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O-Acetylated (1→6)-β-D-Glucan (Lasiodiplodan): Chemical Derivatization, Characterization and Antioxidant Activity

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Abstract: β-Glucans are biomacromolecules that present biological properties of medical and pharmacological interest. The chemical modification of the primary structure of these carbohydrate biopolymers is a way to enhance or achieve new biological properties. Acetylated derivatives of (1→6) -β-D-glucan (lasiodiplodan) with different degrees of substitution (0.48, 0.66, 1.03 and 1.26) were obtained and characterized by infra-red and NMR (nuclear magnetic resonance) spectroscopy, thermal analysis, X-ray diffraction and antioxidant capacity. Acetylation was confirmed by FT-IR, and ^{13}C NMR spectroscopy. Thermal analysis indicated that unmodified lasiodiplodan and the *O*-acetylated β-glucan derivative of degree of substitution 0.48 presented three stages of mass-loss, whereas acetylated derivatives of DS (degree of substitution) of 0.66, 1.03 and 1.26 presented four stages of mass-loss. X-ray diffractograms demonstrated that both native and acetylated lasiodiplodan presented crystalline regions in an amorphous polymeric matrix. Scanning electron microscopy revealed that *O*-acetylation promoted morphologic changes in the biopolymer according to the DS. Acetylation also contributed to improve antioxidant capacity.

Key words: Exopolysaccharide, biopolymer, bioactive carbohydrate, Macromolecules.

1. Introduction

β-Glucans are homogeneous carbohydrate biopolymers composed of D-glucopyranose monomers linked by glucosidic bonds in the β-configuration. The molecular characteristics of biopolymers depend almost exclusively on the source and purification method [1], since the extraction and further processing conditions significantly influence its chemical structure [2].

Most commercially available non-cellulosic β-glucans are extracted from the cell wall of bacteria, fungi, algae and plants including cereals such as barley and oats. Some β-glucans are secreted into the cultivation medium by bacteria and filamentous fungi in which they are grown by submerged fermentation, and some of these are commercially available, e.g., curdlan, scleroglucan. These β-glucans are named EPS (exopolysaccharides) and may show differences in chemical structure, biological functions and physicochemical properties [3]. Recently, β-glucans have aroused much interest because of the chemical structure and biological function relationships, and diverse bioactive properties have been demonstrated such as anti-tumoral, immunomodulatory, hematopoiesis stimulating, antioxidant and anti-inflammatory [4].

Another property that has attracted attention is the fact that they are dietary fibers showing satisfactory

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results in the prevention and treatment of metabolic syndrome and the control of obesity [1].

Chemical modifications in polysaccharides are important tools in obtaining new bioactive agents, which can display changed bioactivities, e.g., botryosphaeran when sulfonylated exhibited anticoagulant and antithrombotic activities [5, 6]. Basically, the bioactivity of derivatized polysaccharides has been assigned to the hydroxyl groups and introduction of new substituent groups such as -SH, -COOH, -C-O- and -NH_2, which changes the chemical structure of native polysaccharides and decreases the intramolecular and intermolecular bonding [7].

Acetylation of polysaccharides such as cellulose and starch using acetic acid and acetic anhydride as derivatizing agents is well known [8]. In this context, acetylation is a kind of modification of polysaccharides that can increase their applications in biomedical and industrial fields, since it can improve the physicochemical and biological properties of such polysaccharides [9]. *O*-acetylation basically consists in the esterification of free hydroxyl groups on the carbohydrate polymer by reaction with acetic anhydride. Hydroxyl groups are therefore converted into ethanoates and this kind of chemical derivatization is influenced by various reaction parameters such as temperature, reaction time, acetic anhydride concentration and solvent types [10-12].

Lasiodiplodan is a linear, non-branched (1→6)-β-D-glucan produced by the ascomyceteous fungus, *Lasiodiplodia theobromae* MMPI, as an extracellular secretion when cultivated in liquid nutrient medium on glucose as carbon source. A wide spectrum of biological properties has been observed for lasiodiplodan such as antiproliferative activity on MCF-7 breast cancer cells mediated by oxidative stress, AMP-activated protein kinase (AMPK) and the Forkhead transcription factor ($FOXO_3$a) [13, 14], antioxidant activity *in vitro* [15] and hypoglycaemic activity [16]. Recently we reported that the chemical modification of lasiodiplodan by carboxymethylation improved its water solubility and enhanced antioxidant capacity [15].

This study reports on the acetylation of (1→6)-β-D-glucan (lasiodiplodan), its chemical and morphological characterization, and evaluation of antioxidant potential using different analytic protocols.

2. Method and Materials

2.1 Production and Purification of Lasiodiplodan

Submerged fermentation of *Lasiodiplodia theobromae* MMPI was conducted in Erlenmeyer flasks (250 mL) containing 100 mL of minimum salts medium [17] and 20 $g{\cdot}L^{-1}$ glucose. Initial pH of the nutrient medium was adjusted to 5.5 and the flasks were incubated on an orbital shaker at 28 °C, 150 rpm for 72 h. Standard inoculum volume (10 mL) was used as described by Cunha et al. [13].

The fermented broth was separated from the fungal mycelium by centrifugation (1,500 × *g*, 20 min) and precipitated with absolute ethanol (4 ºC, overnight). The polysaccharide lasiodiplodan, unacetylated, designated LAS-N was recovered by filtration and solubilized by stirring in distilled water at 60 ºC, followed by exhaustive dialysis (cellulose membrane dialysis tubing (MWCO E 12,000 Da; 1.3 in diam., Sigma-Aldrich, USA) against distilled water, and then dried by lyophilization.

