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Full Length Research Paper

Indigenous West African plants as novel sources of polysaccharide degrading enzymes: application in the reduction of the viscosity of cereal porridges

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Ethnobotanical and biochemical surveys revealed that some local plants from West Africa are novel sources of polysaccharide degrading enzymes such as amylases and glucanases. The study shows that these enzymes could be used for various biotechnological applications. In a crude extract of *Curculigo pilosa*, ß-amylase was the main starch hydrolyzing enzyme. Contrary to other plant amylases, the ß-amylase from *C. pilosa* is able to degrade raw starches from wheat, corn, potato and rice. In the bulbs of *Gladiolus klattianus*, activities of α-amylase and ß-amylase were found. Analysis of the enzyme action pattern showed that it released only maltose units from starch. Activities of α-amylase, ß-amylase, exo-(1→3, 1→4)-ß-D-glucanase and endo-(1→3)-ß-D-glucanase were detected in the leaves of *Boscia senegalensis*. The combined action of a saccharogenic enzyme (ß-amylase) and a dextrinizing enzyme (α-amylase) in *B. senegalensis* was useful to decrease the viscosity of cereal porridges and to increase their reducing sugar contents. The effective technological utilization of these higher plants as sources of carbohydrate degrading enzymes is discussed.

Key words: α-amylase, ß-amylase, ß-glucanase, yeast glucan, infant porridge*, Boscia senegalensis, Gladilus klattianus, Curculigo pilosa.*

INTRODUCTION

In the last decades, enzyme separation and characterization has become increasingly important because of the evolving application of enzymes in the brewing, food, textile, chemical, detergent and pharmaceutical industries. Enzymes (biocatalysts) play a central role in agro-industry and biotechnology. New enzymes from microbes and plants are constantly investigated for food and pharmaceutical industries, and for analytical purposes. Enzymes are involved in all biotechnological processes and food engineering. Our challenge was to investigate for new carbohydrate hydrolyzing enzymes within indigenous West African higher plants for their food applications.

Ethnobotanical investigations revealed that the aqueous extracts of bulbs of *Gladiolus klattianus,* zhizomes of *Curculigo pilosa* as well as leaves of *Boscia senegalensis* (Dicko, 1999) are used in Burkina Faso for the preparation of cereal based food and beverage such as "Zoom-koom", "dêguê" and “dolo”. *B. senegalensis, G. klattianus* and *C. pilosa* are traditional African plants

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Abbreviations: BCA, disodium 2,2’-bicinchoninate; DP, degree of polymerization; HPAEC, high performance anion exchange chromatography; HPSEC, high performance size exclusion chromatography; MALDI-TOF-MS, matrix assisted laser desorption/ionization-time of flight mass spectrometry; PAD-response, pulsed amperometric detection response; and RI-response, refractive index response.

empirically used for human and animal nutrition, protection of cereals against pathogens and for pharmacological purposes (Dicko et al., 1999). In particular, *B. senegalensis* is very well adapted to the semi-arid and arid conditions prevailing over much of the African continent, with unusual drought and high temperature resistance (Kerharo and Adams, 1974; Salih et al., 1991). It is well known that mixing a solution of powder prepared from the leaves or roots of *B. senegalensis,* bulbs of *G. klattianus* or rhizomes of *C. pilosa* with cereal porridge results in the production of sweet products (Kerharo and Adams, 1974). The rationale behind the use of these plant materials for the production of sweet products (oligosaccharides) has been documented (Dicko et al., 1999, 2000, 2001). Furthermore, the applicability and usefulness of these enzymes were investigated in the present study.

Starch degradation in plants is accomplished by α-amylase (α-1,4-D-glucan 4-glucanohydrolase, EC 3.1.1.1), β-amylase (α-1,4-D-glucan maltohydrolase, EC 3.1.1.2), amyloglucosidase (α-1,4-glucan-glucohydrolase, EC 3.1.1.3) and starch phosphorylase (1,4-α-D-glucan:phosphate-α-D-glucosyltransferase, EC 2.4.1.1) action on α-(1→4)-linkages (Manners, 1974). Amylases are hydrolytic enzymes, which depolymerize starch according to a classic acid-base mechanism. α-Amylases are endo-enzymes that randomly split α-(1→4)-linkages in starch with retention of anomeric configuration of glucose residues. β-Amylase is an exoglucosidase acting from the non-reducing end, releasing β-maltose units from starch, hence the name β-amylase (Kaplan and Guy, 2004). The β-maltoses released undergo mutarotation into α-maltose. Both α-amylase and β-amylase cannot split the α-(1→6)-linkages in amylopectin. Therefore, the degradation of starch by these enzymes is incomplete. In addition, plant amylases scarcely hydrolyze raw starch, e.g. their action is lower than 5% hydrolysis (Dicko et al., 1999). In the last decades, notably barley and soya amylases have found application in industry, because of their capacity to produce maltose syrups from starch (Boivin, 1997). There is still a need for new plant sources of highly active and thermostable amylases (Rashad et al., 1995), for formulation of enzymatic complex of starch degrading enzymes for biotechnological applications. The major disadvantage encountered using sorghum as brewing cereal is its low content of ß-amylase (Verbruggen, 1996) causing the incomplete saccharification of the starch. Furthermore, screening for α-amylase and ß-amylase activities in 50 sorghum varieties before and after germination, we have found that ß-amylase specific activity did not uniformly increase in all malted sorghums and even decreased in others (Dicko et al., 2005). This is a general problem of sorghum of sorghum brewing (Dufour, 1992). Thus relatively low cost exogenous sources of ß-amylases need to be investigated for sorghum brewing.

