.01±0.00^{a}	n.d	n.d	n.d
	CGTase	0.39±0.03^{a}	0.64±0.01^{a}	0.57±0.04^{b}	0.67±0.01^{b}	n.d	2.55±0.04^{a}	1.84±0.01^{d}	2.32±0.00^{b}
Cassava	AMG	25.67±0.72^{f}	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	AM	8.48±0.29^{c}	6.61±0.34^{d}	3.15±0.17^{f}	13.05±0.38^{g}	n.d	n.d	n.d	n.d
	CGTase	0.92±0.03^{a}	1.39±0.1^{b}	1.01±0.09^{c}	1.16±0.06^{c}	n.d	2.88±0.01^{c}	0.88±0.01^{b}	2.94±0.00^{c}
P-value	Enzyme type	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000
	Starch source	0.250	0,008	0.009	0.003	0.064	0.008	0.006	0.006

481 n.d. non detected

482 Values followed by different letters within a column denote significant differences ($P < 0.05$) (n = 3).

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Table 2. Effect of enzymatic treatment on amylose content and the water and oil adsorption capacity of the resulting porous starches

Starch source	Enzyme type	Amylose content (%)	Adsorptive water capacity (g/g)	Adsorptive oil capacity (g/g)
Wheat	Native	21.20 ± 0.16 $^{d-f}$	0.77 ± 0.03 ^{b}	0.65 ± 0.01 $^{c-e}$
	AMG	24.39 ± 1.34 $^{f-g}$	1.37 ± 0.04 ^{h}	0.86 ± 0.09 ^{gh}
	AM	19.98 ± 2.60 ^{de}	1.13 ± 0.02 ^{ef}	0.74 ± 0.04 $^{e-g}$
	CGTase	19.93 ± 0.14 ^{de}	1.10 ± 0.02 ^{ef}	0.81 ± 0.06 $^{f-h}$
Rice	Native	13.88 ± 0.98 ^{c}	1.04 ± 0.06 ^{d}	1.10 ± 0.05 ^{j}
	AMG	23.67 ± 3.94 $^{e-g}$	1.15 ± 0.02 $^{e-g}$	0.98 ± 0.01 ^{ij}
	AM	9.49 ± 1.61 ^{b}	1.16 ± 0.02 ^{fg}	0.92 ± 0.01 ^{hi}
	CGTase	3.02 ± 0.69 ^{a}	1.14 ± 0.00 $^{e-g}$	1.37 ± 0.12 ^{k}
Potato	Native	26.53 ± 1.19 ^{g}	0.62 ± 0.02 ^{a}	0.50 ± 0.01 ^{ab}
	AMG	22.81 ± 3.22 $^{e-g}$	1.09 ± 0.05 ^{de}	0.55 ± 0.08 $^{a-c}$
	AM	21.81 ± 1.69 $^{d-f}$	1.20 ± 0.01 ^{g}	0.48 ± 0.03 ^{a}
	CGTase	18.89 ± 1.20 ^{d}	1.20 ± 0.01 ^{g}	0.61 ± 0.04 $^{b-d}$
Cassava	Native	24.66 ± 1.18 ^{fg}	0.67 ± 0.01 ^{a}	0.87 ± 0.08 $^{g-i}$
	AMG	24.63 ± 0.05 ^{fg}	1.13 ± 0.02 ^{ef}	0.72 ± 0.03 $^{d-f}$
	AM	22.70 ± 2.07 $^{d-g}$	0.91 ± 0.02 ^{c}	0.67 ± 0.08 ^{de}
	CGTase	20.09 ± 2.22 ^{de}	0.91 ± 0.04 ^{c}	0.63 ± 0.04 $^{c-e}$
P-value	Enzyme type	0.000	0.000	0.039
	Starch source	0.000	0.000	0.099

Values followed by different letters within a column denote significant differences ($P < 0.05$) (n = 3)

Table 3. Thermal properties of enzymatically modified starches from different botanical sources

Starch source	Enzyme type	T_o (ºC)	T_p (ºC)	T_c (ºC)	ΔH (J/g)
Wheat	Native	53.16 ± 1.74 ^{b}	58.45 ± 1.06 ^{bc}	64.66 ± 0.93 ^{b}	20.88 ± 1.05 ^{ef}
	AMG	58.58 ± 0.10 ^{ef}	60.78 ± 0.12 ^{de}	64.93 ± 0.32 ^{b}	19.33 ± 0.83 ^{cd}
	AM	57.51 ± 0.11 $^{c-e}$	60.37 ± 0.24 ^{d}	64.48 ± 0.37 ^{b}	18.99 ± 0.45 ^{c}
	CGTase	56.68 ± 0.12 ^{cd}	59.62 ± 0.12 ^{cd}	63.49 ± 0.16 ^{b}	18.18 ± 0.59 ^{bc}
Rice	Native	58.84 ± 1.05 ^{ef}	66.62 ± 0.83 ^{h}	75.22 ± 0.28 ^{ef}	14.84 ± 0.17 ^{a}
	AMG	60.83 ± 0.38 ^{g}	66.78 ± 0.12 ^{h}	75.53 ± 0.49 ^{f}	20.62 ± 0.42 ^{de}
	AM	59.37 ± 0.52 ^{f}	67.20 ± 1.18 ^{h}	74.68 ± 1.19 ^{ef}	19.50 ± 0.53 $^{c-e}$
	CGTase	61.74 ± 1.59 ^{g}	64.45 ± 0.35 ^{g}	73.78 ± 0.47 ^{de}	19.34 ± 0.84 ^{cd}
Potato	Native	56.35 ± 0.19 ^{c}	61.79 ± 0.12 ^{ef}	69.12 ± 0.56 ^{c}	27.59 ± 0.54 ^{i}
	AMG	52.60 ± 1.70 ^{ab}	56.20 ± 0.47 ^{a}	61.91 ± 0.51 ^{a}	22.55 ± 0.63 ^{g}
	AM	50.88 ± 0.18 ^{a}	55.37 ± 1.41 ^{a}	61.15 ± 1.71 ^{a}	15.64 ± 0.36 ^{a}
	CGTase	51.03 ± 0.11 ^{a}	57.78 ± 1.06 ^{a}	64.29 ± 0.73 ^{b}	22.22 ± 0.00 ^{fg}
Cassava	Native	57.40 ± 0.02 $^{c-e}$	65.85 ± 0.21 ^{gh}	75.26 ± 0.18 ^{ef}	24.04 ± 1.04 ^{h}
	AMG	58.38 ± 0.32 $^{d-f}$	62.53 ± 0.47 ^{f}	72.32 ± 0.68 ^{d}	17.18 ± 0.57 ^{b}
	AM	57.49 ± 0.14 $^{c-e}$	62.70 ± 0.00 ^{f}	72.53 ± 0.32 ^{d}	18.98 ± 1.11 ^{c}
	CGTase	57.82 ± 0.19 $^{c-f}$	63.03 ± 0.00 ^{f}	72.86 ± 0.70 ^{d}	18,85 ± 0.59 ^{c}
P-value	Enzyme type	0.4431	0.1021	0.0051	0.1191
	Starch source	0.0000	0.0000	0.0000	0.1167

To = onset temperature, Tp = peak temperature, Tc = conclusion temperature, ΔH = enthalpy change. Values followed by different letters within a column denote significant differences ($P < 0.05$) (n = 3).