2.2 Acetylation of Lasiodiplodan and Determination of the DS (Degree of Substitution)

Acetylation of LAS-N was carried out as described by Ma et al. [18] with some modification. Lyophilized exopolysaccharide (1.5 g) was dispersed in 300 mL of pyridine and stirred at 60 °C for 30 min. Then, 300 mL of pyridine-acetic anhydride solutions (180:120, 240:60, 270:30 and 185:7.5 mL/mL) were added and maintained at 60 °C for 4 h under stirring. Distilled water (250 mL) was next added to stop the reaction, and the mixture was concentrated under reduced pressure. Anhydrous ethanol was added and left

overnight at 5 °C. The precipitate was filtered, washed three times with anhydrous ethanol, suspended in distilled water, then exhaustively dialyzed against distilled water and finally lyophilized. These samples were designated LAS-AC.

The percentage of AG (*O*-acetyl groups), and the DS were determined as described by Das et al. [19]:

$$AG\ (\%) = \frac{[(b-s) \times M \times 43]}{W} \times 100 \quad (1)$$

where, b is the volume (L) of HCl (0.2 mol·L^{-1}) used in the blank titration, s is the volume (L) of HCl (0.2 mol·L^{-1}) used to titrate the sample, M is HCl concentration (0.2 mol·L^{-1}), W is sample mass (g, dry basis) and 43 is the molecular weight (g·mol^{-1}) of acetyl group.

The DS was determined from the formula:

$$DS = \frac{162 \times AG}{4{,}300 - 42AG} \quad (2)$$

where, 42 is the molecular weight (g·mol^{-1}) of a deprotonized *O*-acetyl group and 162 is the molecular weight of an anhydro-glucose unit of lasiodiplodan.

2.3 Characterization of Native and Acetylated Lasiodiplodan

2.3.1 Infra-Red Spectroscopy (FT-IR)

Infra-red spectra of LAS-N and LAS-AC were obtained using a FT-IR Spectrometer Frontier (Perkin Elmer, USA) in the region of 4,000-400 cm^{-1}, with a 4 cm^{-1} resolution, and 32 accumulated scans using the KBr disc method.

2.3.2 ^{13}C Nuclear Magnetic Resonance

The ^{13}C solid-state NMR spectra were obtained on a Bruker Avance III 300 Spectrometer (7.1 T), combining the cross-polarization technique with magic angle spinning (CP/MAS) at 10 kHz and proton decoupling in a 5 mm MAS broadband probe. Pulses of 90º were used with a repetition time of 3 s, and CP time of 3 ms.

2.3.3 Thermal Analysis

Lyophilized samples of LAS-N and LAS-AC were subjected to TGA (thermogravimetric analysis), DTG (derivative thermogravimetry) and DSC (differential scanning calorimetry) carried out on a SDT Q600 instrument (TA Instruments, USA). The analysis interval was between 25 °C to 800 °C at a heating rate of 5 °C·min^{-1} and a synthetic air atmosphere with flow of 50 mL·min^{-1}.

2.3.4 Scanning Electron Microscopy

SEM (scanning electron microscopy) was used to examine native and derivatized lasiodiplodans for surface morphology. Micrographs were acquired in a scanning electron microscope (Hitachi TM3000, USA) using lyophilized samples, and images were taken at magnifications of 400, 800 and 1,500×.

2.3.5 X-Ray Diffraction

X-ray diffractogram patterns were recorded on a Rigaku MiniFlex600 diffractometer, using copper radiation font (CuKα = 1.5418Å), 15 mA current, 40 kV voltage, scanning range of 10° to 60° (2θ), a scanning speed of 5º min^{-1} and a step width of 0.02° (2θ).

2.4 Antioxidant Activity

2.4.1 Assessment of FRAP (Ferric Reducing Antioxidant Power)

The FRAP reagent was prepared by mixing 10 volumes of sodium acetate buffer (0.3 mol·L^{-1}, pH 3.6), 1 volume of 2,4,6-tripyridyl-s-triazine (TPTZ) solution (10 mmol·L^{-1}), and 1 volume of an aqueous solution of ferric chloride (20 mmol·L^{-1}). The reagent was freshly prepared before each use. A 50 µL sample (LAS-N, LAS-AC or glucose) at a concentration of 150 mg·L^{-1} was added to a test tube together with 150 µL of distilled water, and 1.5 mL of FRAP reagent. The tubes were shaken and incubated at 37 °C for 30 min in the dark. Analyses were conducted in triplicate and read at 595 nm using the FRAP reagent as the blank. A ferrous sulfate calibration curve was constructed (100-2,000 µmol·L^{-1}) and the results were expressed in µM of $FeSO_4 \cdot 7H_2O$.

2.4.2 Assay of Reducing Power

Reducing power was evaluated according to Liu et al.

[20] using a mixture of 2.5 mL of lasiodiplodan samples or ascorbic acid solutions (150 mg·L^{-1}) incubated with 2.5 mL of potassium ferricyanide (1% w·v^{-1}) at 50 °C for 20 min. The reaction was terminated by adding of 2.5 mL trichloroacetic acid (10% w·v^{-1}) followed by the addition 5 mL of distilled water and 1 mL ferric chloride (0.1% w·v^{-1}). Absorbance was measured at 700 nm. Higher absorbances of the mixture indicated greater reducing power of the sample.

