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Controlled production of oligosaccharides from amylose by acid-hydrolysis under microwave treatment: Comparison with conventional heating

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Abstract

Controlled production of malto-oligosaccharides from pure amylose has been realised in a fast, cheap and easy way with good potentials for later scale up. Pure amylose (20 g L^{-1}) was hydrolysed under dilute acidic conditions (0.45 M HCl, 90 °C) with two sorts of heating: microwave irradiation and conventional heating. The kinetics of oligosaccharide production were followed by high-performance anion exchange chromatography. Microwave treatment was shown to be more efficient. A similar range of oligosaccharides as with the conventional heating procedure was observed, but without any appearance of degradation compounds (brown products) and a 10 times faster production rate leading to very short production times (maximum 15 min).
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Keywords: Polysaccharides; Amylose hydrolysis; Microwave irradiation; Degree of polymerization; Oligosaccharides; High performance liquid chromatography

1. Introduction

Starch is one of the most important and flexible food ingredients possessing attributes for innumerable industrial applications. It is also one of the least expensive agricultural commodity product. As a result, the majority of oligosaccharides used in the food industry are derived from starch (Taniguchi, 2004). Numerous studies have been published about the structure and properties of starch. Starch granules are composed of two types of α-glucans: amylopectin and amylose. Amylopectin consists of very large molecules ($MW = 1 \times 10^7$–1×10^9 g mol^{-1}) with a branched structure built from 95% α-(1 → 4) and 5% α-(1 → 6) linkages. Amylose is a long linear chain (molecular weight = 1×10^5–1×10^6 g mol^{-1}) containing around 99% of α-(1 → 4) and few α-(1 → 6) linkages. Both amylose chains and exterior chains of amylopectin can form double helices which may in turn associate to form crystalline domains. The resulting complex structure is well described in a recent review of Tester, Karkalas, and Qi (2004). The ratio of the two polysaccharides varies according to the botanical origin with various content of amylose from 0% (waxy starches), 20–35% (normal starches) to greater than 40% (high-amylose starches).

Hydrolysis of pure amylose to obtain malto-oligosaccharides can be realised either by enzymic (Kanai, Haga, Akiba, Yamane, & Harata, 2004) or acidic hydrolysis. Malto-oligosaccharides are useful for their low sweetness, low viscosity, high moisturizing effect, high efficiency for digestion and absorption (Kanai et al., 2004), and prevention of sucrose crystallisation (Duedahl-Olesen, Kragh, & Zimmermann, 2000). They are commonly described to be composed of two to ten units of α-D-glucopyranose linked by an α-(1 → 4) bond (Duedahl-Olesen et al., 2000). Polydisperse mixtures of malto-oligosaccharides with greater DP (ranging from 1 to 50) obtained from hydrolysis of

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starch are widely used in pharmaceutical and food technology as syrups (Molinero & Goddard, 2004). In the case of enzymatic hydrolysis, the specific enzymes needed to obtain a precise range of oligosaccharides are expensive and often require the use of a buffer complicating the later product purification (Duedahl-Olesen et al., 2000). The acidic hydrolysis reaction is another way, cheap, simple and easy to stop by neutralisation of the medium. In the past, this production of malto-oligosaccharides was realised under conventional heating but this leads to the parallel formation of brown compounds.

Recent emphasis has been given to other methods to replace the classical ones in the view to develop novel types of chemistry, including microwaves and other unconventional physical actors such as high pressure and ultrasound (Kardos & Luche, 2001). Clean, cheap and a convenient method, microwave irradiation is becoming an increasingly popular heating process. They are extensively employed in food production and preparation. The food industry is the largest consumer of microwave energy, where it can be employed for example in cooking, thawing, heating and re-heating (Oliveira & Franca, 2002).

The application of microwave heating in chemical synthesis reactions appeared only at the end of the 1980s, with the pioneering work of Gedye et al. (1986) and Giguere et al. (1986). Since then, microwave chemistry has become a rapidly developing branch in organic chemistry. Numerous microwave-assisted organic reactions have been performed in the field of carbohydrate chemistry (Satoh et al., 2005; Yoshimura, Shimizu, Hinou, & Nishimura, 2005) because microwaves enhance selectivity, improve reaction rates and give cleaner products with higher yields and shorter reaction times. Microwave heating has actually been shown to be the most useful method to assist manipulation reactions on mono- di- and polysaccharides in the case of synthesis reactions (Corsaro, Chiacchio, Pistara, & Romeo, 2004; Das, 2004; Kunlan et al., 2001; Raghavan, Orsat, & Meda, 2005; Yoshimura et al., 2005).

In recent years, there has been growing interest also in applying microwave heating to rapid thermal digestion prior to elemental (Carrilho, Gonzalez, Nogueira, & Cruz, 2002; de la Fuente, Olano, & Juarez, 2002) and chemical analysis of inorganic and biological samples (Drímalová, Velebný, Sasinková, Hromádková, & Ebringerová, 2005). There have been a few studies on starch degradation with more specific emphasis on physico-chemical properties (Kunlan et al., 2001; Lewandowicz, Jankowski, & Formal, 2000). Only Yu, Chen, Suree, Nuansri, and Wang (1996) have looked at the general impact of microwaves on starch molecular weight in dilute acid conditions. To our best knowledge the production of specific oligosaccharides with a well-defined degree of polymerisation has not been studied so far.

In the current contribution, microwave irradiation is investigated as an alternative approach for obtaining oligosaccharides of a controlled size, in shorter times (compared to acidic hydrolysis under conventional heating or to enzymic treatment) and without contamination by brown reaction products. The hydrolysates were characterised using high performance anion exchange chromatography.

