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Carbohydrate Polymers 71 (2008) 277–286
Carbohydrate Polymers
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Enzymatic and chemical degradation of curdlan targeting the production of β-(1 → 3) oligoglucans

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Received 21 January 2007; received in revised form 13 April 2007; accepted 30 May 2007
Available online 16 June 2007

Abstract

Obtaining biologically active oligosaccharides from hydrolysed polysaccharides is nowadays of great interest. Curdlan is one of the polysaccharides with interesting properties and represents the main source for β-(1 → 3)-oligoglucans. Curdlan degradation remains a challenge due to its high resistance to hydrolysis. In this study, curdlan was hydrolysed both with mild acid treatments (TFA or H_2SO_4, 1 M) as well as with the use of an unpurified enzymic extract produced from *Trichoderma harzianum*. The kinetics of degradation and the production of the β-(1 → 3)-oligoglucans were followed in detail by HPAEC analysis. A comparison of results clearly demonstrated the advantage of the enzymic extract with a complete hydrolysis of the curdlan within 90 h whereas less than 25% hydrolysis yield can be reach with the acidic methodologies. More, the quality of the obtained compounds is particularly dissimilar even between the two acid treatments.

Keywords: Curdlan; Oligosaccharides; β-(1 → 3)-Glucans; Hydrolysis; Endoglucanase; Enzymatic or chemical hydrolysis

1. Introduction

Curdlan is a water-insoluble microbial exo-polysaccharide. Only few strains of bacteria belonging to the *Alcaligenes faecalis* (now reclassified as an *Agrobacterium*) and *Agrobacterium* spp. species have been reported to produce this linear polysaccharide. Production of curdlan also occurs in a few *Rhizobium* strains and in species of *Cellulomonas* (McIntosh, Stone, & Stanisich, 2005). Curdlan is a homopolymer of D-glucose linked β-(1 → 3) without side chains (Fig. 1). The average molecular weight is difficult to estimate as a result of its poor solubility and remains still a challenge (Chan, Chan, & Tang, 2006). However, it has been estimated that the average degree of polymerization (DP) is around 450 and that these macromolecules can possess a maximum of 12,000 units (Futatsuyama, Yui, & Ogawa, 1999). Under alkaline solution (>0.2 M), curdlan is completely soluble and exists as random coils. In this context, its molecular weight has been estimated to be in the range of 5.3×10^4–2.0×10^6 Da (Lee, 2000). After neutralization, the partially opened conformers gradually adopt an ordered state composed of a mixture of single and triple helices with potential hydrophobic interactions in addition (Funami, Funami, Yada, & Nakao, 2000). Therefore, three conformers of soluble curdlan have been reported in alkaline aqueous systems, including single helix, triple helix and random coil conformations (Lee, 2000).

Curdlan can be produced industrially at high production levels from cheap carbon sources such as by-products from the sugar industry (molasses) and therefore constitutes an attractive raw material for the food industry. In fact, one of the unique features of curdlan is that aqueous suspensions can be thermally induced to produce high-set gels, which will not return to the liquid state upon reheating. Curdlan is a member of the limited family of bacterial polysaccharides approved as food additives by the USFDA. It

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doi:10.1016/j.carbpol.2007.05.042

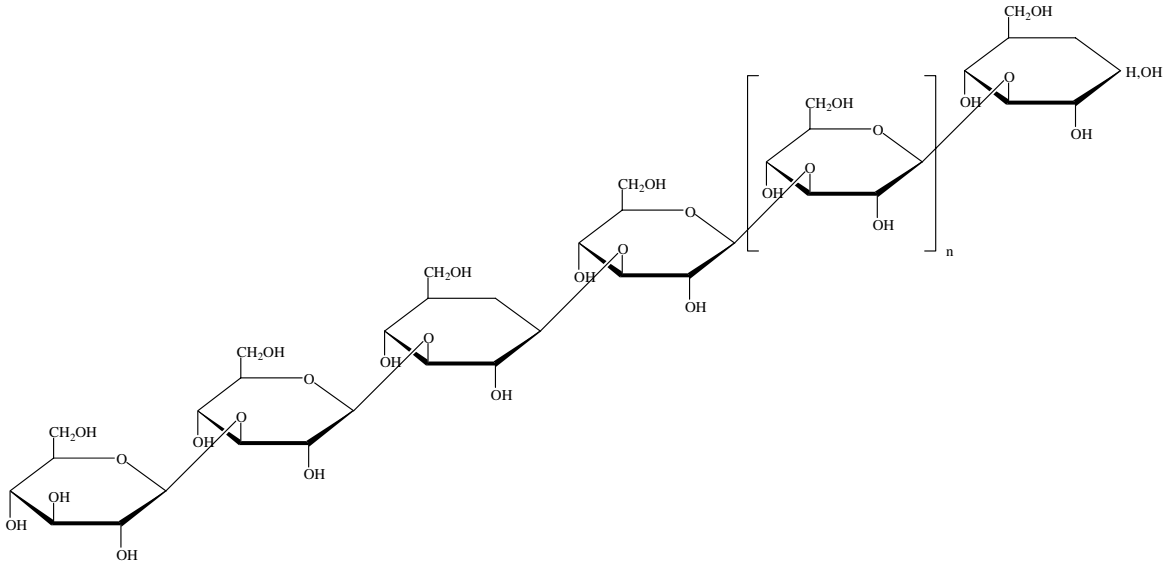

Fig. 1. Curdlan structure.

has been launched in the food area essentially as an additive for improving physical texture (thickener, texture modifier), but also as a formulation aid, processing aid or stabilizer (Shimizu et al., 2001). Various food areas benefit from this such as for example the area of low-fat meat products (Funami, Yada, & Nakao, 1998; Funami & Yotsusuka, 1998).