2.4.3 Hydrogen Peroxide Scavenging Activity Assay

The hydrogen peroxide (H_2O_2) removal capacity was measured according to Liu et al. [20]. The reaction mixture consisted of 1 mL of H_2O_2 (0.1 mmol·L^{-1}, freshly prepared), 1 mL of polysaccharide suspension (150 mg·L^{-1}), 0.1 mL of ammonium molybdate (3 % w v^{-1}), 10 mL of H_2SO_4 solution (2 mol·L^{-1}), and 7 mL of KI solution (1.8 mol·L^{-1}). The mixture was titrated with $Na_2S_2O_3$ (5 mmol·L^{-1}) until the disappearance of the yellow color. Scavenging activity was calculated as follows:

$$\%\ H_2O_2\ \text{scavenging} = \frac{(V_0 - V_1)}{V_0} \times 100\ \% \quad (3)$$

where, V_0 was the volume of $Na_2S_2O_3$ solution used to titrate the control mixture and V_1 was the volume titrated of the mixture containing the samples.

2.4.4 Hydroxyl Radical Scavenging Activity Assay

Hydroxyl radical (HO•) scavenging activity was assessed according to Liu et al. [20] with subtle modification. Hydroxyl radicals were generated from $FeSO_4$ and H_2O_2, and detected by their ability to hydroxylate salicylate. The reaction mixture (2 mL) contained 0.5 mL of $FeSO_4$ (1.5 mmol·L^{-1}), 0.35 mL of H_2O_2 (6 mmol·L^{-1}), 0.15 mL of sodium salicylate (20 mmol·L^{-1}) and 1 mL of sample (150 mg·L^{-1}). Ascorbic acid was used as a positive control. Absorbance of hydroxylated salicylate complex was measured at 562 nm after an incubation period of 1 h at 37 °C.

The percentage of hydroxyl radical scavenging was calculated as:

$$\%\ HO \bullet scavenging = \left[1 - \frac{(A_1 - A_2)}{A_0}\right] \times 100\% \quad (4)$$

where, A_1 is absorbance of sample or ascorbic acid, A_0 is control absorbance and A_2 is reagent blank absorbance with sodium salicylate.

OriginPro 8 software program was used in the construction of the graphs.

3. Results and Discussion

3.1 DS after O-Acetylation

The conditions of *O*-acetylation promoted the formation of *O*-acetylated lasiodiplodans of different degrees of substitution (0.48, 0.66, 1.03 and 1.26) according to the concentration of acetic anhydride used in the derivatizing solution. The maximum theoretical value of DS that can be obtained is 3.0, (maximum of free hydroxyl groups per glucose monomer in lasiodiplodan are available for *O*-acetylation—see Fig. 1).

An estimate of the percent yield of derivatization of lasiodiplodan resulted in different values under the conditions employed for derivatization; 16.0% (DS 0.48), 22.0% (DS 0.66), 34.3% (DS 1.33) and 42.1% (DS 1.26).

The reaction time and the concentration of the derivatizing agent are parameters that strongly influence the degree of substitution of the molecule. Souza et al. [11] evaluating the correlation between acetic anhydride concentration and the DS of derivatized oat β-glucan, found that higher concentrations of acetic anhydride (6%) incorporated more *O*-acetyl groups (DS 0.12) than at a lower concentration (4%) of derivatizing agent (DS 0.06) under the same reaction conditions.

3.2 FT-IR Analysis

Infra-red spectra of LAS-N and LAS-AC are presented in Fig. 2.

There is a wide band in the LAS-N spectrum that shows strong intensity in the region of 3,362 cm^{-1} that is assigned to -OH stretching vibration [11, 21-23]. The

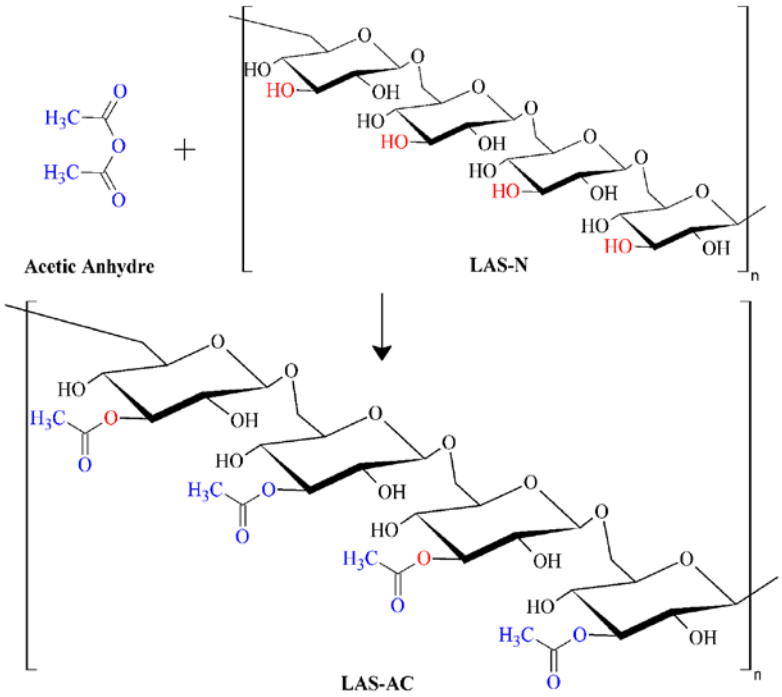

Fig. 1 Schematic representation of the *O*-acetylation reaction of LAS-N.