2. Experimental

2.1. Sample preparation

The procedure for polysaccharide hydrolysis using conventional or microwave heating were largely similar. Amylose (Fluka, 25 mg) was put in a Pyrex tube containing 650 μL of deionised water.

2.2. Conventional heating procedure

After 60 min in a water-bath at 90 °C (pre-heating step), a 0.98 M solution of hydrochloric acid was added to obtain a final concentration of 0.45 M HCl in the amylose sample (total volume 1.2 mL). The tube was put back into the water bath after mixing and fractions were analysed at different times using high performance anion exchange chromatography (HPAEC) (vide infra).

2.3. Microwave heating procedure

Different types (time and power) of pre-warming microwave treatments with 3 s pulses were applied. The microwave oven used was an EMS 820 of 1000 W (Electron Microscopy Sciences, Pennsylvania, USA). Similarly to the conventional heating, a 0.98 M solution of hydrochloric acid was added to have a final concentration of 0.45 M HCl in the amylose sample (total volume 1.2 mL). After mixing, an additional microwave irradiation (hydrolysis period) was performed at 25% power. Fractions were then analysed by HPAEC.

2.4. Degree of hydrolysis

The remaining amylose (Y) was recovered by centrifugation (7000 rpm, 10 min, 25 °C) of the sample (Yu et al., 1996). The supernatant was removed and the precipitate was re-suspended in water and centrifuged again. This procedure was repeated 3 times and the final precipitate was freeze-dried to weigh the non-hydrolyzed fraction. The hydrolysis extent X was determined as $X = [(Y/\text{initial stock}) \times 100]$.

2.5. High performance anion exchange chromatography (HPAEC)

The kinetics of the amylose hydrolysis reaction were studied using HPAEC with pulsed amperometric detection (PAD) with a method adapted from Hanashiro, Abe, and Hizukuri (1996). After centrifugation to recover the supernatant, 10 μL fractions were diluted in 140 mM sodium hydroxide to a total volume of 1 mL. Twenty microlitres of this solution was injected into two Carbopac PA-1 analytical columns (Dionex, Bavel, The Netherlands,

4 × 250 mm) connected in series following a PA-1 guard column (Dionex, 4 × 50 mm). HPAEC was performed with a gradient chromatography system equipped with an eluent degasser module. The pulse potentials and durations of the electrode were: $E_1 = +0.05$ ($t_1 = 400$ ms); $E_2 = +0.75$ ($t_2 = 200$ ms); $E_3 = -0.80$ ($t_3 = 300$ ms). The response time and the sensitivity of the detector were set to 900 ms and 5 k nA, respectively. Gradient elution was performed at a flow rate of 1 mL min^{-1} with eluent A (140 mM NaOH) and eluent B (140 mM NaOH + 500 mM sodium acetate). Each run was carried out using the gradient detailed in Table 1.

2.6. Appearance of brown compounds

Measurements of the absorbance in the visible range from 400 to 500 nm were performed on the supernatant recovered after centrifugation of the samples. The spectra were recorded using an Opsys MR spectrophotometer (Dynex technologies, VA, USA).

3. Results and discussion

Two percent w/v amylose was added to a water solution and hydrolysed under diluted acidic conditions (0.45 M HCl) with either conventional or microwave heating. Amylose is known to be difficult to solubilize (Autio, Suortti, Hamunen, & Poutanen, 1996) and basically remains as a suspension at room temperature (Manelius, Nurmi, & Bertoft, 2005). Thus, we applied a pre-heating treatment to allow molecules swell making them more accessible for the acid. A temperature of 90 °C was chosen for the conventional heating as this was the maximum temperature measured after the longest microwave irradiation. Hence, the way of applying the heat should be more important in this study than the actual temperature itself.

3.1. Kinetics of hydrolysis in conventional heating

Each sample was centrifuged following a variable time of acid treatment and the composition in terms of oligosaccharides present in the supernatant was analysed using HPAEC-PAD (Table 2). For clarity reasons and no additional benefit to the discussion, the amount of each oligosaccharide was reported up to DP 17. After application of just the pre-heating treatment (1 h, 90 °C), only a small amount of glucose was found to be present (Fig. 1A). Most likely, this glucose was already present in the commercial amylose. Another possibility would be the liberation of a small amount of glucose during the pre-heating period.

Table 1
Gradient procedure used to separate malto-dextrins by DP

Time (min)	0	3.0	6.8	11.0	16.0	27.0	39.0
Eluent A	70	70	60	50	40	30	80
Eluent B	30	30	40	50	60	70	20

