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Cassava Starch Maltodextrinization/ Monomerization Through Thermopressurized Aqueous Phosphoric Acid Hydrolysis†

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Abstract

Kinetic conditions were established for the depolymerization of cassava starch for the production of maltodextrins and glucose syrups. Thin-layer chromatography and high-performance liquid chromatography analyses corroborated that the proper H_3PO_4 strength and thermopressurization range (e.g., 142–170°C; 2.8–6.8 atm) can be successfully explored for such hydrolytic purposes of native starch granules. Because phosphoric acid can be advantageously maintained in the hydrolysate and generates, after controlled neutralization with ammonia, the strategic nutrient triplet for industrial fermentations (C, P, N), this pretreatment strategy can be easily recognized as a recommended technology for hydrolysis and upgrading of starch and other plant polysaccharides. Compared to the classic catalysts, the mandatory desalting step (chloride removal by expensive anion-exchange resin or sulfate precipitation as the calcium-insoluble salt) can be avoided. Furthermore, properly diluted phosphoric acid is well known as an allowable additive in several popular soft drinks such as colas since its acidic feeling in the mouth is compatible and synergistic with both natural and artificial sweeteners. Glycosyrups from phosphorolyzed cassava starch have also been upgraded to high-value single-cell protein such as the pigmented yeast biomass of *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*), whose astaxanthin (diketo-dihydroxy-β-carotene) content may reach 0.5–1.0 mg/g

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of dry yeast cell. This can be used as an ideal complement for animal feeding as well as a natural staining for both fish farming (meat) and poultry (eggs).

Index Entries: Starch; hydrolysis; phosphoric acid; maltodextrinization; astaxanthin; byproducts.

Introduction

Following cellulose, starch is the most widespread natural source of the monosaccharide glucose in a polymeric form. Chitin, the second most abundant glycopolymer in nature, bears a modified monomeric unit (2-deoxy-2-acetylamino-D-glucopyranose) *(1)*. Although fully linear as a homo-β-D-glucopyranan, cellulose is exceptionally resistant to strong acid hydrolysis with mineral acids owing to the maximized inter- and intramolecular hydrogen bonding that holds the chains together in bundles. Even enzymatic depolymerization through fungal and bacterial cellulolytic complexes seldomly leads to complete monomerization. These optimized cellulase complexes, at the most, accumulate cellobiose since β-glucosidases are not a dominant or balanced component within naturally occurring cellulolytic complexes, mainly composed of isoforms of endo- and exoglucanases including cellobiohydrolases *(2)*. By contrast, starch is amenable to both mineral acid and enzymatic hydrolyses since the same kind of glycosidic linkage is built on the α-anomer in both the linear homopolymeric 1,4-linked amylose fraction and the 1,6-ramified fraction amylopectin, thus favoring helicoidal conformations rather than the particularly rigid crystalline structure found in cellulose.

A number of enzymatic preparations are commercially available for starch bioprocessing and upgrading. The most intensively employed enzymes are the thermoresistant α-amylases (maltogenic for amylose, malto- and dextrinogenic for amylopectin) and amyloglucosidases or glucoamylases (glucogenic for both starch fractions). The combination of both types of enzyme, taking advantage of the thermostability of *Bacillus* spp. amylases, allows for the simultaneous gelatinization and saccharification of starch granules. At lower temperatures, amyloglucosidases complete the splitting of any remaining 1,4- and 1,6-linkages (branching points) of short dextrins, branched oligosaccharides, and maltose *(3)*.