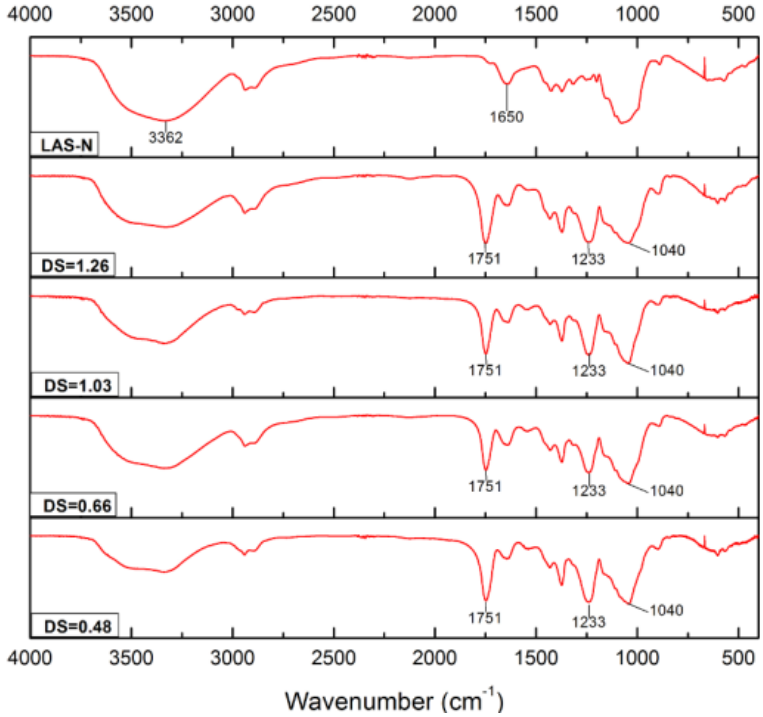

Fig. 2 FT-IR spectra of LAS-N and LAS-AC of different DS (1.26, 1.03, 0.66 and 0.48).

band present in the region of 1,650 cm^{-1} corresponds to water adsorption [24-26]. In the spectral regions of 1,541 cm^{-1}, 1,428 cm^{-1}, 1,316 cm^{-1}, 1,202 cm^{-1} and 1,152 cm^{-1}, there are bands arising from deformation vibrations of C-H and -OH, which are typical in carbohydrates. These may present few variations such as wavenumber and intensity depending on the nature of the polysaccharide [23]. Bands with medium and

low intensity at 1,076 cm^{-1} and 1,029 cm^{-1}, respectively, are attributed to stretching vibration of C-O that occurs in the pyranose ring present in the monomers comprising glucans. In the region of 888 cm^{-1} there is a band with low intensity indicating β configuration of lasiodiplodan [23, 27-29].

FT-IR spectra of LAS-AC show a band with high intensity in the 3,343 cm^{-1} region corresponding to stretch vibrations of -OH [11, 22, 29]. The high intensity band at 1,751 cm^{-1} arises from carbonyl groups on glucose monomer linked by acetate ester bonds -O (C=O) [11, 22, 23, 29].

The decrease in the intensity of bands assigned to -OH groups in the LAS-AC samples compared to those of the LAS-N spectrum occurred due to the substitution of the hydroxyls by *O*-acetyl groups [22]. Bands in the regions of 1,233 cm^{-1} and 1,041 cm^{-1} showed more intensity in the LAS-AC spectra compared to LAS-N. This is attributed to acetate ester groups -O (C=O) (1,233 cm^{-1}) and the increase in C-O vibration (1,041 cm^{-1}) that appeared in the *O*-acetylated lasiodiplodan derivatives.

3.3 ^{13}C NMR Analysis

The ^{13}C MAS NMR spectra from LAS-N and LAS-AC are presented in Fig. 3.

^{13}C MAS NMR analysis showed that LAS-AC has a β-configuration as judged by a typical chemical shift at δ104.2. The chemical shifts observed in the LAS-AC sample at δ86.7 and δ62.5 were attributed to carbons 3 and 5, respectively. The C-2 signal was only identified in the LAS-N spectrum (δ76.6), while in the LAS-AC spectrum this specific signal was not observed, suggesting a shift to higher field in this particular resonance which caused an overlap with the signals from C-4 and C-6. The shift of only one signal is a strong indication of *O*-acetylation at that position. The ^{13}C MAS NMR spectrum of LAS-AC showed that the δ171.1 chemical shift was attributed to the carbonyl group and that at δ20.8 to the methyl group, confirming the biopolymer was *O*-acetylated lasiodiplodan as indicated by FT-IR analysis.

3.4 Thermal Analysis

Fig. 4 shows the TG (thermogravimetric), derivative thermogravimetric and DSC (differential scanning calorimetry) curves for LAS-N and LAS-AC. According to thermoanalysis data, LAS-N showed three stages of mass-loss. The first event of mass-loss occurred up to 127 °C, being attributed to water elimination as water