A wide range of oligosaccharides (up to DP = 37) appeared after 5 min hydrolysis (Fig. 1B) with a big proportion of oligosaccharides with DP > 17 (24.4%). The levels of these oligomers ranged from 2.2% for DP 17 to 0.6% for DP 37 respectively (data not shown). The levels of the shorter oligomers are more or less constant. The difference in relative amount between maltotriose (DP = 3) and maltodecaose (DP = 10) is only 3.4%. During the short 5 min treatment, the amylose is rapidly and randomly hydrolysed resulting in the appearance of a wide range of oligosaccharides, all present at more or less equal levels. After an additional 2 min (i.e. a total hydrolysis time of 7 min), the difference between DP 3 and DP 10 diminished slightly to 3.3% whereas the overall level of this group ($3 \leqslant DP \leqslant 10$) increased to 44.8% (instead of 36.7% as seen after 5 min hydrolysis). This confirms the random hydrolysis of amylose. Between 7 and 15 min of hydrolysis (Fig. 1C), the intensity of the subsequent oligomers follows a more or less Gaussian distribution. This implies that the insoluble amylose continues to be hydrolysed with this degradation being accompanied by a decrease of the maximum observed DP from 32 to 20. In addition, the total proportion of DP > 17 decreases from 14.7% to 1.1%, respectively. The shift towards a lower maximum DP starting from 7 min hydrolysis suggests that the degradation of the available amylose (insoluble) became more difficult. Consequently, lower quantities of oligosaccharides with DP > 32 are produced despite the fact that there is a still visible residual insoluble fraction (high molecular weight amylose potentially available for degradation). Then, the longest oligosaccharides already present in the solution are hydrolysed faster than they are provided which results on higher proportion of lower MW fractions. This process continues in the same way after 30 (Fig. 1D) and 45 min hydrolysis (Fig. 1E). After 1 h hydrolysis (curve not shown), we obtained almost the same results as at 45 min (maximum oligomer size of DP 7). Glucose, maltose and maltotriose are now the major hydrolysis products in the solution (total sum = 84.6%). From this time onwards changes are much slower as compared to the rapid initial changes in the DP distribution. Apparently, the acid hydrolysis of amylose entered a slower second phase after 1 h. At 2 h (Fig. 1F), glucose became the major species in the oligosaccharide distribution representing 66.8% of the original mass, which increased to 88.1% after 4 h (with persistently maltose and maltotriose), and to 98.2% after 8 h (Fig. 1G). Starting from 2 h of hydrolysis, a peak appeared before the glucose. Even if this peak stayed unknown, it is assume to be a furfural type degradation product (5-hydroxymethylfurfural) as described by Corsaro et al. (2004). In our experiments, complete conversion of the soluble part of amylose into glucose was achieved only after 8 h heating whereas Yu et al. (1996) found the major amylopectin starch to be hydrolysed within 60 min. High amylose starches are reported to display B-type double helices that are usually less susceptible to acid hydrolysis than normal starches with low amylose contents (Gérard, Colonna, Buléon, &

Table 2
Detailed composition of oligosaccharides obtained after a constant pre-heating time (1 h, 90 °C) followed by different times of acidic hydrolysis (HCl 0.45 M) under conventional heating

Hydrolysis time (h)	Degree of polymerisation (DP) of malto-oligosaccharides[a]																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	>17
0	100																	
0.08	12.1	6.6	6.8	5.7	5.0	4.1	3.9	3.8	4.0	3.4	3.2	3.1	2.9	3.6	2.5	2.4	2.6	24.4
0.12	10.8	7.5	7.8	6.6	6.1	5.9	5.1	4.7	4.1	4.5	3.6	3.5	3.2	2.9	3.0	2.5	1.7	14.7
0.25	19.4	13.4	14.9	11.7	8.6	6.7	5.1	4.5	3.5	2.4	2.1	1.7	1.5	1.1	0.8	0.8	0.3	1.1
0.5	26.0	18.2	19.9	14.6	8.6	5.1	3.1	2.0	1.2	0.6	0.4	0.3						
0.75	38.1	23.6	22.3	10.4	3.6	1.4	0.6											
1	39.1	23.6	21.9	9.7	3.5	1.5	0.9											
2	66.8	26.1	5.9	1.2														
3	85.4	12.8	1.4	0.4														
4	88.1	10.5	1.3															
6	97.7	2.1	0.3															
8	98.2	1.8																

[a] Area ratio was calculated from respective peak areas of HPAEC-PAD.

Planchot, 2002). In fact, a visible insoluble part of amylose remained at 8 h. It even remained unchanged after 20 h of treatment (data not shown) whereas the colour of the samples changed from slightly yellow to dark brown indicating the degradation of glucose.