The most important classes of ß-glucanases in plants are (1→ 3, 1→ 4)-ß-D-glucanase (EC 3.2.1.73), (1→4)-ß-D-glucanase (EC 3.2.1.4) and (1→3)-ß-D-glucanase (EC 3.2.1.39). Oligosaccharides obtained from the degradation of fungal (1→3)-ß-D-glucan by ß-glucanase are associated with antitumor, antibacterial, anticoagulatory and wound healing properties (Bohn and BeMiller, 1995; Săndula et al., 1999). Therefore, the research of a novel source of (1→3)-ß-D-glucan converting enzymes or chemical modifications of these polymers has been our challenge (Dicko et al., 2001).

The overall objective of this study is to screen for enzyme activities of agro-industrial and pharmaceutical interests in traditional West African plants based on ethnobotanical surveys. The specific purpose would be the use these plants extract as exogenous sources of enzymes in cereal-based food processing.

MATERIALS AND METHODS

Plant materials

Green leaves of *Boscia senegalensis* (Pers.) Lam. ex. Poir (Capparaceae), harvested in the rainy season at Ouagadougou were generously provided by the National Centre for Scientific and Technological Research of Burkina Faso (CNRST). Ungerminated fresh bulbs of *Gladiolus klattianus* Hutch *(Iridaceae)* were used. Tubers of *Curculigo pilosa* (Schum. et Thonn.) Engl. (Hypoxidaceae or Liliaceae) were harvested at Ouagadougou (Burkina Faso) and after thoroughly washing kept in the dark at 4°C for one month before use.

Chemicals and reagents

Amylose and amylopectin were from Avebe (Veendam, The Netherlands), soluble starch from Merck (Darmstadt, Germany) and raw starches of wheat, corn, potato and rice from BDH Chemicals Ltd. (The Netherlands). Flours of maize, white sorghum, red sorghum, and millet are bought in the local market of Ouagadougou. The composite flour was Kasono was obtained from a private cooperative Centre Féminin d'Aliments de Sevrage at Ouagadougou, Burkina Faso. The composite flour is manufactured from the mixture of millet (68%, w/w), soya (17%, w/w), peanut (5%, w/w), sucrose (9%, w/w) and salt (1%, w/w). Chemicals for electrophoresis, columns for chromatography, ampholites, molecular mass marker proteins (14.4-94 kDa) and p*I* marker proteins (p*I* 3.5-9.3) were supplied by Amersham Pharmacia Biotech (Uppsala, Sweden). *p*-Nitrophenyl maltopentaoside, azurine-crosslinked-amylose and *p*-nitrophenyl-α-D-glucopyranoside were purchased from MegaZyme (Sydney, Australia). 2,2'-disodium bicinchoninate was from Pierce (USA). Laminarin (degree of polymerization about 25), bovin serum albumin and Coomassie Brillant Blue G-250 were purchased from Sigma (St. Louis, MO). Maltodextrins MD05 were obtained from Spreda (Burghof, Switzerland). Sorghum (1→3, 1→4)-ß-D glucan has been prepared as previously described by Verbruggen et al. (1996). Water insoluble yeast cell wall glucan (from *S. cerevisiae*) was generously provided by Professor F. M. Rombouts, of the

laboratory of Food Microbiology at Wageningen University (The Netherlands). All other chemicals were analytical grade.

Enzymes characterization

Unless indicated otherwise, all separation steps were carried out as previously described (Dicko et al., 1999, 2000, 2001). The degree of purification and the molecular masses of the enzymes were determined by SDS-PAGE in a 10-15% polyacrylamide gradient gel under reducing conditions with the PhastSystem (Amersham Pharmacia Biotech). The gel was stained by Coomassie Brilliant Blue. Low molecular mass standard proteins (14.4-94 kDa) were used for calibration. Fergusson equation ($\log M_r = a + b \times Rf$, where a and b are constants and Rf the frontal reference) was used for M_r calculation. The p*I* of the enzyme was estimated with the PhastSystem using standard proteins (p*I* 3.5-10.3). Proteins were detected by silver staining. Electrophoretic titration curves were performed in the same type of gels to monitor the purification procedure.

Enzyme assays and protein determination

Enzymatic release of reducing sugars from glucan substrates was measured by 2,2'-di-sodium bicinchoninic acid assay (BCA) in a microtiter plate format (Dicko et al., 2001). Activities of ß-amylase, α-amylase and α-glucosidase were determined by using specific substrates as previously described (Dicko et al., 1999). Total protein was quantified using Coomassie Brillant Blue G-250 according the method of Bradford, using the ratio of A_{620}/A_{450} versus protein concentration (Zor and Selinder, 1996). Bovin serum albumin was used a standard protein.