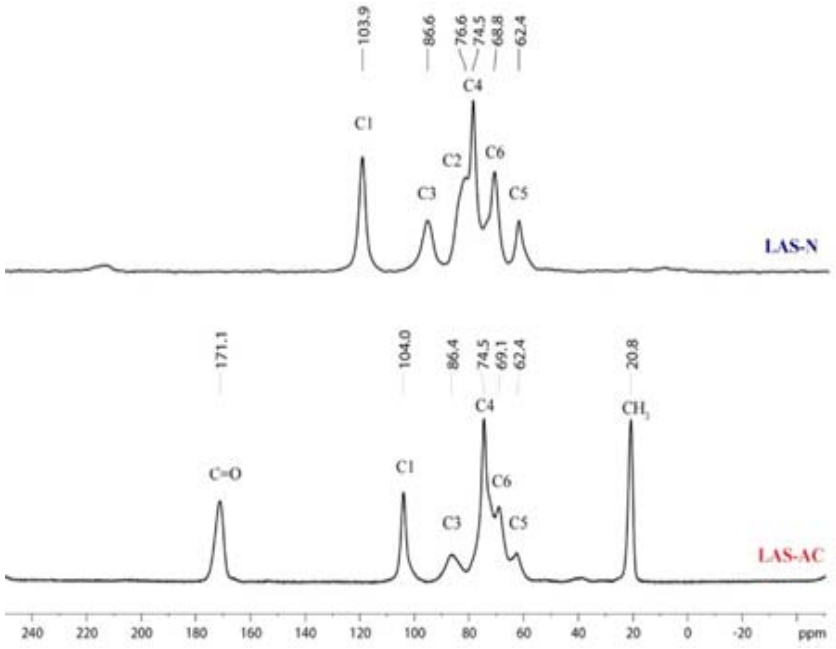

Fig. 3 Solid state ^{13}C NMR spectra of native (LAS-N) and acetylated (LAS-AC) lasiodiplodan.

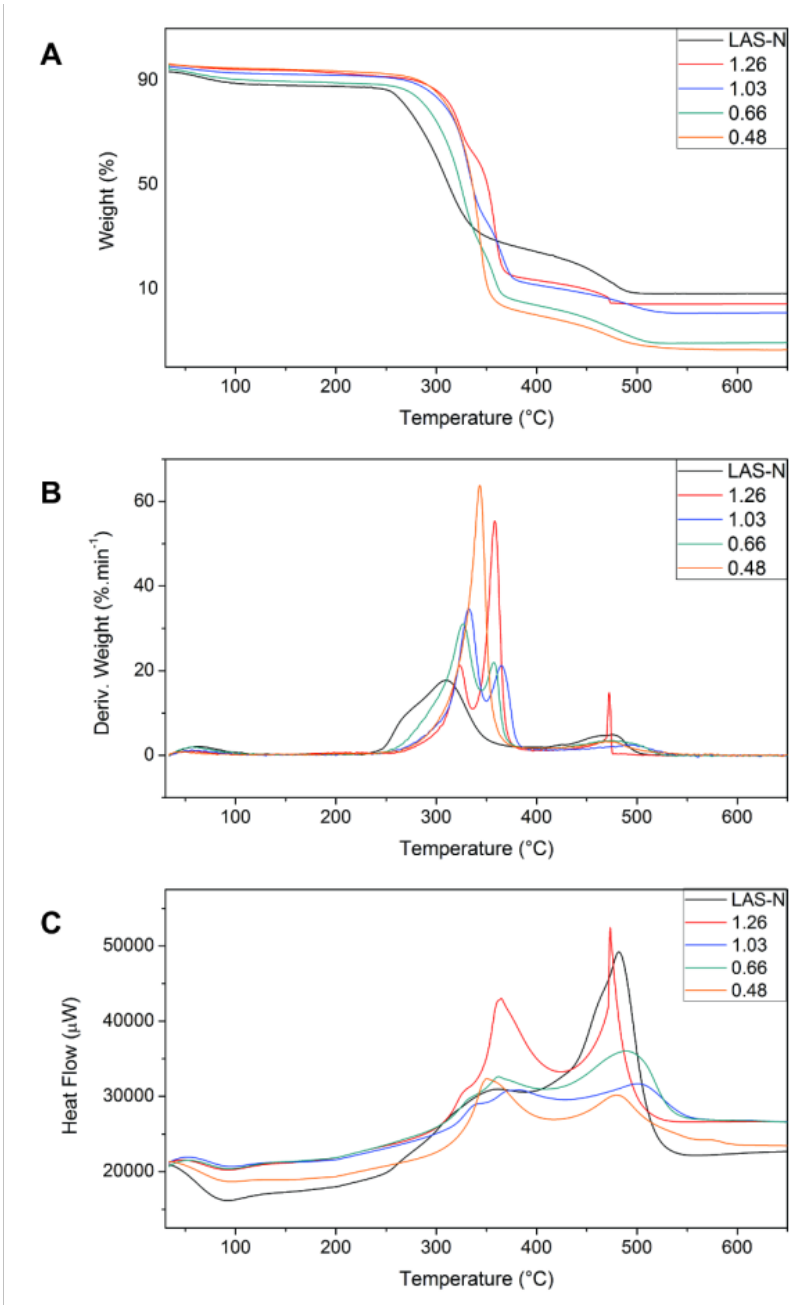

Fig. 4 Thermogravimetric (A), thermogravimetric derivative (B) and differential scanning calorimetry (C) curves of LAS-N and LAS-AC of different DS (1.26, 1.03, 0.66 and 0.48).

of hydration. Such water loss is confirmed through an endothermic peak at 63 °C in the DSC curve (222.55 $J \cdot g^{-1}$). Thereafter, two consecutive events of mass-loss occurred between 200 °C and 400 °C, which were confirmed by two exothermic peaks (5,752 $J \cdot g^{-1}$) at 310 °C and 475 °C in the DSC curve, corresponding to thermal degradation and carbonization of LAS-N.

Acetylated derivatives (DS 1.26, 1.03 and 0.66) showed four mass-loss stages. The first event occurred between 30 °C and 130 °C corresponding to water loss with endothermic peaks observed at 55.38 °C (85.28 $J \cdot g^{-1}$), 58.01 °C (155.6 $J \cdot g^{-1}$) and 56.61 °C (155.5 $J \cdot g^{-1}$) in the DSC curves. The second stage of mass loss occurred between 200 °C and 393 °C, corresponding to the decomposition of samples with exothermic peaks at 323.6 °C, 332.2 °C and 326.5 °C in the DSC curves.