As one of its numerous properties described in the literature, curdlan has been reported to favour the growth of *Bifidobacterium* bacteria in rats *cecum* (prebiotic action). Bifidobacteria, known with lactobacilli species to have probiotic property, furnishes beneficial physiological effects to the host. By analogy with fructo- and xylooligosaccharides, both described as prebiotics, hypotheses have been made that β-(1 → 3)-gluco-oligosaccharides from curdlan could also present prebiotic properties and would therefore be a potentially new ingredient for the nutraceutical market (Shimizu et al., 2001).

To further demonstrate the putative prebiotic activity of oligo β-(1 → 3)-glucans, it is firstly necessary to produce them in larger quantities. Degradation of the curdlan polysaccharide can be realised in two different ways: enzymic or acidic treatment. Partial hydrolysis of curdlan has also been achieved by formolysis. During this process, a good result was obtained in terms of reaction time required (around 3 h) and the resulting oligosaccharide distribution. But, this method is almost impossible to scale-up to industrial level due to the stringent experimental conditions of this two-steps procedure: (i) 90% formic acid, 90 °C, 60 min; (ii) 0.1 M TFA, 100 °C, 60 min (Koizumi, Kubota, Tanimoto, & Okada, 1989; Ohno et al., 1995).

Alternatively, curdlan can be degraded by the action of glucanases. The filamentous mycoparasitic fungus *Trichoderma harzianum* (TH) has been widely studied for its ability to produce a large variety of enzymatic actions such as cellulase, chitinase, protease, β-(1 → 3)- and β-(1 → 6)-glucanase activities. These β-(1 → 3)-glucanases are divided into two groups with regard to their possible mechanisms of action: (i) exo-β-(1 → 3)-glucanases (or β-(1 → 3)-glucan glucohydrolase, EC 3.2.1.58) hydrolyse β-glucans by sequentially cleaving glucose residues from the non-reducing end with only glucose as hydrolysis products; (ii) the endo-β-(1 → 3)-glucanases (or β-(1 → 3)-glucan glucanohydrolase, EC 3.2.1.6 or EC 3.2.1.39) cleave β-linkages at random sites along the polysaccharide chain releasing smaller oligosaccharides (de la Cruz, Pintor-Toro, Benŕtez, Llobell, & Romero, 1995). Even though the modes of action of these enzymes have been well described, data relative to the production of oligosaccharides are not available. In this study we will investigate the controlled large scale production of β-(1 → 3)-gluco-oligosaccharides through enzymatic or chemical degradations of curdlan.

2. Experimental

2.1. Production and isolation of enzymatic extracts

Trichoderma harzanium CECT 2413 was obtained from the Spanish Type Culture Collection (Burjassot, Valencia, Spain). Glucose–potato agar was used to maintain cultures.

For the production of the β-(1 → 3)-D-glucan hydrolase extract, the fungal strain was grown in two-steps liquid cultures according to the procedure described by Deane, Whipps, Lynch, and Pederby (1998). Cultures were first performed in 100 ml of *Trichoderma* complete medium (TCM) supplemented with glucose (0.1% w/v) in a 500 mL flask shaken at 100 rpm and incubated at 25 °C during 72 h. This culture was used to inoculate 900 mL of mineral medium (MM – Penttilä, Nevalainen, Rättö, Salminen, & Knowles, 1987) supplemented with curdlan from Rhodia (0.4% w/v) in a 3 L flask shaken at 100 rpm for 96–120 h at 25 °C.

The culture was filtered successively through 160–0.2 μm filters to take off mycelia. Proteins in the culture filtrate were recovered by concentration on a 1×10^4 nominal molecular weight cut-off (NMWCO) polyethersulfone membrane (Amicon, Beverly, MA) in a stirred Amicon cell. After a wash step with H_2O, the enzymatic fraction (final volume 35 mL) was dried under vacuum.

2.2. Characterisation of the total enzymatic extract by polyacrylamide gel electrophoresis (SDS–PAGE)

Sodium dodecyl sulphate (1%) PAGE and silver staining (after 20 min of proteins migration) were carried out with the Multiphor II Flatbed electrophoresis System (Amersham Biosciences) according to the supplier manual using an ExcelGel SDS gradient 8% (separating)–18% (stacking) gel (GE Healthcare, UK). Proteins from the total extract were separated and their molecular masses were estimated using a low molecular weight calibration kit (Amersham Biosciences, NJ, USA). The proteins were visualised by silver staining.

2.3. Enzymic degradation of curdlan

Curdlan (Takeda Chemical Industries Ltd., Osaka, Japan) is solubilised in an alkaline aqueous solution (pH 11.0) at a concentration of 10 g L^{-1} under strong agitation (500 rpm). 2.5 mM of sodium azide (NaN_3) is added to avoid possible contamination. After 10 min of solution stabilisation, the pH is adjusted to 5.5 with a solution of acetic acid (1.5 M). The freeze-dried enzymatic extract is dissolved in pure water to the concentration of 3.3 mg mL^{-1}. One milliliter of this liquid is added to the curdlan solution (total volume = 25 mL). Aliquots are taken at different degradation times and are heated 5 min at 95 °C to inactivate the enzyme(s). After centrifugation at 5,000 rpm during 10 min, the supernatant is filtered (0.22 μm, Millipore) and analysed by HPAEC–PAD (vide infra).