Carbohydrate hydrolysis

The specificity of the enzyme for carbohydrate substrates was studied by measuring the release of reducing sugars after incubation of the enzyme (0.5 unit) with various polysaccharides (5 mg), in 100 mM sodium acetate buffer pH 4.5 (total volume of 1 ml) at 40°C for 30 min. After centrifugation of the incubation mixtures, the carbohydrates present in the supernatant were analyzed. Analytical high performance anion exchange chromatography (HPAEC) was performed on a Dionex Bio-LC HPLC system (Sunnyvale, CA, USA) as previously described (Dicko et al., 1999, 2000, 2001). Analytical high performance size exclusion chromatography (HPSEC) was carried out on a SP8800 HPLC (Spectra Physics, San José, CA, USA) as previously described (Dicko et al., 2001). Matrix-assisted laser desorption/absorption mass spectrometry (MALDI-TOF-MS) analysis was performed with the Voyager-DE RP Biospectrometry Workstation (Perspective Biosystems, Inc., Framingham, Manchester, England), using maltodextrins (MDO5) for calibration (Dicko et al., 2001). Michaelis-Menten constants were calculated from Lineweaver-Burk plots of the initial rate of hydrolysis of polysaccharides under standard assay conditions with carbohydrate concentrations ranging from 0.1 to 5 mg/ml. The assays were performed at least in triplicate using the BCA assay. Changes of optical rotation of sugars formed by the α-amylase and ß-amylase hydrolysis of starch were determined as a function of time as previously described by Kohno et al. (1989) using a Ceti Polaris polarimeter (Belgium), with tube length of 10 cm.

Porridges preparation and measurement of viscosity

To study the effect of added enzymes on the paste viscosity of cereal-based weaning food, the flour was mixed with partially purified (ammonium sulfate fractionation, dialysis and lyophilization) enzymatic proteins from different plant extracts. The slurry was stirred and then heated slowly until gelatinization of starch (75 ± 2°C). The porridge is stirred for about 5 min, then further heated up to ebullition, for 5 min. Next, the porridge was cooled to 45 ± 2°C with occasional stirring to measure the viscosity in a Brookfield viscometer (HBDV-II+ model), using appropriate spindle (HB 7, 100 rpm).

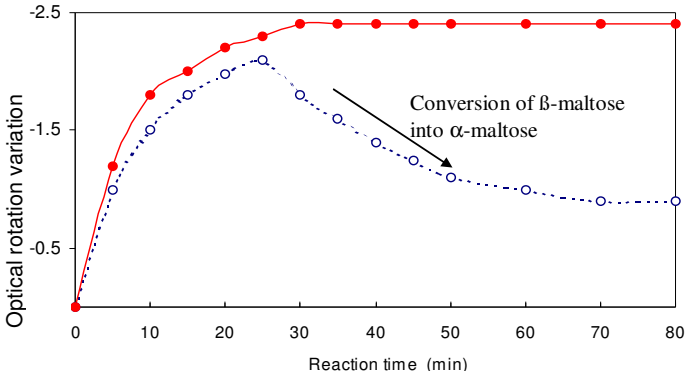

Figure 1. Mutarotation of realized oligosaccharides from starch hydrolysis by *C. pilosa* ß-amylase. Incubation was performed in 50 mM sodium acetate pH 5 at 30°C. The mixture of the enzyme and starch was performed in a 10 cm length polarimeter. *C. pilosa* ß-amylase (○) and commercial porcine pancreas α-amylase (●).$\Delta\alpha = \alpha - \alpha_0$ where α_0 is the initial optical rotation angle and α the optical rotation angle during the course of starch hydrolysis by amylase.

RESULTS AND DISCUSSION

Characteristics of a raw starch degrading amylase from rhizomes of *C. pilosa*

The purified ß-amylase from *C. pilosa* had a specific activity of 5650 U/mg (Dicko et al., 1999). This is much higher than the specific activities in the range of 1000-2500 U/mg that are mostly found for plant ß-amylases (Houvet 1986, Rashad et al., 1995), and also compares favorable to those reported for very active ß-amylases from activated sweet potatoes (4664 U/mg) (Chang et al., 1996) and the enzyme from *Clostridium thermosulphurogenes* (4215 U/mg) (Shen et al., 1988). The enzyme had a molecular mass of 64 kDa, which is in the range of monomeric ß-amylases from plants (60 ± 5 kDa) (Monroe and Preiss 1990; Rashad et al., 1995). The p*I* is 4.2, a value lower than reported for barley, wheat