The third mass-loss event has been attributed to decomposition (oxidation) of the derivatized macromolecules and occurred at the temperature range between 334 °C and 392 °C. The last stage of mass loss was attributed to the final molecule decomposition observed between 381 °C and 480 °C, and indicated by exothermic peaks in DSC curves observed at 472.6 °C, 491.8 °C and 473.6 °C. The *O*-acetylated derivative with less substituted hydroxyl groups (DS 0.48) presented only a three-stage mass-loss as was observed for non derivatized lasiodiplodan.

3.5 X-Ray Diffraction

Fig. 5 shows the X-ray diffraction profile of LAS-N and LAS-AC samples. Three diffraction peaks at 2θ with values near to 20.9°, 23.4° and 39.4° were identified in all diffractograms (*O*-acetylated and non-acetylated samples). Such peaks indicate that lasiodiplodan has crystalline regions in an amorphous matrix, which can be seen from the high intensity of the peaks. Anusuya and Sathiyabama [30] described the X-ray diffraction pattern of β-glucan nanoparticles presenting three peaks (23.2°, 32.5°, 46° at 2θ), which were attributed to its crystalline nature.

Peaks observed at 20.9° (2θ) and 23.4° (2θ) were more pronounced and intense in the LAS-AC samples, probably because in those regions of the macromolecule there are new functional groups (*O*-acetyl groups) positioned. The introduction of *O*-acetyl groups may have induced an increase in the degree of crystallinity, although the profile pattern of the X-ray diffraction of native molecule was maintained.

3.6 Scanning Electron Microscopy

The SEM micrographs of LAS-N and LAS-AC are shown in Fig. 6. LAS-N presented morphological structure in the form of thin films with folds along their lengths and translucent appearance. Granular structures along the film surfaces were not verified as previously observed in micrographs of LAS-N produced in a stirred tank bioreactor [28]. Possibly, the different conditions of fungal cultivation (shake-flask vs. STR fermenter) may have influenced the morphologic structure of the biopolymer produced.

The degree of *O*-acetylation apparently influenced the morphological aspect of the biopolymer. Morphological structure in the form of films remained in the acetylated derivatives regardless of the degree of substitution, but with differences in thickness and

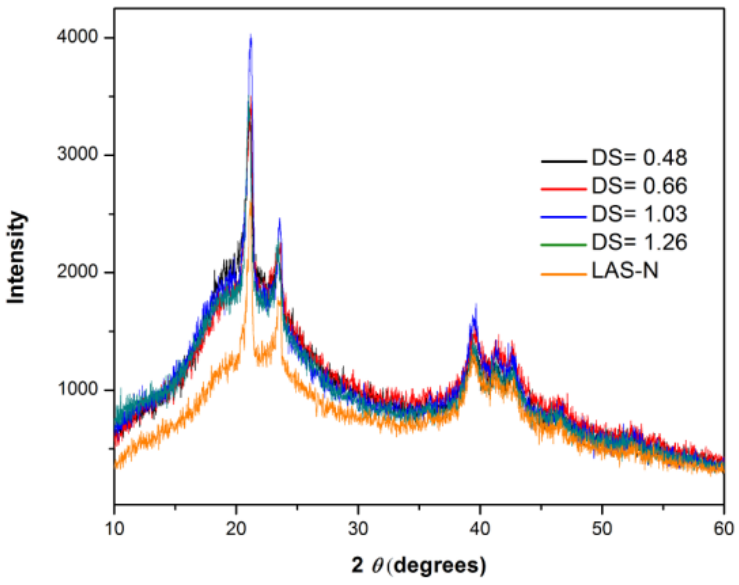

Fig. 5 X-ray diffractogram of LAS-N and LAS-AC.

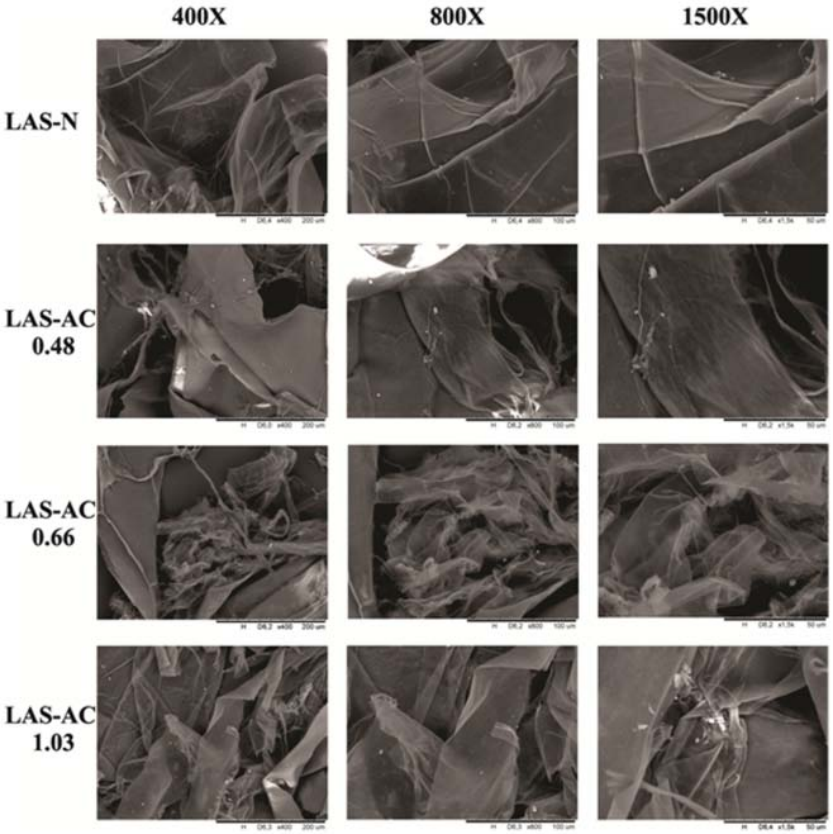

Fig. 6 Micrographs (SEM) of LAS-N and LAS-AC with different degree of substitution (1.26, 1.03 and 0.48) at 400×, 800× and 1,500× magnification.

translucency. Such differences probably are due to changes in the chemical structure of the macromolecule through derivatization.