2.4. Chemical degradation of curdlan

Curdlan is added to pure water at room temperature at a concentration of 10 g L^{-1} under strong agitation (500 rpm). This suspension is warmed up to 60 °C. After 15 min, either sulphuric acid (H_2SO_4) or trifluoroacetic acid (TFA) (both final concentrations at 1 M) is added to the curdlan solution with a final volume of 50 mL. Aliquots are withheld regularly, cooled for 5 min on ice and neutralised with NaOH. After centrifugation at 5,000 rpm during 10 min, the filtered supernatant (0.22 μm, Millipore) is analysed by HPAEC–PAD (vide infra).

2.5. High performance anion exchange chromatography (HPAEC)

The kinetics of the curdlan hydrolysis reaction were studied using HPAEC with pulsed amperometric detection (PAD) using a method adapted from Hanashiro, Abe, and Hizukuri (1996). The recovered aliquot was diluted 20 times in pure water and 20 μL 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: $E1 = +0.05$ ($t1 = 400$ ms); $E2 = +0.75$ ($t2 = 200$ ms); $E3 = -0.80$ ($t3 = 300$ ms). The response time and the sensitivity of the detector were set to 900 ms and 5 μA, respectively. The separations were carried out at the flow rate of 1 mL min^{-1} following the gradient program detailed in Table 1. The run time was 65 min. The mobile phase was composed of 140 mM NaOH solution as eluent A and 140 mM NaOH solution containing 500 mM sodium acetate as eluent B. The results are analysed with Total Chrom software (Perkin-Elmer, Norwalk, CT, USA).

Table 1
Gradient procedure used to separate β-(1 → 3)-gluco-oligosaccharides by DP

Time (h)	Ratio A/B
0	100/0
3.0	90/10
6.8	90/10
14.0	71/29
18.0	63/37
18.8	60/40
22.5	50/50
27.5	40/60
38.5	30/70
50.5	20/80
60.0	14/86
60.1	100/0
65.0	100/0

3. Results and discussion

Several concentrations of curdlan were tested (data not shown). At concentrations above 20 g L^{-1}, a high jellification of the media appeared. This eventually led to a poorer access of the degradation agent to the molecules. As a result of this, only the surrounded molecules were degraded and the core of the jelled solution remained mostly native. At these high concentrations, very low degradation yields were seen both for enzymatic and acidic treatment. In the view to observe the real hydrolysis mechanism of the polymers (not only the surrounded part) and to obtain a maximum yield, a concentration of 10 g L^{-1} was chosen for this study to stay under this jellification state.

3.1. Production of β-(1 → 3)-gluco-oligosaccharides by acid hydrolysis of curdlan

To our best knowledge, the acid degradation kinetics of curdlan with the view to produce oligosaccharides have not