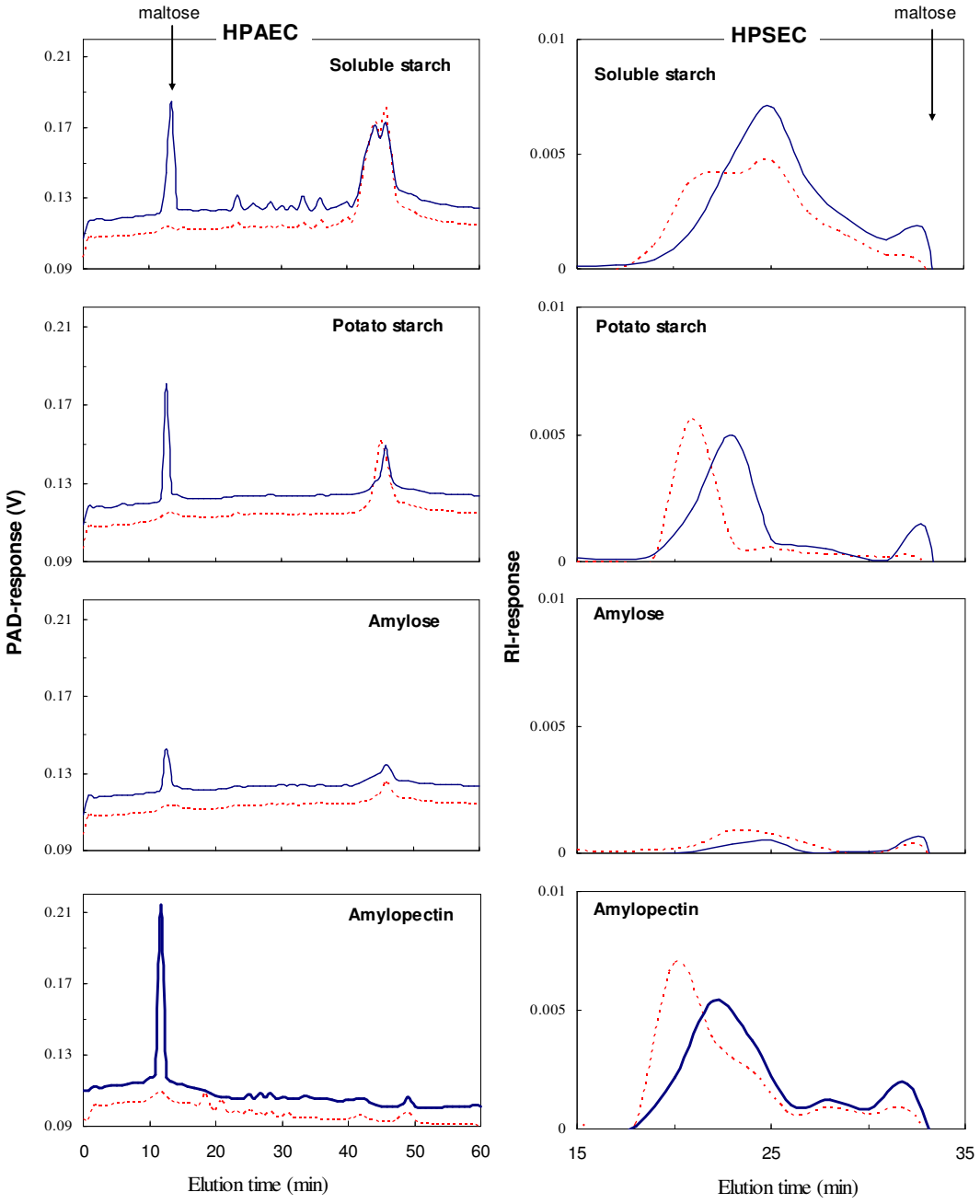

Figure 2. HPAEC and HPSEC elution patterns of hydrolysis products of starches by *C. pilosa* ß-amylase. (----) Substrate alone, (—) substrate + enzyme

and soybean ß-amylases but comparable to pea epicotyl ß-amylase (p*I* 4.35) (Lizotte et al., 1990) and maize ß-amylase (p*I* 4.2-4.4) (Laurière et al., 1992). The enzyme was identified as an exo-acting amylase based on the release of ß-maltose units, which undergo mutarotation into α-maltose (Yamazaki et al., 1989) (Figure 1). The enzyme had high activities with soluble starch, potato starch and amylopectin, but low activity with amylose (Figure 2). The hydrolysis of amylose can be limited by the low availability of non-reducing ends but also by its solubility in water (Lizotte et al., 1990). The K_m and k_{cat} for soluble starch were similar to those of ß-amylase from *C.*