Alternatively, the acid-catalyzed depolymerization of starch, most often using HCl (and less often H_2SO_4), is in fact still applied extensively in starch-processing factories around the world, despite the full commercial availability, at higher costs, of several enzymatic preparations for starch hydrolysis. HCl-mediated hydrolysis, depending on the acid strength and temperature (pressurization), may convert the slurry from native starch granules to either maltooligosaccharides (maltodextrins) or glucose *(3)*. In the latter case, owing to the partial overlapping of the kinetic constants for the breakdown of glycosidic linkages *(4)* and for the subsequent free hexose dehydration/degradation, glucose syrups are undesirably accompanied by some nonsugar byproducts. In addition, if the starch prepara-

tions (commercial feculas) have some degree of contamination with (hemi)cellulosic fibers (e.g., arabinoxylans), the level of degradation products increases with the accumulation of another dehydration byproduct from pentoses—furfural. These parallel color- and taste-generating reactions require additional industrial steps for clarification of glucose syrups (e.g., activated charcoal), a situation different from the kitchen caramels *(5)* resulting from light or deep browning of sucrose on excessive heating in which color and a particular taste are intended to replace or combine the basic sweetening power of table sugar.

This article deals exactly with the replacement of strong mineral acids—HCl or H_2SO_4—by a milder catalyst, H3PO4. The pursued advantages for this new technology are broached in the Results and Discussion.

Materials and Methods

Starch Sources

Fresh tubers of cassava (variety Fibra Branca) were obtained in a local market, peeled, and quickly submerged in cold water to avoid the browning phenolization. Each root segment was coarsely sliced with a sharpened knife. The slices were combined with 2 to 3 vol of cold water and triturated in a Waring blendor for 30 s. The thick and milky suspension of starch granules, containing (hemi)cellulosic fibers and released protein, was filtered through a double layer of cheesecloth in which most of the foam material was retained along with coarser fragments. Cassava starch granules settled very quickly as a deep white and firm bed, leaving most of the protein in the yellowish supernatant, which was then discarded. To complete the removal of residual free protein and also the smallest and less dense starch granules, the referred bed was resuspended in an excess of water, agitated to disperse the starch granules, and filtrated through a fourfold bent layer of cheesecloth repeatedly until there was no noticeable turbidity in the supernatant. The final stock solution of cassava starch was shown to have at least 35% solids concentration on dry basis, as determined by freeze-drying of the starch slurry.

In some cases, cassava and potato feculas from local producers were used, and for comparative purposes, soluble starch (Reagen) and wet-milled corn kernels (no SO_2 addition) served as representative samples of purified tuber (potato) and crude grass starch, respectively.

Acid and Enzymatic Hydrolyses

The aforementioned starch slurries were acidified with 1:10 and 1:100 dilutions of commercial phosphoric acid (85% [w/w]) (Merck, Brazil) under strong agitation (magnetic bar) to avoid settlement of starch granules. This addition of acid corresponded to a range of 12.5–425 mg of acid/g of starch (dry basis). The minor buffering interference of the residual protein from the intact starch granules was taken into account, and this

motivated the extensive granule washing procedure described in the previous section. Reference incubations of soluble starch (Reagen) with amylolytic enzymes from Novo Industri (*Fungamyl*® and amyloglucosidase) followed the experimental procedure recommended by the supplier.

Thermopressurized Acid Hydrolysis

A homemade steel reactor vessel (V_t = 15 L), equipped with both temperature and pressure control systems and a purge valve, was used for the hydrolysis operations. After hydrolysis, starch suspensions were contained in Pyrex glassware with loosely fitted caps. The heating period to reach the desired peak temperature (or corresponding pressure) corresponded to about 20 min. The employed thermopressurization ranges varied from 2.8 to 6.8 atm, and the residence time at the peak temperature was from 5 to 10 min.

Analytical Procedures

Partially or completely phosphorolyzed starch slurries were diluted from 1:10 to 1:100 for the chromatographic analyses. Thin-layer chromatography (TLC) was carried out on silica gel 60 chromatoplates (Merck-Darmstadt, Germany) irrigated with isopropanol:ethyl acetate: nitroethane:acetic acid:water (30:5:5:0.5:7 [v/v]) as the mobile phase for double developments. Free glucose and maltooligosaccharides were revealed by first spraying the plates with orcinol (0.5 g% [w/v]) in a sulfuric acid:methanol solution (5:95 [v/v]) and then heating them until full color development (blue violet) was achieved. Densitometry of developed TLC plates was carried out in a CS-9301PC from Shimadzu (Tokyo, Japan) under the flying spot operational mode.