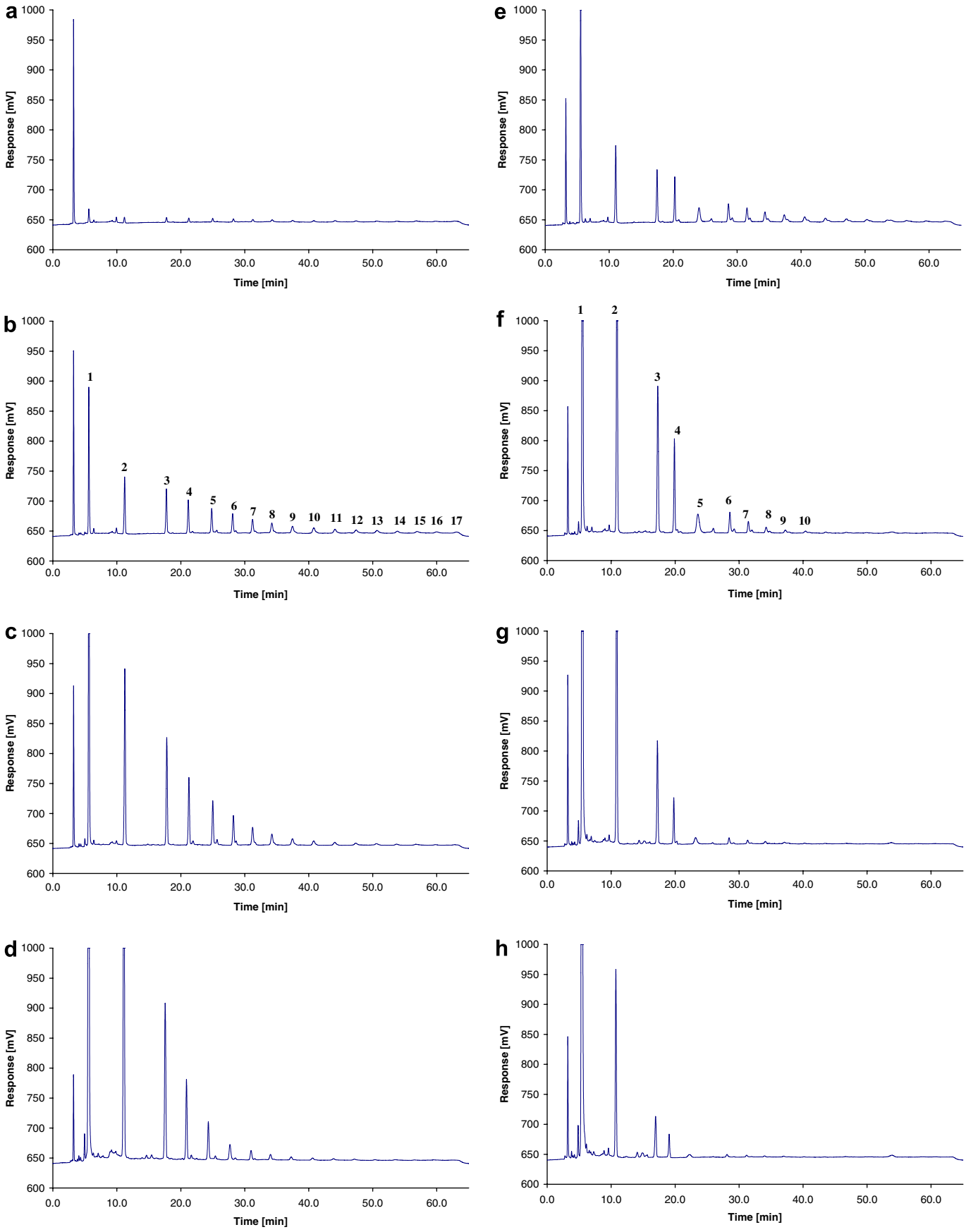

Fig. 2. Analysis by HPAEC of acid hydrolysed curdlan with [TFA (represented by a, b, c, d) and H_2SO_4 (represented by e, f, g, h)] obtained at increasing treatment times: 6, 24, 48 and 90 h respectively (from up to down). Respective oligosaccharide DP are indicated in b (TFA) and f (H_2SO_4).

been studied so far. In our study, H_2SO_4 and TFA (1 M) are used to hydrolyse the curdlan at 60 °C and the resulting reaction mixtures was analysed. Several HPAEC profiles obtained at different treatment times (6, 24, 48 and 90 h, respectively) are reported in Fig. 2 (Fig. 2a–d for TFA; Fig. 2e–h for H_2SO_4, respectively). Astonishingly, the results obtained for the two acids are widely different both in terms of speed as well as with regard to the type of action. After 6 h of hydrolysis (Fig. 2a), the TFA presents a limited action whereas the H_2SO_4 treatment has already released a wide range of oligosaccharides (up to DP 17) (Fig. 2e). The amount of β-(1 → 3)-glucan obtained is reflected by the sum of the total peak areas (Fig. 3). Clearly, H_2SO_4 presents a quicker and stronger impact on the curdlan than the TFA when compared at identical concentration. This difference in speed of hydrolysis could be expected due to the different pK_a of the acids, but not in term of oligosaccharide distribution. This effect is still visible after 24 h even though TFA at this reaction time has also begun to provide a wide range of oligosaccharides (Fig. 2b). This variety of oligosaccharides is represented by a semi-Gaussian distribution whereas clearly DPs greater than DP4 represents a minor part in the case of H_2SO_4 (less than 15%) (Fig. 2f). After 48 h of degradation, the maximum amount of oligosaccharides is obtained in the H_2SO_4 treatment (Fig. 2g). The highest DP recovered decreases gradually to DP12 (24 h treatment) and further to DP8 (48 h treatment) even if there remains a large visible non-degraded mass of curdlan in the media (respectively Fig. 2g and h). Apparently, the H_2SO_4 (rapidly) reacts with only a part of the curdlan stock, the remainder keeping a strong resistance to hydrolysis. This difference of resistance is probably due to the presence of different curdlan conformations as explained in the Section 1. Single helices are easier to degrade than the triple coils or when hydrophobic interactions occur (Funami et al., 2000). The total peak area for the oligosaccharides decreases. The already obtained oligosaccharides are degraded to smaller DP, eventually giving glucose. In the case of TFA, the initial behaviour of heterogeneous hydrolysis giving a semi-Gaussian dispersity is still being observed after 48 and 90 h treatment (Fig. 2c and d, respectively). The amount of oligosaccharides obtained is now equivalent and after 90 h it is even higher than for the H_2SO_4 treatment (Fig. 3), but their DP distribution is extremely dissimilar.

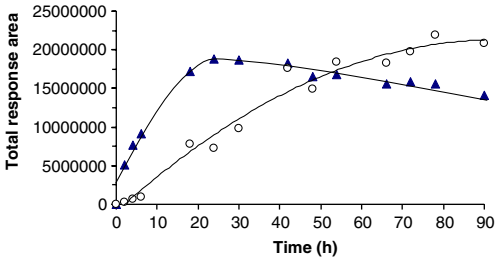

Fig. 3. Evolution of the total response area of obtained oligosaccharides by degradation with TFA (○) and H_2SO_4 (▲).

We have therefore compared for both acid treatments (with a and b for TFA and H_2SO_4, respectively) the