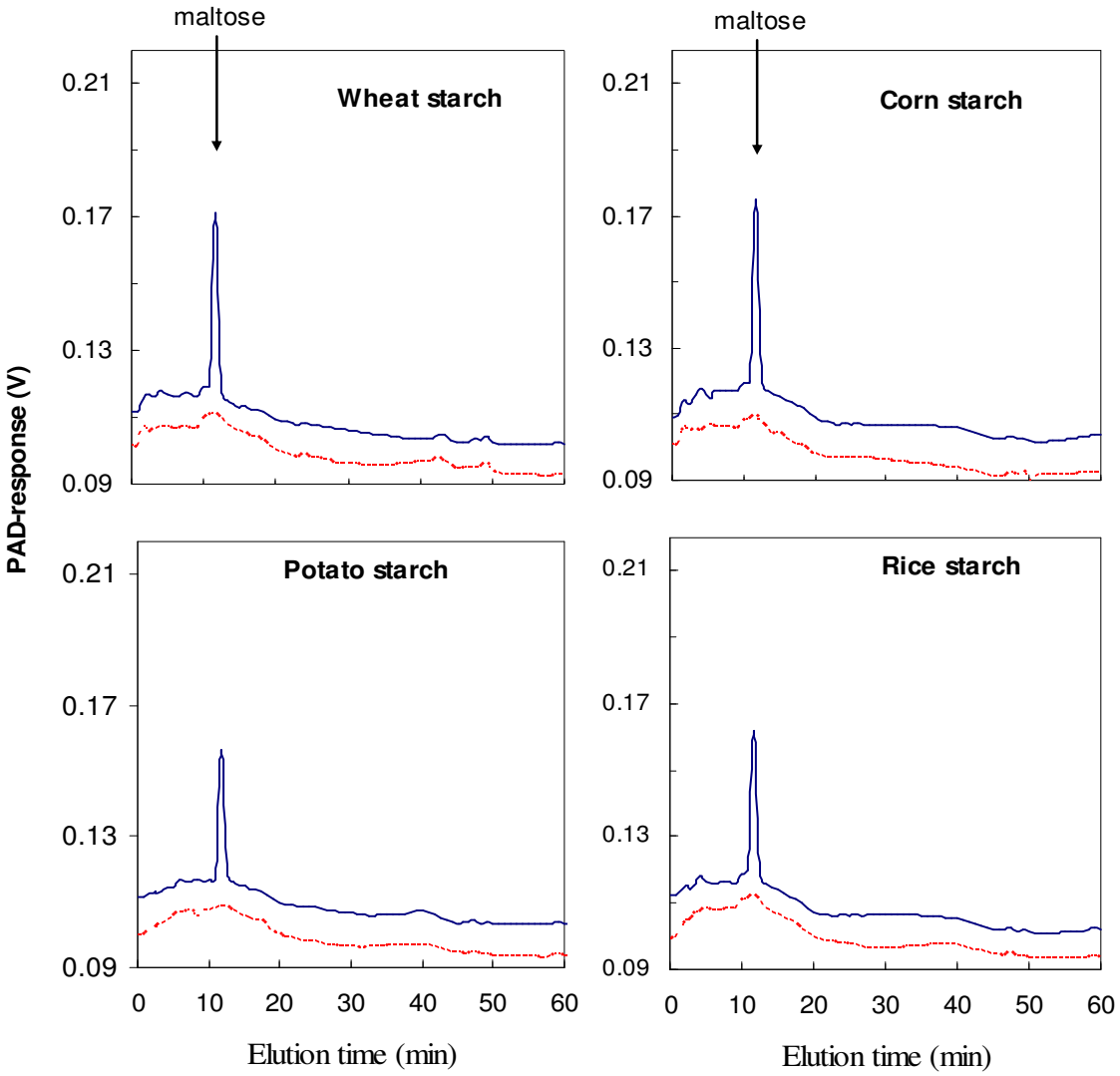

Figure 3. HPAEC elution patterns of hydrolysis products of raw starches by *C. pilosa* ß-amylase. (----) Substrate alone, (—) substrate + enzyme.

thermosulphurogenes (1.6 mg/ml and 6.66 10^3 s^{-1}, respectively) (Shen et al., 1988). The K_m's for soluble starch, boiled potato starch and amylose were similar (1.5-1.8 mg/ml) and are lower than those of sweet potato ß-amylases (3 mg/ml) (Chang et al., 1996), indicating a high affinity of our enzyme for these substrates. The K_m for amylopectin was 2.3 mg/ml, higher than the 1 mg/ml found for *Arabidopsis thaliana* but was not reflected in a lower k_{cat} for this substrate. The *C. pilosa* ß-amylase displayed hydrolytic activity towards raw starches from wheat, corn, potato and rice (Figure 3), whereas other plant ß-amylases (Lizotte et al., 1990) were unable to do so. The digestion of raw starches by ß-amylase from *C. Pilosa* is comparable to raw starch degrading amylases from microbial sources (Sohn et al., 1996; Saha et al., 1987; Nagasaka et al., 1998). Degradation of raw starch has not been reported for plant ß-amylases before, but it might explain the absence of α-amylase and α-glucosidase activities in the tuber *C. pilosa* (before germination).

The presence of high amylolytic activity in extracts of *C. pilosa* explains it traditional use in the preparation of easily digestible infant food and in the traditional method for the preparation of sorghum beer. In Nigeria, sorghum has become the predominant cereal for industrial scale malting and brewing, following legislation banning the importation of barley and wheat. The major disadvantage encountered using sorghum as brewing cereal is its low