3.2. Kinetics of microwave hydrolysis

The same procedure as that used in the conventional heating study was applied with microwave irradiation to compare the impact of the treatment. The composition of the reaction mixture in terms of oligosaccharide (obtained by HPAEC-PAD) in the supernatant of centrifuged samples is reported in Table 3. After a pre-heating treatment of 10 min (20% power), only a small amount of glucose and no oligosaccharide were found (Fig. 2A), demonstrating that the pre-warming treatment by microwave irradiation gives little or no effect on the hydrolysis of amylose. Nevertheless, it is possible that the polymer has been cut into very large fragments. After 1 (Fig. 2B) and 2 min of treatment, the level of oligosaccharides present is very low, with DP 2 and 3 representing 39.5% of the entire amount suggesting that amylose resists to acidic treatment, a hypothesis further supported by the fact that amylose rich starches are difficult to hydrolyse. On the other hand, the results obtained with conventional heating revealed that the first phase of hydrolysis is relatively fast and starts as soon as the acid is added to the solution. In fact, possible degradation of the amylose macromolecule might have appeared yielding lower molecular weight fractions, but still with a MW higher than DP 80 which made them undetectable in our method proven previously to be efficient (separation and analysis of oligosaccharides) up to that DP (Hanashiro et al., 1996). The chromatogram obtained after 3 min of hydrolysis (Fig. 2C) confirmed this hypothesis with the appearance of a wide range of DP > 17, up to DP 35 [total proportion of DP > 17 = 26.2% ranging from 2.47% to 0.6% (data not shown)]. The signal intensity remains low in the chromatograms. Under the conventional heat treatment, a similar distribution with a slightly higher yield was obtained after 5 min hydrolysis (Fig. 1B). If the microwave acidic hydrolysis would follow the same kinetic, a two phase process should occur also here. In this case, the first phase of the kinetic presents the same characteristics of the obtained distribution but is quicker. Then, with this hypothesis, the mode of heating would have an impact more on the speed of the hydrolysis than on the resulting types of oligomers. After 4 min, the maximum DP decreases to 22, and then to 18 after 5 min (Fig. 2D). In contrast to the observation made by Yu et al. (1996) after irradiation for 5 min, our solution was still clear (supernatant) despite the presence of insoluble amylose. In their study, no retrograded starch remained in suspension. This difference is probably due to the fact that these authors used starches rich in amylopectin and also applied a different microwave frequency which has been pointed out to have an influence (Raghavan et al., 2005). Supporting our results, starches containing bigger proportions of amylose have been shown to be more difficult to degrade, which is generally believed to be caused by a strong association between amylose/amylopectin or amylose/amylose (Gérard et al., 2002). To our knowledge, degradation of pure amylose under acidic conditions by microwave heating has never been studied and consequently it is not possible to compare our results with other microwave treatments. When Yu et al. (1996) re-dissolved the isolated retrograded starch in a more dilute solution and irradiated again, the material became soluble again. This result led them to the hypothesis that the B-structure of the retrograded starch was deformed by microwave irradiation (atomic rotation), allowing the penetration of water molecules into the double-helices, making the starch soluble in the solution and therefore, subject to hydrolysis. However, only a pre-heating treatment did not reduce the amount of insoluble amylose recovered (data not shown). So, if the microwave irradiation really had this type of impact, it seems to be reversible causing the B-structure to be formed again when the treatment is stopped.

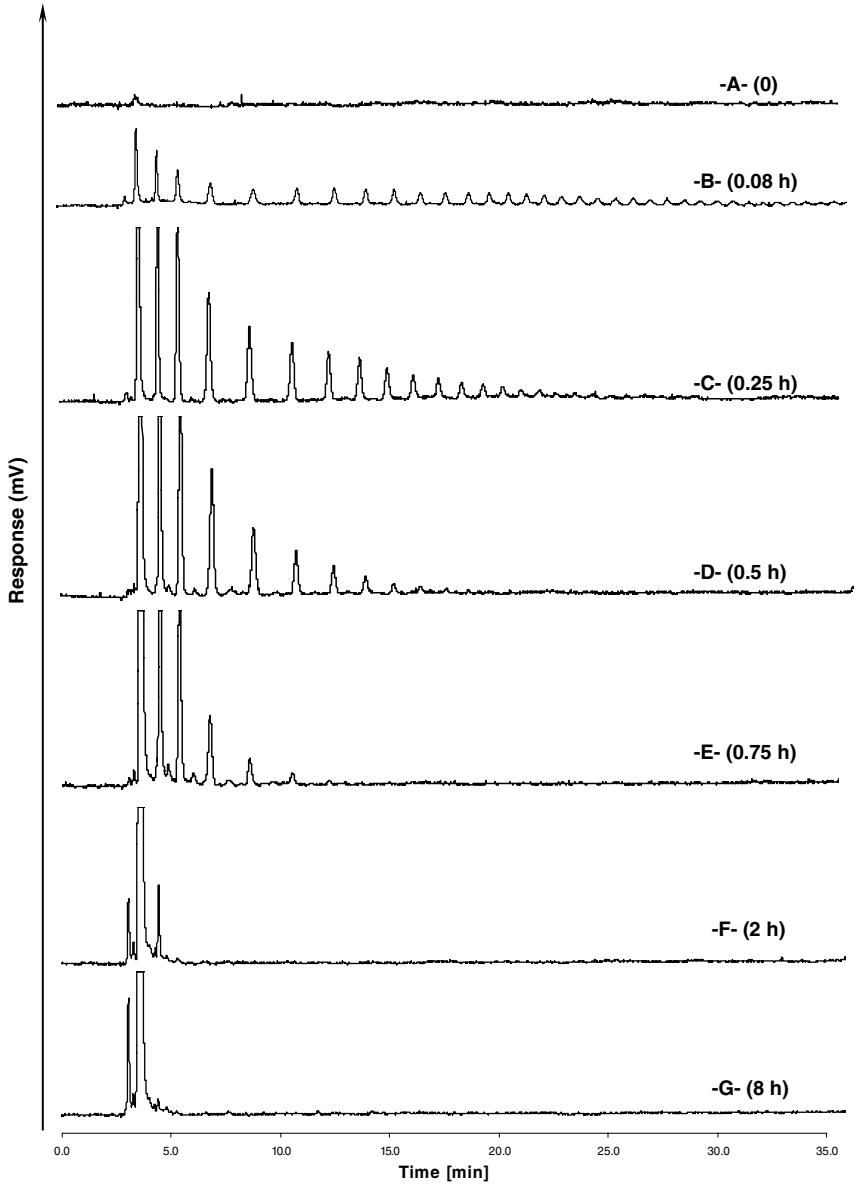

Fig. 1. Analysis by HPAEC of hydrolysed amylose obtained at different heating times (h) in a water bath (90 °C) after 1 h of pre-heating reaction times from A to G: 0, 7 min, 15 min, 30 min, 45 min, 2 and 8 h (HCl 0.45 M).