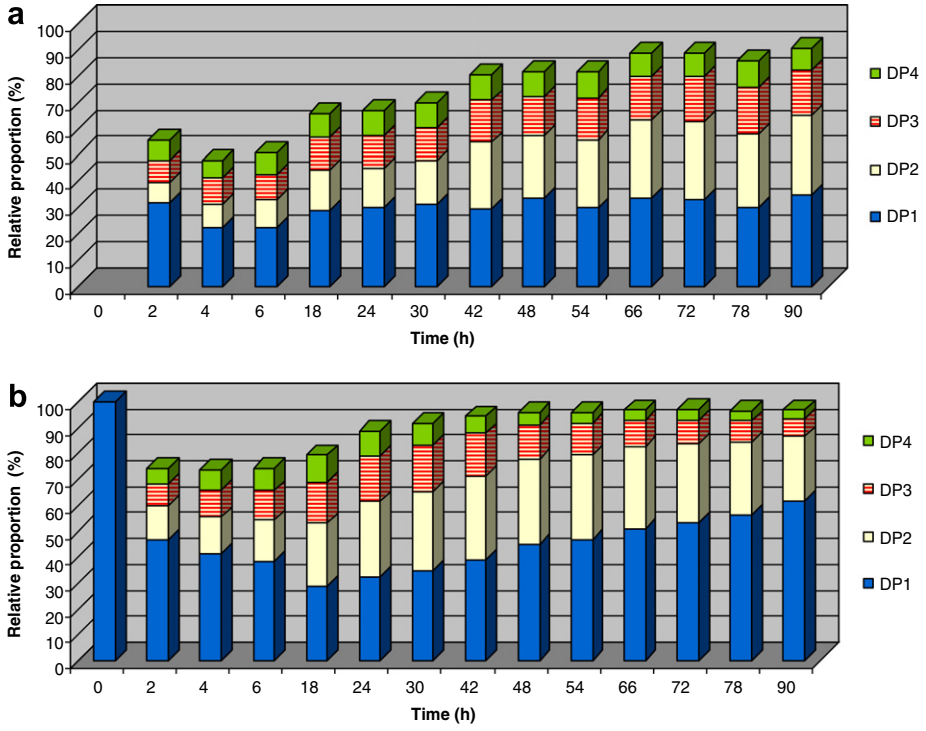

Fig. 4. Evolution of the relative representation in the degraded sample (% of the total oligosaccharides) of DP1–DP4 versus the degradation time with TFA (a) and H_2SO_4 (b), respectively.

evolution of each DP until DP4 in regard of two different parameters: their relative proportion in the total obtained oligosaccharides and their respective areas (Fig. 4 and Fig. 5, respectively). The DP1–DP4 oligosaccharide fraction increases gradually in total amount (93 and 85% for TFA and H_2SO_4, respectively) but its DP distribution varies. Just after H_2SO_4 addition, glucose is already present in the solution. In fact, it is necessary to wait a few seconds between the actual acid addition and the homogenisation, time enough for the curdlan degradation to start due to the strong acidity of H_2SO_4. This observation needs however to be relative due to a low quantity of oligosaccharides at this time as shown in Fig. 5b. Whereas the proportion of the fraction increased (Fig. 5b), the relative amount of DP1 decreased (Fig. 4b). When the easy degradable fraction of curdlan is hydrolysed, the proportion of DP1 constantly increased as a result of the hydrolysis of the higher DP (2–4) to represent after 90 h an important proportion of the fraction (61%) (Fig. 4b). In the case of TFA treatment, the appearance of DP1 is stabilised after 24 h treatment mainly to the benefit of the DP2 and DP3 oligomers (Fig. 4a). The curdlan hydrolysis rate reduces in the case of the TFA with the reach of a putative plate without any further decrease (at the contrary of H_2SO_4). The obtained level of oligosaccharides is higher than in the case of H_2SO_4 treatment (Fig. 5). The polydispersity is maintained up to DP11 (Fig. 2d) whereas for H_2SO_4 DP1 and DP2 represent 85% of the total (Fig. 4b). It is hence clearly possible to influence the proportion and quality of the β-(1 → 3)-oligoglucans by choosing a special type of acid and treatment time at a fixed temperature.

3.2. Enzymatic degradation of curdlan

When the maximum of curdlan has been degraded under the two acid treatments (Fig. 3), an important stock of curdlan remains non-degraded, demonstrating that this polysaccharide is highly resistant to acid degradation. Higher concentrations of the acid could be a solution, but this would make the process difficult to scale-up in an industrial environment. Therefore, we decided to compare these results with the degradation kinetics obtain in enzymic treatment.

The production of β-glucanases by fungus, and especially TH, was described to depend on the type and amount of supplementation of the growth medium (Giese et al., 2005; Noronha, Kipnis, Junqueira-Kipnis, & Ulhoa, 2000; Roldán, Palacios, Peñate, Benítez, & Pérez, 2006). In the view to obtain the maximum β-(1 → 3)-glucanase specific activity, the growth media used was supplemented with curdlan as inducer.

Experimental conditions were chosen based on previous findings in reported literature. The optimal activity of fungal β-(1 → 3)-glucanases usually occurs in the range of pH 4.0–6.0 (Pitson, Seviour, & McDougall, 1993). Rana, Thèodore, Naidu, and Panda (2003) experimentally determined the optimal combination pH/temperature concerning a β-(1 → 3)-glucanase from *T. harzianum* NCIM 1185 to be 5.0/41 °C. The glucanase activity increases by more than a factor two as the temperature is reduced from 65 to 41 °C under different pH conditions. Surprisingly, no further investigations have been performed at temperatures below 41 °C. Two β-(1 → 3)-glucanases described in *Trich-*