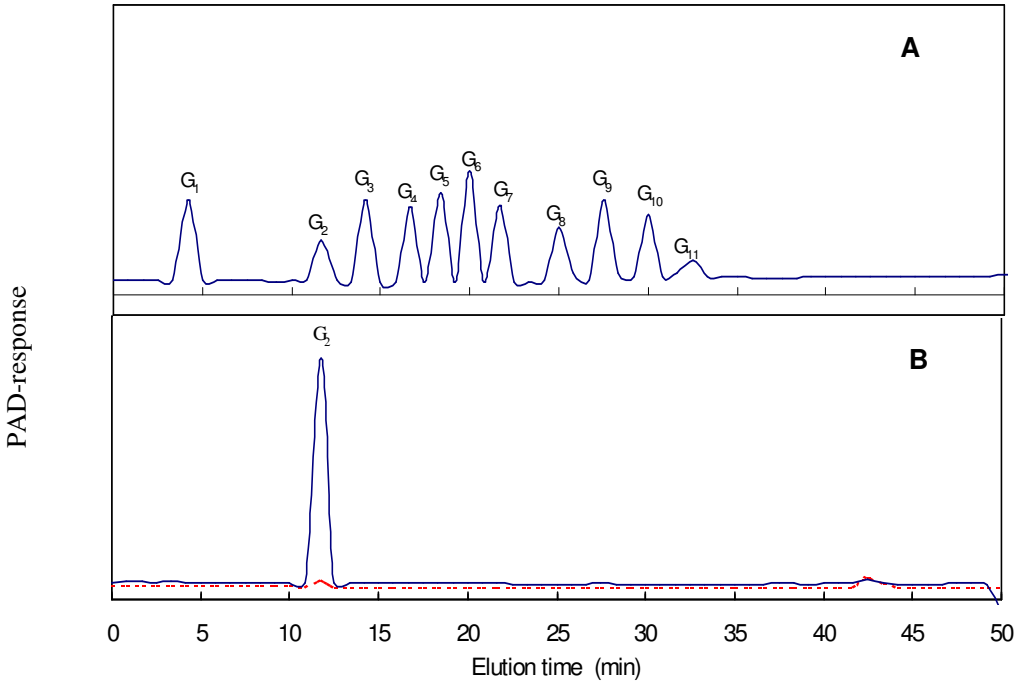

Figure 4. HPAEC elution patterns of hydrolysis products of raw starches by *G. klattianus* ß-amylase. (----) substrate alone, (—) substrate + enzyme. (A). Elution profile of maltodextrin standards. (B). elution profiles of the incubation mixture of the enzyme with the substrate.

content of ß-amylase (Verbruggen, 1996) causing the incomplete saccharification of the starch. This is a general problem of sorghum, for comparison of more than sixty sorghum malts in laboratory scale brewing experiments has shown clearly that in most cultivars ß-amylase was low or absent (Dufour, 1992). To increase the diatasic power of sorghum malt, cloning the ß-amylase gene from barley or microbes into sorghum is been envisaged. But alternatively to these techniques involving genetic engineering, the use of partially purified *C. pilosa* ß-amylase as exogenous source of enzyme in sorghum beer processing may be expected.

Characteristics of the most abundant starch degrading enzyme in the bulbs of *G. klattianus*

ß-amylase appears to be the major starch degrading enzyme of the bulbs of *G. klattianus* (Dicko et al., 2000) and its activity in the crude extract was approximately 440 U/g of fresh matter; that value is similar to the activity of ß-amylase from barley malt (400-500 U/g of fresh matter; Boehringer, Mannheim, Germany). The final specific activity was 2360 U/mg. The enzyme is a dimeric protein constituted of two protein subunits of 12 and 60 kDa, with an estimated total molecular mass of 72 kDa. The enzyme exhibited optimum activity for hydrolysis of starch at pH 5.5. It was stable in pH range of 4.5 to 8. This behaviour is similar to most of plant ß-amylases. The enzyme displayed optimum activity at 55°C. Analysis of the action pattern of the enzyme by high performance anion exchange chromatography showed that it produced only maltose after 5 min incubation with potato starch (Figure 4). This indicates the exo-fashion mode of hydrolysis of the enzyme. The high activity of ß-amylase within these bulbs explains their empirical use for food processing in West Africa, by starch conversion to maltose. The high activity of ß-amylase could make *G. klattianus* an interesting novel source of ß-amylase that could be used for high maltose syrup production and in brewery (Boivin, 1997). This study shows that a ß-amylase is abundant in a plant species of local importance, widely distributed in West Africa, and which should be expected to be useful in the future for biotechnological applications.

Among carbohydrate degrading enzymes in *B. senegalenis* the ß-1,3-glucanase has unusual properties

The presence of several carbohydrate hydrolases in the leaves of *B. senegalensis* (Table 1) could justify their traditional use in food processing (Dicko et al., 2001).

Table 1. Polysaccharide hydrolases in the 20-70% ammonium sulfate fraction obtained from the leaves of *B. senegalensis*.

Target enzyme	Substrate used	Specific activity* (U/mg)	Total activity (U/g of leaves)
α-Amylase	Azurin-cross-linked-amylose	4.2 ± 0.2	12.8 ± 0.3
ß-Amylase	p-nitro-phenyl-maltopentaoside	51.7 ± 0.8	158.1 ± 4.2
α-Glucosidase	p-nitro-phenyl-α-D-glucoside	0	0
Exo-(1,3;1,4)-ß-D-glucanase**	Barley (1,3; 1,4)-ß-D-glucan	2.4 ± 0.1	7.3 ± 0.2
Endo-(1,3; 1,4)-ß-D-glucanase	Barley (1,3; 1,4)-ß-D-glucan	0	0
Exo-(1,3; 1,4)-ß-D-glucanase	Sorghum (1,3; 1,4)-ß-D-glucan	3.6 ± 0.1	11.0 ± 0.4
Endo-(1,3; 1,4)-ß-D-glucanase	Sorghum (1,3; 1,4)-ß-D-glucan	0	0
Endo-(1,3)-ß-D-glucanase	Laminarin	8.9 ± 0.3	27.3 ± 1.0

*Total protein in the ammonium sulfate fraction was approximately 306 mg per 100 g of leaves.
**The mode of action was analyzed by HPAEC; exo-glucanases released only glucose monomers whereas endo-glucanases released oligosaccharides with different DP. Total activity was determined by the BCA assay. Data are from Dicko et al. (2001).