Films with lower translucency and apparently thicker were observed in the micrographs of the LAS-AC with DS of 0.48. Films with enhanced translucency, apparently thinner and agglomerated were observed in samples with DS 0.66 compared with the sample of DS 0.48. Samples with DS 1.03 presented sections with disruption of the film along its extension, which was not observed in the other micrographs.

Hu et al. [22] reported that the nanofiber structure of cellulose was modified upon *O*-acetylation. They found the surfaces of non-acetylated fibers were rather smooth and clean, while the acetylated fibers were rougher. Souza et al. [11] analyzed the ultrastructure of native and *O*-acetylated β-glucan from oat, showing that derivatization by acetylation led to a decrease in porosity, compaction and smoothness in the surface of the *O*-acetylated sample compared to the unmodified native β-glucan.

3.7 Antioxidant Capacity

Some reports in the scientific literature on antioxidant activity of polysaccharides indicate that these biomacromolecules exhibit notorious and promising results especially when in the native state, they contain along the primary (backbone) chain other molecules such as peptides, proteins, lipids, pigments, flavones and phenolic compounds, which could be partially responsible by this activity. Whereas, the same polysaccharides in the pure state (not derivatized) showed moderate antioxidant activity [31].

Chemical derivatization can be an alternative tool to potentiate antioxidant activity of polysaccharides. The introduction of *O*-acetyl group increases the electronic cloud along the polymer structure, making the

hydroxyls more “active”, and as a consequence the potential for hydrogen donation is increased. The introduction of the *O*-acetyl groups into the main chain leads to weaker dissociation energy of the hydrogen bond. Therefore, the hydrogen donating ability of polysaccharide derivatives could be increased [31].

3.7.1 Reducing Power

The reducing power assay is based on measuring the ability of electron donation from sample to potassium ferricyanide ($K_3Fe(CN)_6$), which is converted to potassium ferrocyanide ($K_4Fe(CN)_6$), generating Perl’s Prussian blue color that can be measured spectrophotometrically [32].

The reducing capacity of all the *O*-acetylated derivatives was higher than native lasiodiplodan (Fig. 7A). The chemical derivatization of macromolecules leads to increasing reducing power that appears to be associated with the degree of substitution. LAS-AC derivatives with DS of 1.26, 0.66 and 0.48 showed reducing abilities of 46.73%, 71.35% and 118.59%, respectively, which were higher than LAS-N. Interestingly, the *O*-acetylated derivative with the lower degree of substitution (0.48) demonstrated the best reducing activity. The derivatization experimental protocol contributed to improving ferric ion reducing ability as measured by reducing power method. However, the reducing ability of the *O*-acetylated derivatives was lower than that of the standard antioxidant ascorbic acid at the same concentration (150 $mg \cdot L^{-1}$).

A similar behavior was described by Song et al. [10], who found lower reducing activity in polysaccharides extracted from pumpkin (*Curcubita pepo*, lady godiva) and chemically derivatized by acetylation when compared to ascorbic acid. They also reported that reducing activity improved after derivatization and there was a correlation between degree of substitution and antioxidant activity.

3.7.2 Hydrogen Peroxide Scavenging

LAS-N samples in the presence of H_2O_2 showed scavenging activity of 6%, while this could be increased by the LAS-AC derivatives from 18.0% (DS 1.03) to 36.0% (DS 0.48) as the DS decreased. Glucose, which is a monomeric unit of lasiodiplodan, presented scavenging activity of 44.0%, while for ascorbic acid (standard antioxidant for comparison purposes) it was 86% (Fig. 7B). Higher hydrogen peroxide scavenging capacity was verified in *O*-acetylated derivative with the lowest DS (0.48), and similarly to what was observed in the reducing power assay, the degree of substitution appears to influence antioxidant capacity of the macromolecule.

Antioxidant activity of scavenging H_2O_2 has also been assessed in other fungal β-glucans. For example, Giese et al. [15] evaluated H_2O_2 scavenging activity of botryosphaeran and algal laminarin at different concentrations (500 to 3,000 $mg \cdot L^{-1}$), and reported that botryosphaeran was more effective with a scavenging activity of 38% as opposed to 19% for laminarin; antioxidant activity observed for ascorbic acid was 60%.

3.7.3 Hydroxyl Radical Scavenging

The assay performed to determinate scavenging activity of HO• radical is based on the well-known Fenton reaction. This reaction occurs when hydrogen peroxide is in an acid environment with transition metals as catalyst, generally Fe^{2+} ions, producing hydroxyl radicals [33, 34]. Fig. 7C shows the results of hydroxyl radical scavenging of LAS-N, LAS-AC, glucose and ascorbic acid.