High-pressure liquid chromatography (HPLC) was performed in a Shimadzu HPLC system, model LC10AD, provided with an SIL10A autosampler and an RID10A refractive index detector. HPLC analysis was performed at 65°C in an Aminex HPX-87H column (Bio-Rad) eluted with 8 m*M* H_2SO_4 at a flow rate of 0.6 mL/min, a condition that does not lead to any on-line hydrolysis of maltooligosaccharides. Soluble sugars were quantified by calibration using maltose (retention time, R_T, of 6.9 min), glucose (8.7 min), and acetic acid (14.7 min) as external standards.

Upgrading of Phosphoric Hydrolysates to Carotenoid-Enriched Biomasses

Following pH adjustment of starch digests with aqueous ammonia to 5.0 and dilution to a theoretical total sugar content of 4 g%, the substrate was sterilized and supplemented with 75 mg/L of yeast extract. All individual assays were normalized with respect to the final content of ammonium phosphate. Inoculation proceeded with the basidiomycetous yeast *Xanthophyllomyces dendrorhous* (ATCC *Phaffia rhodozyma* strain 24202) or with an amylolytic yellowish bacterium isolate from rot dahlia tubers

(*AMRL*). Cultures were grown in 50-mL Erlenmeyer flasks (medium:total volume = 1:5) for 5 d at 25 to 26°C at moderate oxygen transfer rate (100 rpm on an orbital shaker).

The yeast or bacterial cell mass was collected by centrifuging at 3000*g*, lyophilized, and then treated with dimethyl sulfoxide (2 vol for good swelling) for carotenoid extraction with an excess of acetone (two times). Comparative yields of astaxanthin (yeast) or β-carotene plus xanthophylls (bacterium) were evaluated in each 10-mL normalized organosolvent extract by spectrophotometry at 470 nm, assuming that 0.25 U of absorbance corresponds to 1 µg of carotenoid/mL *(5)*.

Results

The cassava business currently is an important world concern. Although Nigeria (Africa) is the biggest producer (31 million t/yr), Thailand occupies the leading position for exports of native and modified cassava starches. In Brazil (12% of the world production), cassava is mainly cultivated in the southern state of Paraná and in the Amazon region (Pará State), each affording crops of about 3.5 million t/yr. In the former state, an industrial plant (INDEMIL, Paranavaí, PR) is reported to be the only one producing glucose syrups from cassava in the Occident (about 100 t/mo). Catalysis for this is carried out with HCl, the same acid procedure also utilized by larger plants operating with corn starch.

Figure 1 demonstrates the effectiveness of bringing cassava starch slurries to dextrinization (lanes 1 and 2), oligomerization (lanes 3 and 4), or monomerization (lane 5) using phosphoric acid as the acid catalyst. It is readily observed that the progressive increase in acid strength favored monomerization for any prefixed thermopressurization condition (160°C and 5.1 atm, in the present case, but with a residence time at the peak temperature enlarged to 10 min). Minor amounts of degradation products were detected in the most severe hydrolytic condition, together with the progressive accumulation of glucose (R_f = 0.6). The amount of released glucose increased accordingly (Fig. 2; analyzed samples from TLC of Fig. 1 were those corresponding to lanes 10 and 12), as measured by densitometry, and the amount of degradation products (main peak at $R_f > 0.8$) accounted for <5% of the recovered free monosaccharide in the case of the strongest hydrolysis condition. In one case, even a trace amount of maltononaose was visible, but by increasing the catalyst concentration, the detection or recording limit shifted to maltopentaose. The distribution ratio of glucose:maltose:maltotriose for four phosphoric strengths, except for the mildest condition, are reported in Table 1; it increased (proportionally with acid strength) from 35:31:34 to 61:17:22. Also, the hydrolysis products profile obtained with phosphoric acid superseded that obtained with *Fungamyl*® (an *Aspergilllus* sp. amylolytic preparation from Novo Nordisk, Denmark) and approached that provided by amyloglucosidases (last two lanes in Fig. 1).