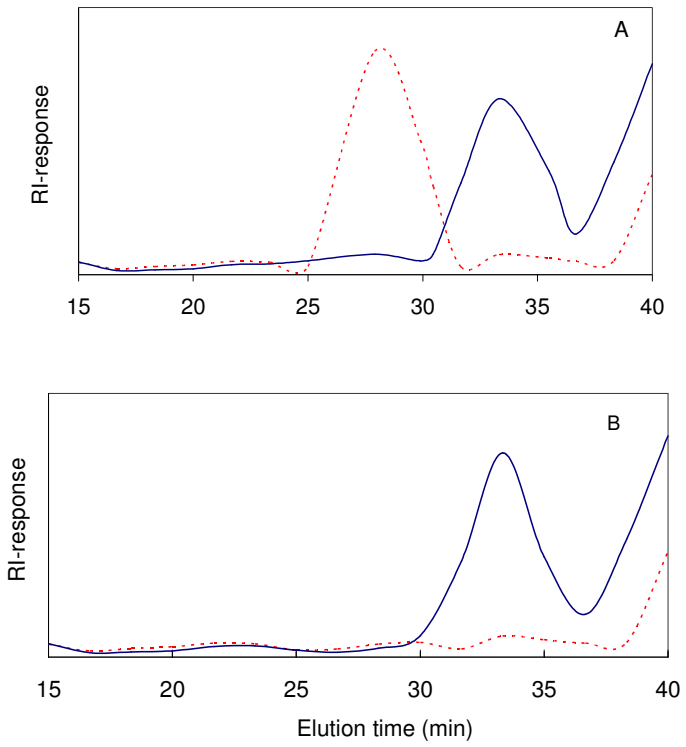

Figure 5. Analysis of the action mode of *B. senegalenis* (1→3)-ß-D-glucanase par HPSEC. A) hydrolysis products of laminarin. B) Hydrolysis products of insoluble yeast glucans. Incubations were performed in 100 mM sodium acetate buffer pH 4.5 at 40°C, fort 30 min.(----) Substrate alone, (—) substrate + enzyme.

These enzymes could allow the use of the extract of the leaves for the formulation of cereal based weaning flour, in order to increase the energy intake of infants, by reducing the dietary bulk of cereal porridges. Alternatively to the currently used amylases from micro-organisms and barley malt, the battery of carbohydrate hydrolases from *B. senegalensis* could be applied for this purpose. The production of sweet products after incubation of the leaves with cereal flour could be explained by the conversion of starch and cell wall ß-glucans into fermentable sugars by the glucosyl hydrolases present in the leaves. The depolymerization of ß-glucans and starch during sorghum beer preparation may improve the filtration of wort and the production of fermentable sugars. That may justify the rationale behind the empirical use of the leaves of *B. senegalensis* in sorghum beer manufacturing. The basic 36-kDa (1→3)-ß-D-glucan endohydrolase purified from the leaves of *B. senegalensis* has a similar molecular mass of 36 kDa. The enzyme is thermostable and the K_m for laminarin (0.42 mg/ml) was substantially lower than reported for (1→3)-ß-D-glucanases from other plants (Hrmova et al., 1997). The turnover number (k_{cat} = 395 s^{-1}) is higher than those of the (1→3)-ß-D-glucanase isoenzymes from barley leaves. The substrate specificity and action pattern of *B. senegalensis* (1→3)-ß-D-glucanase showed that the enzyme split, in an endo-fashion, exclusively linear (1→3)-ß-glucosyl linkages in polysaccharides (Dicko et al., 2001; Figure 5). The HPSEC analysis was useful to show the complete degradation of laminarin and also the solubilization of yeast glucan, after the enzyme's action (Figure 6). Nevertheless, the high sensitivity of the MALDI-TOF-MS allowed identifying more accurately the DP of the released (Dicko et al., 2001) products after the action of the enzyme on laminarin and yeast glucan.

In contrast to the (1→3)-ß-D-glucanases from plants, the purified enzyme showed activity in an endo manner on insoluble yeast glucan. The property of the *B. senegalensis* enzyme differs from plant endo-(1→3)-ß-D-glucanases characterized so far, in its ability to hydrolyze yeast cell wall glucan. The relatively high abundance of the enzyme in the leaves of *B. senegalensis* and its biochemical properties could make it as a candidate endo-(1→3)-ß-D-glucanase for applications such as the

Table 2. Reduction of the viscosity of cereal pastes with enzymatic proteins from the leaves of *B. senegalensis*.

Source	Viscosity of 25% (w/v) flour (Pa.s)				
	Maize	White sorghum	Red sorghum	Millet	Composite flour
Control	70	71	80	55	40
Control + 0.08% (w/w) of enzymes	2.5	3	2.5	2.1	1.5