Oppositely, in Yu's experiments the remaining retrograded starch can be solubilized in a more dilute solution where more water molecules are present per macromolecule. These results are then probably caused by the loss of the association between the two macromolecules (loss of the crystalline conformation due to the formation of an open network structure in dilute solution) which is favoured by heating (Richardson, Kidman, Langton, & Hermansson, 2004). In addition, working with a more dilute solution increases the number of water molecules available to the action of microwaves and hence the heat transfer which in turn favours the action of hydrochloric acid.

After 7 min of microwave treatment (Fig. 2E), oligosaccharides with DP $\leqslant 4$ represent approximately 85.7% of the soluble fraction. This even increases further to 92.2% after 9 min of irradiation (Fig. 2F). As in the case of classical heating, the major part of amylose (easily-degradable) is already transformed into oligosaccharides at that point. The small amount of remaining amylose is acid resistant and hence provides only small oligosaccharides. In addition, the longer oligosaccharides obtained earlier in the hydrolysis process are cut into smaller ones. After 10 min of hydrolysis (Fig. 2G), mainly glucose remains (97.2%).

3.3. Determination of the easily degradable fraction (EDF)

The two heating procedures, classical or microwave irradiation, seemed to demonstrate two phases in the degradation of the polymer. To further support this, hydrolysis curves were constructed. These curves are plotted as semi-logarithmic graphs [$\log(100/100 - X)$] (where X is the degree of hydrolysis) vs. time. After different times of

Table 3
Detailed composition of oligosaccharides obtained after a constant pre-heating time (10 min, 20% power) followed by different times of acidic hydrolysis (HCl 0.45 M) with microwave heating

Pre-heating treatment	Hydrolysis time (min)	Degree of polymerisation (DP) of malto-oligosacharides[a]																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	>17
10 min 20% power	0	100																	
	1	71.6	28.4																
	2	60.5	21.6	17.9															
	3	11.3	5.8	5.3	6.1	5.6	4.2	4.3	4.1	4.2	3.3	3.1	3.3	2.8	2.8	2.5	2.7	2.3	26.2
	4	17.7	12.2	12.8	10.8	8.0	7.0	5.7	4.7	4.2	3.1	2.6	1.9	2.0	1.5	1.1	1.3	0.7	2.7
	5	18.9	13.3	14.9	12.3	9.0	7.0	5.3	4.6	3.5	2.4	2.3	1.6	1.5	1.0	0.9	0.8	0.4	0.5
	6	19.7	13.4	13.6	11.3	8.3	7.0	5.3	4.5	3.9	2.9	2.2	1.6	1.8	1.2	1.2	0.9	0.7	1.0
	7	30.8	20.9	21.1	12.9	6.5	4.0	1.8	1.4	0.6									
	8	31.2	21.0	21.2	14.4	6.5	3.5	1.9	1.0	0.5									
	9	35.8	22.0	22.6	11.8	4.1	2.5	0.7	0.5										
	10	97.2	2.8																

[a] Area ratio was calculated from respective peak areas of HPAEC-PAD.

hydrolysis, the fractions were centrifuged and the solid pellet was recovered, freeze-dried and weighed. The two curves (Figs. 3A and B) effectively showed two regions of first-order kinetics. This confirms the hypothesis of a varying behaviour of acid on two different structural organizations in amylose: a first rapid stage during which amorphous layers within the amylose are assumed to be eroded and a second slower stage where crystallites are degraded. These observations are similar to those reported by Gérard et al. (2002).

After 45 min the second phase of the degradation began in the conventional heat treatment. This confirmed our observations based on the oligosaccharide distribution with the presence of two distinct phases during the hydrolysis process. The EDF (88.9%), determined by the extrapolation of the second phase to the initial time, has been completely hydrolysed at this time (Fig. 3A). After that point, the speed of hydrolysis decreases strongly. After 1 h, the EDF (amorphous region of amylose) has been completely hydrolysed. At that point, the degradation of the remaining amylose (crystallites) is very slow and no longer contributes to the appearance of new oligosaccharides of DP > 7. The putative structure in double helices of the crystalline amylose does not allow the acid to act in. Therefore, it works only on the outer end of the chains yielding only glucose or small molecular weight oligosaccharides. On the contrary, as shown previously by the oligosaccharide studies, there was still a perceivable evolution from 94.5% of hydrolysis of the starting material after 8 h to 98.5% after 20 h. This step was accompanied by the transformation of glucose into furfural type molecules (supported by the colour of the samples).

Yu et al. (1996) found in their study that following 60 min of a traditional heating of starch at 100 °C, the soluble starch was completely converted to glucose whereas the retrograded starch remained suspended in the solution. This retrograded starch has been recovered for four different types of starch and represented between 2.5% and 9.8% of the initial amount.

When microwave irradiation is applied, this second phase began after only 4 min (Fig. 3B), which is around 10 times faster than with conventional heating. Nonetheless, the type of kinetics was similar irrespective of the treatment method applied. This speed difference reflects the capability of microwave irradiation to allow water molecules to rapidly enter the B structure of the amylose and speed up the heat exchange. The EDF after hydrolysis under microwave irradiation is calculated to represent 81.7% of the total amount. The explanation for this 7.2% variation in comparison with the results obtained with classical heating is yet unclear. A possible explanation could be the longer presence (time effect) of amylose at high temperature during the conventional heating. Thus, the resistant amylose fraction could already begin to be hydrolysed before the EDF has actually disappeared resulting in an overestimation in the calculations.