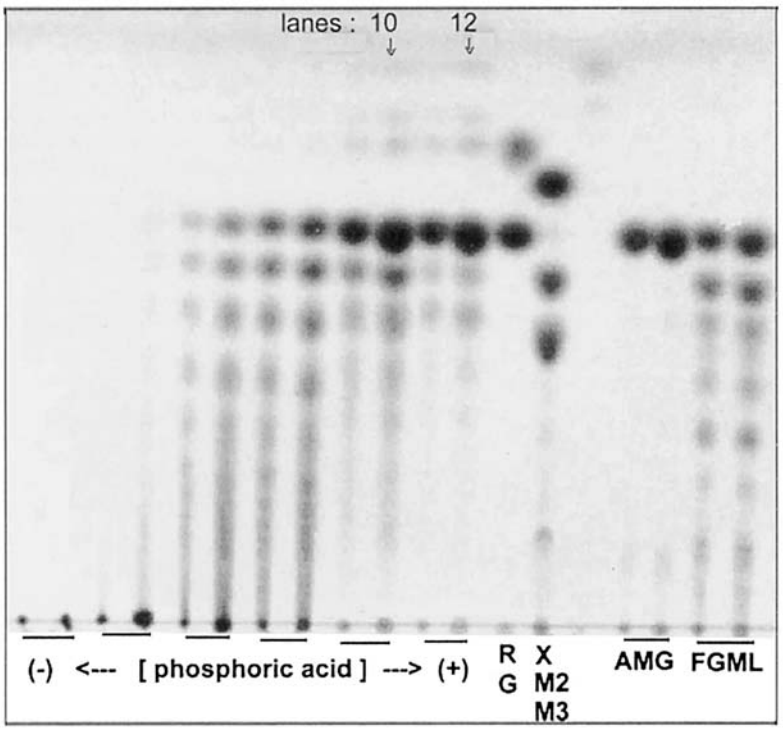

Fig. 1. Thin-layer chromatogram of cassava starch hydrolysis with thermopressurized aqueous phosphoric acid. (–) <—— [phosphoric acid] ——> (+) = hydrolysis with increasing phosphoric acid strength from 10.8 to 425 mg of acid/g of dry cassava native starch; R, G = rhamnose and glucose standard, respectively; X, M2, M3 = xylose, maltose, and maltotriose standard (in order of decreasing mobility, respectively); AMG = hydrolysis with amyloglycosidase; FGML = hydrolysis with *Fungamyl*, the latter two incubations using soluble starch as substrate.