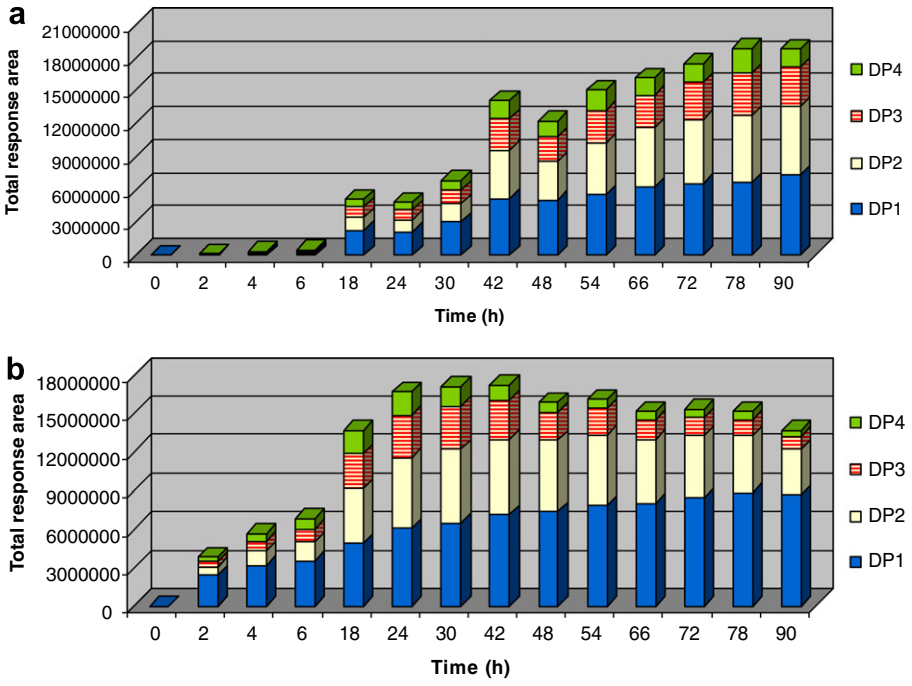

Fig. 5. Evolution of the total response area of DP1–DP4 in the degraded sample versus the degradation time with TFA (a) and H_2SO_4(b), respectively.

oderma loose a great part of their activity (from 50% to 100%) at temperatures above 45 °C after only 45 min (Noronha & Ulhoa, 2000). In this experiment, pH 5.5 was used to increase the amount of dissolved curdlan and to stay close to the optimum conditions described above. The temperature was arbitrary set at 25 °C to keep the enzyme stable as long as possible. Further analysis in the view to obtain a comprehensive knowledge of these parameters with a prediction/optimisation of the oligosaccharide distribution results in function of pH, temperature and time represent the next main step.

The kinetic of degradation has been followed by retro-weighting the remaining curdlan after centrifugation and comparison with the initial mass. The result of this comparison with H_2SO_4 treatment is shown in Fig. 6. A similar graph was also constructed for TFA (result not show). The differences in hydrolysis are tremendous. After only 20 h, almost 90% of the initial curdlan mass is already degraded by the enzymic extract whereas the maximum of degradation is obtained after 70 h for H_2SO_4 with only 20% being degraded. After 90 h, the complete stock of curdlan is hydrolysed in the enzymic treatment. Clearly our enzymatic extract is much more efficient than the acid treatment. We performed HPAEC analyses to observe the type of β-(1 → 3)-oligosaccharides formed during the enzymic treatment. The results of these experiments are shown in Fig. 7.

The obtained oligosaccharide profile is semi-Gaussian from the start of the degradation (6 h) as it is shown in Fig. 7a. Contrary to the situation with the acid treatments, this result tended to be reinforced later on (Fig. 7b, c and d) even if the proportion of degraded curdlan is already 86% after 24 h (Fig. 6). Instead of loosing the majority of the higher DP to the benefit of DP1 and DP2, the whole range of DP until DP5 increased widely. In addition, the higher DP observed vary from 14 (24 h, Fig. 7b) to 16 (90 h, Fig. 7d). This is confirmed by the profile of the total relative amount of oligosaccharides (Fig. 8a) which increased constantly to stabilise at 90 h hydrolysis time demonstrating a stop in the hydrolysis reaction. A difference in the

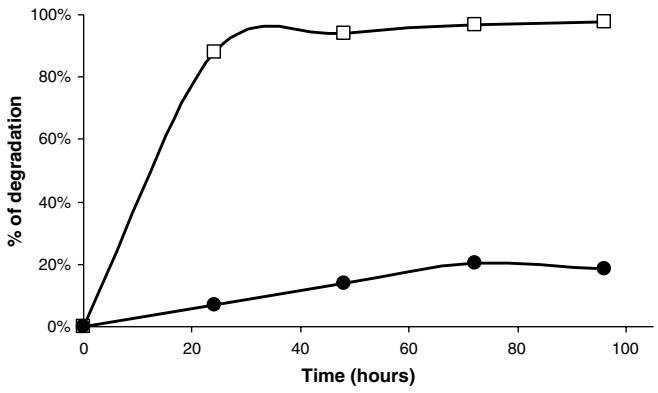

Fig. 6. Evolution of the curdlan degradation (% of the initial stock) as a function of the degradation time with H_2SO_4 (●) and enzymic extract (□).