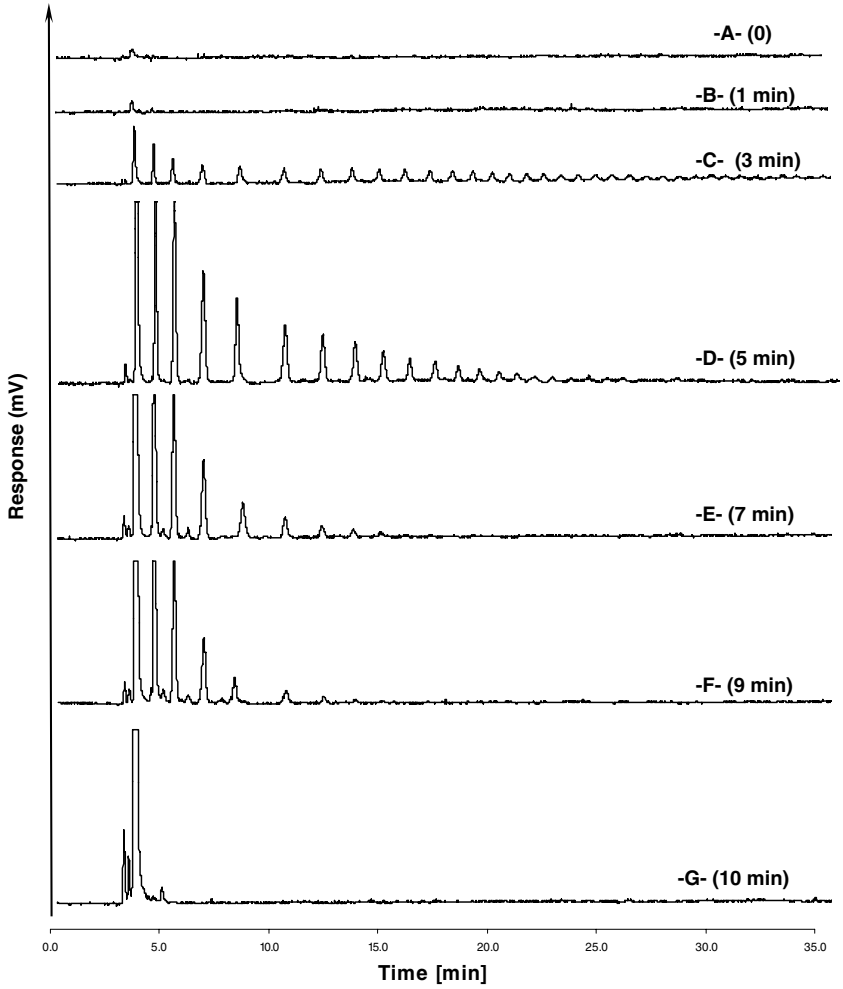

Fig. 2. Analysis by HPAEC of hydrolysed amylose obtained at different microwave treatment times after 10 min of pre-heating (20% power) reaction times from A to G: 0, 1, 3, 5, 7, 9 and 10 min (HCl 0.45 M, 25% power).

Gérard et al. (2002) found lower EDF values (maximum = 67%) for different starches with variable amounts of amylose. It is possible that this difference is due to the use of a lower temperature (35 °C) during their treatment which might counteract the action of the HCl (even at the higher concentration of 2.2 N used). A likely hypothesis is that the resistance to acidic hydrolysis of amylose-rich starch is due more to a close association of amylopectin and amylose (with formation of crystalline areas), than to interactions of amylose with itself. Whatever the heating treatment, we reached a high degree of hydrolysis of the pure amylose (96.5% and 98.5% for microwave and conventional heating respectively) which is close to the levels reported for pure amylopectin (Gérard et al., 2002). This importance of the proportion of amylose in the starch on physico-chemical properties after microwave irradiation has been previously discussed (Lewandowicz, Jankowski, & Formal, 2000). Thus, resistance of starch to acid hydrolysis is greater when the starch variety comprehends both amylose and amylopectin than when it contains only one of each (starches with pure amylose or with pure amylopectin). This resistance becomes even greater when the ratio of amylose to amylopectin is higher.

3.4. Appearance of brown compounds

To study the putative impact of the molecules conversions, the appearance of brown compounds was also followed. Even after 10 min of microwave treatment with HCl, any brown compound was observed (no difference with the initial supernatant, data not shown). Apparently, this type of treatment allowed the hydrolysis of amylose to the same extents as with conventional heating (comparable or better yields) without a noticeable formation of degradation molecules. As described by Corsaro et al. (2004) the shorter reaction time during the microwave treatment prevents decomposition of glucose.

On the other hand, when the classical thermo-hydrolysis was prolonged at high temperature, the reaction solution became coloured and the absorbance of the solution increased significantly at wavelengths between 400 and 500 nm (Fig. 4A). Similar findings were also reported by Yu et al. (1996). In the sample heated at 90 °C, the absorbance increased with increasing incubation times. This phenomenon appears most pronounced close to 400 nm. If time curves of the variation of absorbance at 400 and 500 nm versus hydrolysis time were plotted (Fig. 4B),