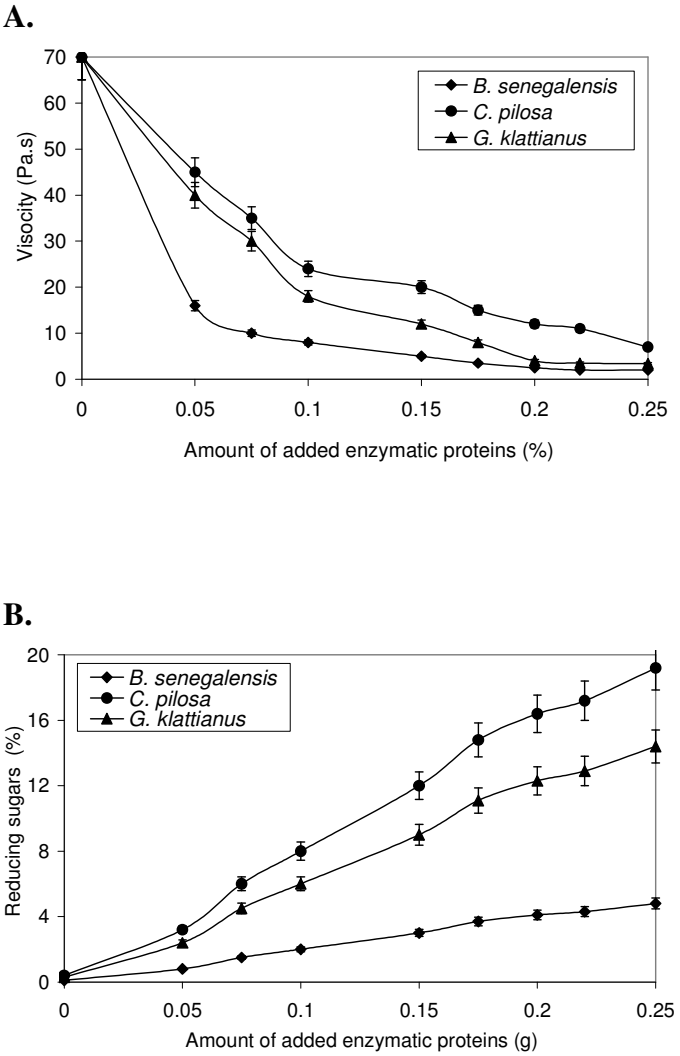

Figure 6. Effect of addition of enzymatic proteins on paste viscosity (A) and reducing sugars (B) of 25% (w/w) maize porridge. Maize porridges at 25% (w/w) were prepared using freeze-dried enzymatic proteins from *B. senegalensis* (◆), *C. pilosa* (●), *Gladiolus klattianus* (▲). The bars represent the standard error for each experiment.

biotechnological conversion of (1→3)-ß-D-glucans for food or pharmacological purposes.

Reduction of paste viscosity using the batteries of carbohydrate degrading enzymes from *C. pilosa*, *G. klattianus* and *B. senegalensis*

To study the effect of exogenous enzymes on the reduction of the viscosity of cereal porridges, partially purified enzymes from the plants extracts were used. The partial purification involved ammonium sulfate fractionation in the early purifications steps as previously described (Dicko et al., 1999, 2000, 2001) followed by dialysis and lyophilization. The powders containing enzymatic proteins were then mixed with cereal flour prior to starch gelatinization as descried in Materials and Methods. The viscosity of a 25% (dry weight) of the control cereal porridges was ranging from 40-80 Pa.s depending on the cereal (Table 2). However, there was a progressive reduction in viscosity with increasing the amount of enzymatic proteins in the porridge (Figure 6A). Results show that the enzymes from *B. senegalensis* are more effective in the reduction of porridge viscosity than those of *G. klattianus* and *C. pilosa* (Figure 6A). This is due to the fact the extracts of *B. senegalensis* contained a higher diversity of glucosyl hydrolases (notably α-amylase) than the other plants. The higher reduction of porridge viscosity of the *G. klattianus* enzymatic extract than *C. pilosa* enzymatic extract may be justified by the presence of α-amylase in the former plant. Indeed, the reduction of porridge viscosity is more governed by the endo-hydrolysis of starch by α-amylase than the other carbohydrates hydrolases such as ß-amylase. Addition of the enzymatic extract from *B. senegalenis* to various porridges of local cereals commonly used (Table 2), allows the preparation porridges of low viscosity (1-3 Pa.s) with desirable energy density (25% dry weight). Porridge of viscosity between 1 to 3 Pa.s is the consistency generally suitable for infants' consumption (Malesshi and Desikachar, 1988). That suitable viscosity was obtained by addition of 0.2 g of enzymatic proteins of *B. senegalensis* (e.g. 0.08%). From this, it could be inferred that the combined actions of a saccharogenic enzyme (ß-amylase) and a dextrinizing enzyme (α-amylase) in *B. senegalensis* could be useful in the decrease of the viscosity of cereal pastes and to increase their reducing sugar contents. ddition of enzymatic

proteins from leaves of *B. senegalensis* to weaning food definitely reduces it dietary bulk and therefore could enable infants to consume food with more energy density (Table 2).

On the other hand, the amount of reducing sugars has much more increased in the porridge containing the enzymatic proteins from *C. pilosa* than the other plants (Figure 6B). This could be attributed to the high ß-amylase activity displayed by *C. pilosa* extract and also by the ability of this enzyme to hydrolyze raw starch. In addition, these results show that the extracts of *C pilosa* and *G. klattianus* can effectively be used as source of ß-amylase for the production of maltose syrup or to increase the levels of reducing sugars in cereal based-foods and beverages. That increase of reducing sugars is mainly due to the conversion of starch into maltose by the ß-amylase, which is a maltogenic amylase.

CONCLUSION

Since polysaccharide degrading enzymes such as amylases and glucanases are nowadays at the center of food process engineering, evolving enormous applications in biotechnology, the discovered enzymes from local West African plants, could be used for several purposes. This finding could also be a way to enhance the value of industrial enzymes from local higher plants from West Africa. Furthermore, the present work has shown that these enzymes could be used for the formulation of weaning food with low viscosity to allow infant to consume porridges with low viscosity and high energy intake. The characterized endo-1,3-ß-glucanase from *B. senegalensis* is an unusual enzyme with respect to its ability to degrade insoluble yeast glucans and to yield valuable oligosaccharides. These properties open a wide array of application of this enzyme for the biotechnological synthesis of specific oligosacharides with special features in pharmacology. In addition, low cost glycosyl hydrolases have interesting features for biotechnological applications such as food processing in low income countries.

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