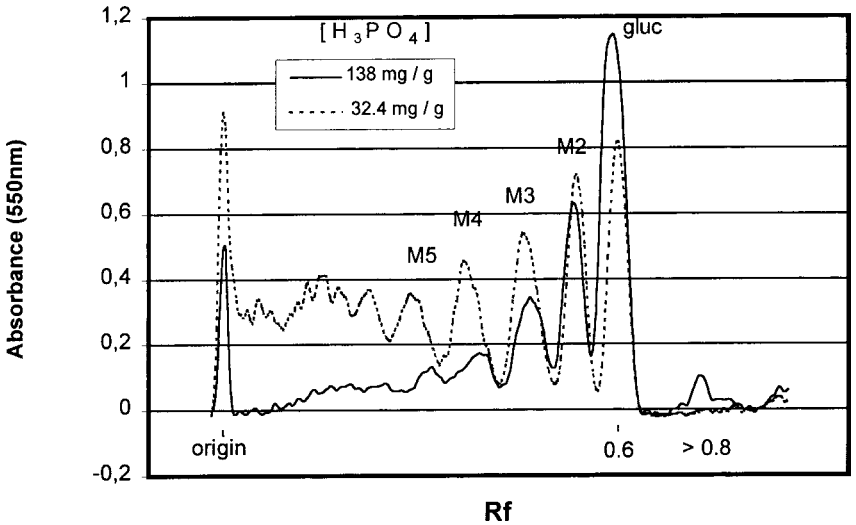

Fig. 2. Densitometric analyses at 540 nm of the developed thin-layer chromatogram (see Fig. 1, lanes 10 and 12) from the thermopressurized aqueous phosphoric digest of cassava starch. Apparatus: Shimadzu CS-9301PC densitometer; multipeak dashed and solid lines: starch (dry basis) processed at acid catalyst ratios of 32.4 and 138 mg/g, respectively.

Table 1
Monomeric to Trimeric Percentage Distribution of Reducing Sugars After Thermopressurized Diluted Phosphoric Acid Hydrolysis of Cassava Starch

Acid addition (mg/g starch)	Glucose	Maltose	Maltotriose
32.4	35.0	31.2	33.8
138	54.4	24.4	21.2
425	60.7	17.2	22.1

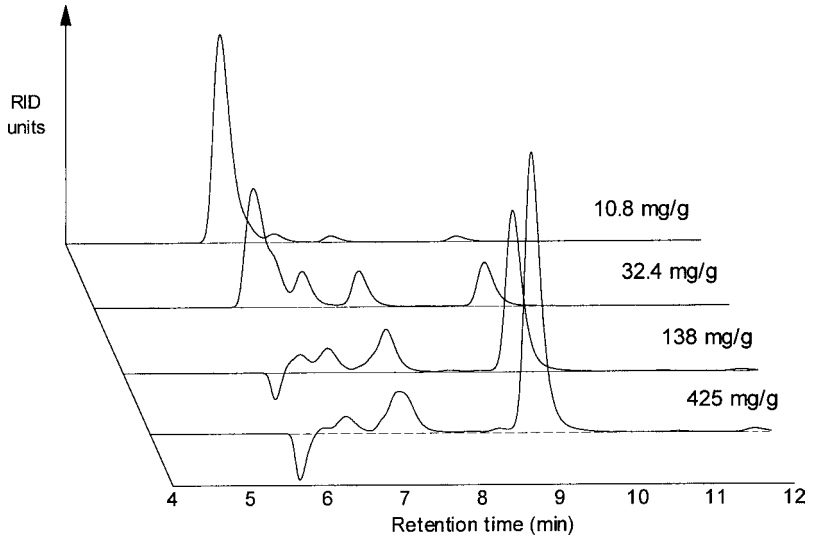

Fig. 3. HPLC of cassava starch hydrolysis with thermopressurized aqueous phosphoric acid (from top to bottom: increasing [phosphoric acid] from 10.8 to 425 mg of acid/g of dry cassava native starch). Chromatographic conditions are as described in Materials and Methods. RID, refractometer index detector.

HPLC analysis (Fig. 3) of starch hydrolysates corroborated the aforementioned interpretation, despite the lower resolution for maltooligomers with degree of polymerization (DP) higher than 3 (maltotriose). Similar results were obtained when commercial feculas, soluble starch, or even corn starch were used in substitution for our laboratory-made native starch granules preparation. The purity and granule integrity of the prepared starch were assessed by optical microscopy of the fresh material or following staining of the intact granules with iodine/iodide or Coomassie blue. Transmission electron microscopy has also been used for this purpose *(6)*.

The suitability of phosphoric acid–hydrolyzed starch for fermentation procedures was successfully verified with two different microorganisms: the astaxanthinogenic GRAS yeast *X. dendrorhous* (formerly *P. rhodozyma*) and a bacterial isolate. In both cases, the usual level of micro-