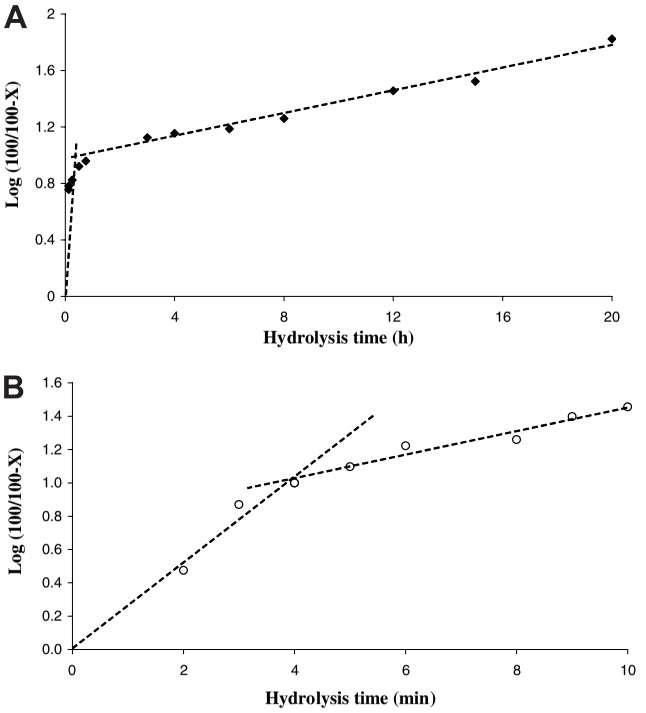

Fig. 3. Kinetics of diluted acid hydrolysis (HCL, 0.45 M). X: degree of hydrolysis (for definition see text). (A) Conventional heating (◆) and (B) microwave irradiation (○).

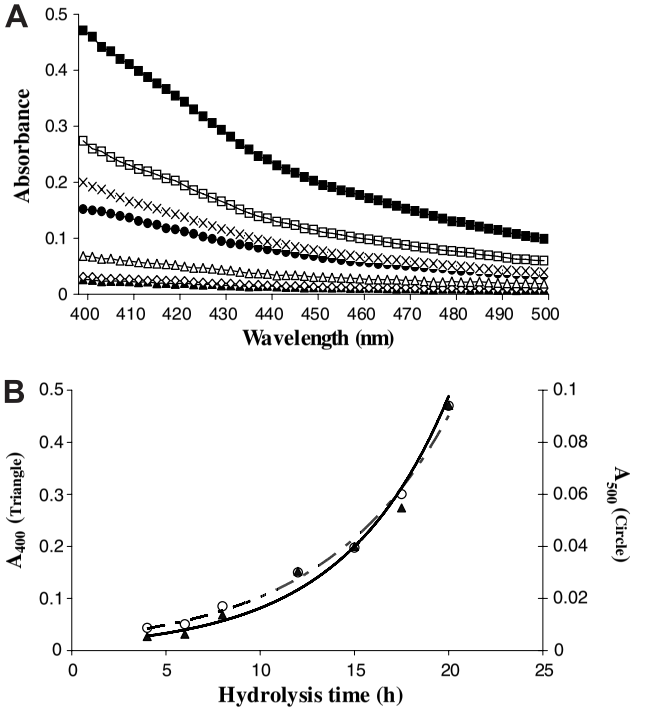

Fig. 4. Determination by spectrophotometric measurements of brown compounds formed during acidic hydrolysis of amylose under conventional heating conditions. (A) Evolution of absorbance in the range of 400–500 nm of the supernatant after centrifugation (7000 rpm, 4 min) for different treatment times (h): 4 (▲), 6 (◇), 8 (△), 12 (●), 15 (×), 17.5 (□) and 20 (■). (B) Evolution of absorbance at 400 (▲) and 500 (○) nm at different treatment times of samples.

Table 4
Detailed composition of oligosaccharides obtained after variation of the pre-heating time (20% power) or the pre-heating power (5 min) followed by acidic hydrolysis (HCl 0.45 M) with microwave heating (5 min, 25% power). 40 °C: 20 min cooling period between the pre-heating and the acid hydrolysis stage

	Hydrolysis treatment	Degree of polymerisation (DP) of malto-oligosaccharides[a]																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	>17
Pre-heating time (min) at 20% power	0	13.3	7.4	7.8	7.2	6.5	5.5	5.1	4.5	4.3	3.6	3.2	3.1	3.0	2.8	2.4	2.4	2.6	15.4
	5	19.4	12.1	12.4	10.5	8.5	7.0	5.4	4.9	4.0	3.1	2.5	2.1	1.7	1.2	1.0	0.8	0.8	2.8
	10	18.9	13.3	14.9	12.3	9.0	7.0	5.3	4.6	3.5	2.4	2.3	1.6	1.5	1.0	0.9	0.8	0.4	0.5
Pre-heating power (%) during (5 min)	20	19.4	12.1	12.4	10.5	8.5	7.0	5.4	4.9	4.0	3.1	2.5	2.1	1.7	1.2	1.0	0.8	0.8	2.8
	30	18.6	11.9	13.1	11.1	8.7	7.1	5.7	4.6	3.4	3.3	2.2	1.9	1.5	1.2	0.8	0.8	0.7	0.9
	40	33.2	21.1	18.7	12.4	6.8	3.1	2.2	1.2	0.7	0.5								
	40 °C	25.3	15.2	15.5	11.8	8.7	6.0	4.7	3.3	2.9	1.9	1.6	0.9	0.8	0.4	0.5	0.4		

[a] Area ratio was calculated from respective peak areas of HPAEC-PAD.