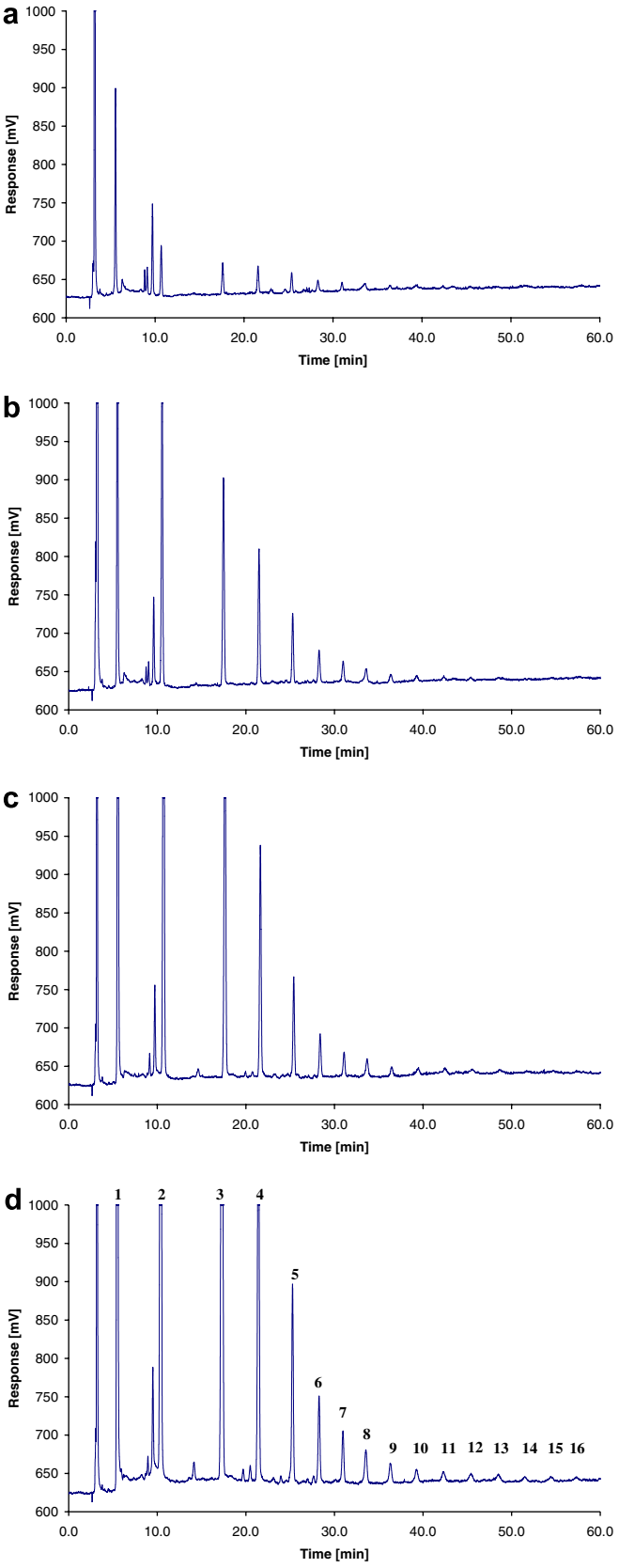

Fig. 7. Analysis of enzymically degraded curdlan obtained at different treatment times from a to d: 6, 24, 48 and 90 h by HPAEC. Respective oligosaccharide DP are indicated in d.

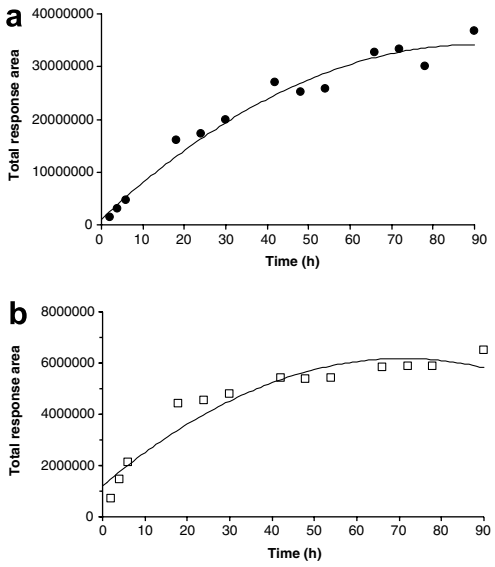

Fig. 8. Evolution of the response area of the total obtained oligosaccharides (●) and DP1 oligomer (□) after treatment with the enzymic extract.

beginning of this stable phase is observed with the study of the curdlan mass disappearance (Fig. 6) where no further evolution is noted already after 24 h of treatment. This difference is explained by the difference of each DP in relative response surface factor in the PAD. Koizumi et al. (1989) have demonstrated that this coefficient is relatively stable between DP1 and DP4, but can more than double when increasing to DP7. Unfortunately, no data are reported for β-(1 → 3)-oligoglucans with higher DP but this trend is strongly believed to continue. In this case, higher DP oligomers are not observed in our method because they are soluble enough to be present in the dried pellet, but big enough to have a low detector response in the HPAEC analysis. Therefore, we focus on the evolution of the major fraction represented by DP1 to DP4 (stable relative response coefficient) which is also a great indicator of this higher DP parent stock (soluble) degradation.

In Fig. 9a, this stabilisation in the appearance of oligomers with DP < 4 is confirmed. The production of glucose (DP1) diminished regularly during the process to stabilise around 15% of the total amount of oligosaccharides after 48 h treatment (Fig. 9b). Moreover, DP2, DP3 and DP4 are not degraded as it was seen in the case of acid treatments. Seventy-hours treatment seems to be the best hydrolysis time under our experimental conditions regarding the yield and quality of the recovered oligosaccharides (with the aim of producing oligosaccharides with DP < 5). The stock of curdlan is hydrolysed to 97.0% (Fig. 6) with a total of 82.9% for this DP range (19.5%, 22.6%, 23.8% and 17.0% for DP1–DP4, respectively).

The results obtained with our enzymatic extract are highly promising. To characterize the different types of protein present in our fungal enzymatic extract, the freeze-dried extract was studied by SDS–PAGE (Fig. 10). As the freeze-drying processes have been reported to affect the proteins (Roy & Nath Gupta, 2004), homogeneity and stability of several batches as well as the repeatability of the SDS–PAGE separation itself were studied. Two examples are reported (A and B).