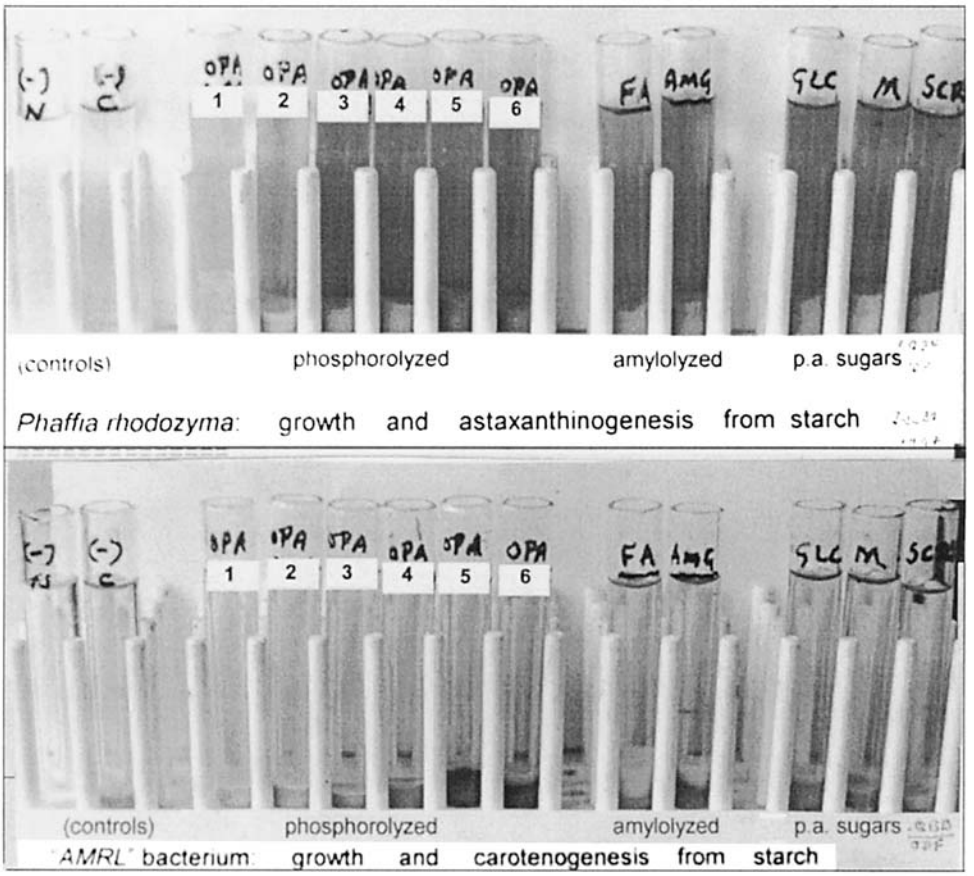

Fig. 4. Carotenogenesis from phosphoric acid–hydrolyzed starch with *X. dendrorhous* (**top**) or with the bacterial isolate *AMRL* (**bottom**). (–)N and (–)C denote the controls where nitrogen or carbohydrate were omitted in the fermentation. Assays 6, 5, 4, and 3 refer to starch thermopressurized phosphoric pretreatments with 425, 138, 32.4, and 10.8 mg of acid/g of dry starch at 160°C/5.1 atm, respectively. For assay 2, starch was mildly acidulated to pH 3.85 before digestion, and assay 1 is the control with no acid addition. FA and AMG correspond to enzymatic digests with *Fungamyl* or amyloglucosidase. GLC, M, and SCR are the fermentative runs carried out with glucose, maltose, or sucrose standard sugars, respectively. Supernatants contain the carotenoid material after organosolvent extraction of each formed yeast or bacterial biomass. Precipitates contain either only depigmented microbial cells (**right**) or the same with residual starch (**left**). See Materials and Methods for other details.

bial biomass production was obtained, and, more important, carotenoid pigmentation (Fig. 4) was indeed attained just after the onset of the stationary phase of growth.