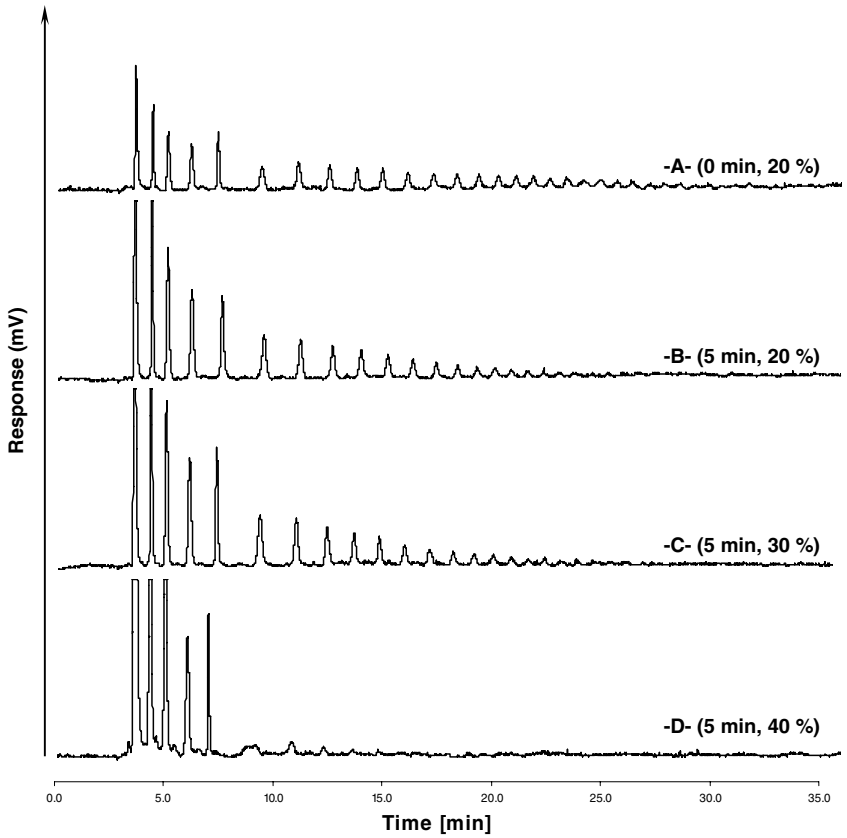

Fig. 5. Analysis by HPAEC of amylose hydrolysed by microwave treatment (HCl 0.45 M, 5 min, 25% power) after different conditions of pre-heating. (A) 0 min; (B–D) 5 min with 20%, 30% and 40% power, respectively.

similar results were obtained but with a lower amplitude in the case of the absorbency measurements at 500 nm (note differences in scaling on Y axis). These results can be fit with an exponential curve ($R^2 = 0.9753$ and 0.991, respectively). The appearance of brown compounds increases strongly after 7 h of warming with an even faster progression from 10 h onwards. This coincides with the observation by HPAEC of a peak before glucose after 8 h of hydrolysis (Fig. 1G).

3.5. Impacts of the time effect or the heat transfer on the hydrolysis

The application of microwave heating gave similar results in terms of the maximum degree of hydrolysis that can be obtained. The kinetic of hydrolysis are faster than in conventional treatment and any brown compound appears. In both studies, a pre-warming period has been applied. To study to which extent this pre-heating contributed to the hydrolysis process, we varied the microwave irradiation (intensity and time). Conditions for the actual heat treatment remained the same (5 min, 25% power).

Firstly, at 20% power, the pre-heating time was varied (Table 4) to study the time effect. When the hydrolysis was started directly (i.e. pre-heating time = 0), we observed a comparable result (Fig. 5A) with more intensity than that obtained after 3 min hydrolysis with 10 min (20% power) pre-heating (Table 3 and Fig. 2C). This is confirmed by the respective hydrolysis extent with 86 (10 + 3 min) and 87% (5 min). The pre-heating period seems to have a real impact in terms of heat transfer and/or on the availability of water molecules as discussed previously. The increase of the pre-heating time to 5 min (Fig. 5B) resulted in an increase in the degree of hydrolysis (91%) expressed by the amount of oligosaccharides recovered (signal intensity) and in lower DP fragments. A further increase to 10 min (Fig. 2D) did not cause a further change in terms of dispersity but resulted in a slight increase in the amount of oligosaccharides (92% of hydrolysis extent). The initial temperature of the sample before the acidic hydrolysis clearly is a preponderant key variable. This is confirmed by experiments in which the power applied at constant time was varied (Table 4). With the same 5 min time, the increase from 20% to 30% power applied during the pre-heating (respectively Figs. 5B and C) led to a higher degree of hydrolysis (92.5%) even if this is less pronounced compared to a 40% power treatment (95%) (Fig. 5D). Moreover, the same treatment as the latter was realised but with a short cooling period at room temperature for 20 min between the pre-heating and the acid hydrolysis stage (40 °C – Table 4). The acidic hydrolysis of amylose was less pronounced demonstrating that the effect of the

microwave treatment (before hydrolysis) was apparently reversible after a cooling period which was too short in our case to conclude the process to be absolute reversible. That confirms the hypothesis discussed previously (Palav & Seetharaman, 2006; Raghavan et al., 2005) that microwave treatment acts through the transfer of heat and not via a chemical modification of the molecules.

4. Conclusion

The distribution of oligosaccharides (and their evolutions) obtained in acid hydrolysis with conventional heating and microwave treatments were the same with the two physical treatments. The calculated EDFs were also similar with the two treatments. In regard of that and because acidic hydrolysis is random, the microwave irradiation seems to act only on the speed of heat transfer without any specific effect on the amylose. With microwave treatment, the temperature is then faster reached and heat transfer in the medium is more efficient than the conventional heating process, avoiding the appearance of brown compounds and yielding shorter reaction times. Thus, we demonstrated in this study that it is possible to produce by acidic hydrolysis under microwave treatment a desired mixture of malto-oligosaccharides in a short time, with a high yield, and without undesired side reactions.

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