Ten distinct bands are visible in the electrophoregram and their respective apparent molecular weights are

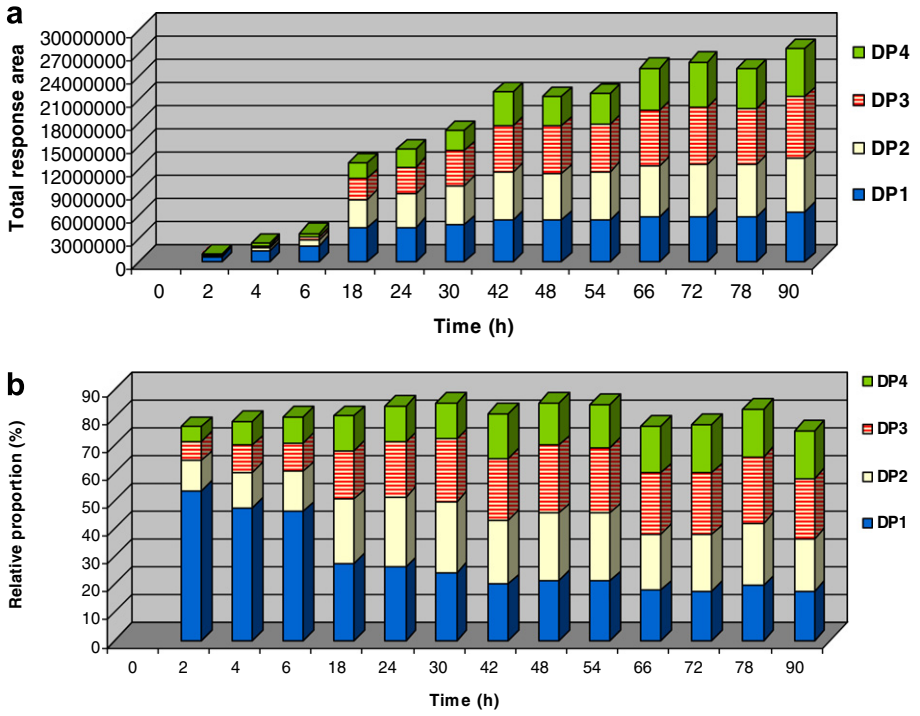

Fig. 9. Evolution of the total response area (a) and the relative fraction (b) (% of the total oligosaccharides) of DP1–DP4 in the degraded sample versus the degradation time with the enzymic extract.

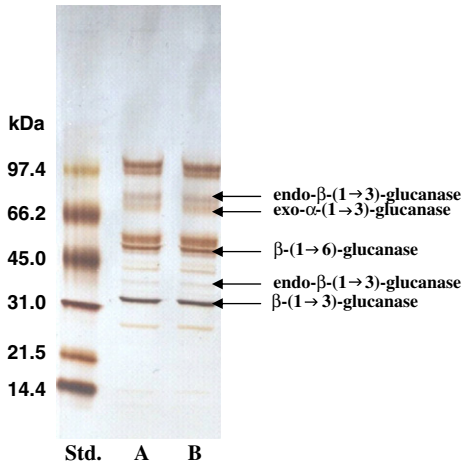

Fig. 10. SDS–PAGE of the raw enzymatic extract from *Trichoderma harzianum* of different batches of production (A and B). Std: standard MW markers. The numbers on the left of the panel indicate the respective molecular weight (kDa) of the markers including phosphorylase B (97.0), albumin (66.0), ovalbumin (45.0), carbonic anhydrase (30.0), trypsin inhibitor (20.1) and α-lactalbumin (14.4).

reported in Table 2. The results are analogous to those obtained from previous studies carried out to characterised the β-(1 → 3)-glucanases of *T. harzianum* (de la Cruz et al., 1995; Noronha & Ulhoa, 2000; Noronha et al., 2000). These latter β-(1 → 3)-glucanases were purified from the supernatant of TH culture with respective molecular masses of 29 (activity not yet described), 36 and 78 kDa (endo-β-(1 → 3)-glucanases activity). We found in this study proteins with similar molecular weight as the β-(1 → 3)-glucanases described previously (31, 36 and 76 kDa, respectively) even if we used different main carbon source for the production of our enzymatic extract (curdlan instead of chitin). In addition, the excretion of β-(1 → 6)-glucanases (with no activity on curdlan) by TH is common even if the media is supplemented by only β-(1 → 3)-glucan. A protein with an MW of 46 kDa has been reported by Montero, Sanz, Rey, Monte, and Llobell (2005), which is probably identical to our protein band at 48 kDa.

Another protein band separated by electrophoresis presents an MW of 69 kDa, a mass close to that of the exo-α-(1 → 3)-glucanase (MW estimated to be 72 and 67 kDa by SDS–PAGE and SEC, respectively) described in TH which does not degrade the β-glucan molecule of curdlan (Ait-Lahsen et al., 2001). This homology is further supported by the fact that even if glucose is produced at the beginning of the hydrolysis, its production slowed down rapidly and the level eventually became stable (Fig. 8b). Also, DP2, DP3 and DP4 which represents a major fraction of the produced oligosaccharides are not degraded further (Fig. 9a and b).

The other bands observed in our electrophoresis experiments have not yet been described in literature as being glucanases, indicating the possible presence of additional endoglucanases (exoglucanases not probable due to the explanations mentioned above) or other enzymes (e.g. protease or chitinase), which can accompany the production of high amounts of β-glucanase (Roldán et al., 2006).

Table 2
Characterisation of the protein molecular weights from the fungal extract separated by SDS–PAGE

Band	MW (kDa)
1	25.1
2	31.2
3	36.5
4	41.7
5	48.0
6	52.6
7	68.7
8	75.7
9	94.3
10	99.9

4. Conclusion

Enzymic degradation of curdlan demonstrated to be the best solution to produce low DP (<6) β-(1 → 3)-oligoglucans. It is cheap and efficient route. Both TFA and H_2SO_4 can not reach the same yield of hydrolysis and quality of resulting oligosaccharides under mild conditions. Optimisation of the enzymic reaction parameters is the next step to further improve the yield and scale-up the process.

Acknowledgement

The authors gratefully acknowledge Cees van Vliet for technical assistance on electrophoresis.

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