Discussion

Starch hydrolysis with HCl or H_2SO_4 requires, for purposes such as food application of glucose- or maltose-enriched syrups, catalyst removal

by anion exchange or precipitation of insoluble salts (e.g., calcium sulfate), respectively. Also, in the former case, the chloride anion is seldom beneficial (if not inhibitory) as compared to the sulfate anion when syrups are used for microbial upgrading to solvents, organic acids, antibiotics, and related fermentation goods. Here resides the main advantage of using phosphoric acid as an alternative catalyst because it may be directly incorporated, regardless of the particular concentration, to both food and fermentative applications. A partial neutralization with ammonia is recommended both to increase the pH to a more appropriate physiologic condition for fermentation and to provide an input of N source. In the case of lower sweetening syrups enriched in maltooligosaccharides or short dextrins (obtained with more dilute catalyst for food uses), no neutralization step would be required as long as the final acid concentration is <1.5 m*M*. Ammonium phosphate, together with the released reducing sugars themselves, constitutes the basic triplet of mandatory macronutrients in any fermentation procedure (C, N, and P). Also differentiating it from the stronger mineral acids, phosphoric acid (and its salts) may be considered by far more (if not exclusively) compatible with human physiology, acting as a "physiologic catalyst." Although HCl plays a role in stomach digestion, no consumer would accept any food acidified with HCl because both the mouth and pylorus lack the natural protection available in the stomach walls for this particular acid. Furthermore, the presence of small amounts of phosphoric acid (final pH of about or above 3.0) in food materials is widely accepted in the market, such as in the case of established soft drinks or colas.

Fermentation results matched those obtained in parallel runs with pure glucose, maltose, or sucrose. No attempt was made for the exact gravimetric expression of biomass yield (Y) because, in those runs in which starch was barely hydrolyzed (e.g., in the case of 11.25 mg of catalyst/g of starch), the removal of residual starch from the centrifuged cells proved very tedious. Conversely, the biosynthesized carotenoid (mostly if not completely remaining associated to the microorganism cells) was estimated, after organosolvent extraction, to be between 0.5 and 1.0 mg/g of dry cells (results not shown) based on previously reported literature procedures *(7,8)*, and, as expected, astaxanthin was the major highly oxygenated carotenoid (>70%) produced by *X. dendrorhous*.

The strategy described herein for the phosphoric acid–mediated hydrolysis of starch confirmed our pioneering work carried out on sugarcane and sorghum bagasses *(9)* (xylan as the selectively hydrolyzed target) and dahlia inulin *(10)*. Xylose and fructose were the respective dominant and sole hydrolytic products in those preliminary studies.

If no quantitative yield of glucose is pursued (which would demand much larger amounts of phosphoric acid as compared to HCl, since the pK_{a1} of the former is 2.15), phosphoric acid catalysis looks rather advantageous. And in dealing with acid catalysis, HCl being the most often employed at a final concentration of 20 m*M*, the higher price for phosphoric acid would

not account for significant differences for two reasons: (1) the commercial provision refers to 37% HCl and to 85% H_3PO_4, both expressed as weight/weight; and (2) the acid catalyst is itself a secondary component of the cost breakdown. In fact, a much higher cost component is the removal of degradation products, which also brings additional advantages to phosphoric acid, since it leads to less colored starch hydrolysates. Taking into account the local market prices for bulk purchases for 32% HCl (w/w) and 85% H_3PO_4 (w/w) and computing the effective and respective dry acid concentrations, the cost is very similar for both catalysts. Work is in progress to refine the kinetic aspects of this chemical technology, whereas the improved enzymatic technology employing unusual isolates (e.g., extremophiles) or novel enzymes (e.g., maltogenic amylases from lactobacilli acting on crude nongelatinized starch granules *[6]*) goes to maturity.

Conclusion

Phosphoric acid was successfully tested as an alternative acid catalyst for starch depolymerization to glucose and maltooligosaccharides. Fermentation culture media based on these hydrolysates, with no need for catalyst removal, led to good biomass generation and normal physiology of the tested microorganisms. *X. dendrorhous* experienced the usual pigmentation expressed mainly as astaxanthin (diketo-dihydroxy-β-carotene), which increased in proportion to starch DP reduction.

Acknowledgments

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