LAS-N presented hydroxyl radical scavenging activity of 27.38%, while the *O*-acetylated lasiodiplodan samples demonstrated different scavenging activities: 26.19% (DS 1.26), 23.02% (DS 1.03), 24.21% (DS 0.66) and 45.63% (DS 0.48). Additionally, glucose presented a scavenging activity of 13.89%; data were normalized with ascorbic acid set as 100%. LAS-AC sample with a DS value of 0.48 resulted in the highest HO• scavenging activity (66%) with respect to the native polysaccharide, again indicating that DS can influence the antioxidant potential of the macromolecule. Taken together, the

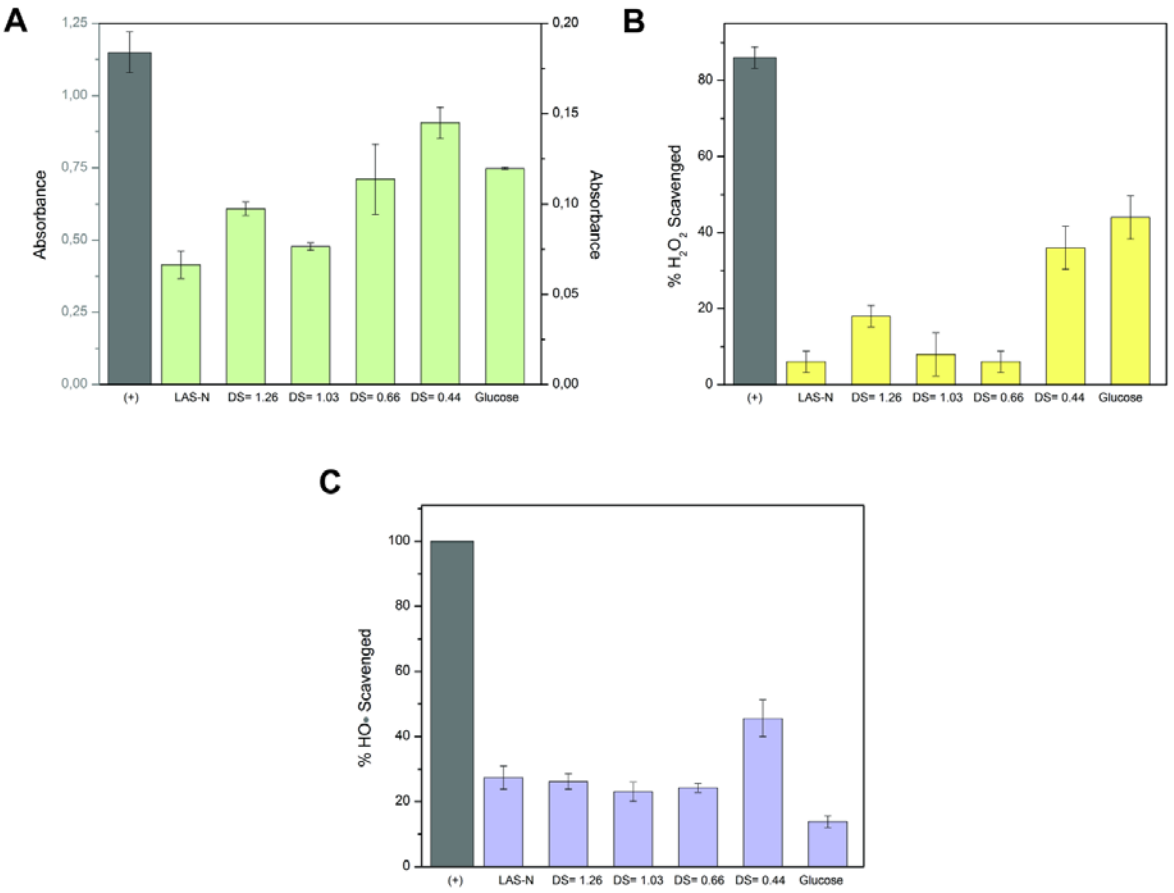

Fig. 7 Antioxidant activity of LAS-N, LAS-AC, glucose and ascorbic acid (+ control) measured by (A) reducing power, (B) H_2O_2 scavenging, and (C) HO• scavenging.

reducing power, and the H_2O_2 and HO• scavenging methods have demonstrated that the derivatized samples with the lowest DS value (0.48) of those examined, presented higher antioxidant activities than the un-derivatized and acetylated lasiodiplodans of DS > 0.48.

Maity et al. [35] evaluated antioxidant potential of fungal β-glucan extracted from *Entoloma lividoalbum* by the HO• scavenging ability. The assays were conducted in a concentration range of 100 to 800 $mg{\cdot}L^{-1}$ using BHT (butylated hydroxytoluene) as the standard, and antioxidant activity were found to be dose dependent. They suggested that the mechanism of antioxidant is attributed to the hydrogen supply by polysaccharide, which combines with radicals to form stable structure to terminate the chain reaction.

4. Conclusions

The derivatization protocol allowed the *O*-acetylation of lasiodiplodan with DS of 1.26, 1.03, 0.66 and 0.48 according to the concentration of the derivatizing agent. FT-IR spectral analysis confirmed that lasiodiplodan was acetylated as revealed by specific bands of carbonyl groups as well as increased C-O vibration band. High thermal stability was evidenced in both native and acetylated lasiodiplodan, and the X-ray diffraction indicated that LAS-N and LAS-AC have typical structures of amorphous compounds containing some crystalline regions. Acetylation leads to morphological changes in the biopolymer structure that resulted in different aspects of thickness and translucency according to the degree of substitution. Antioxidant capacity of the native

polysaccharide was enhanced after derivatization and seems to have a correlation between DS and antioxidant activity. The acetylated derivative (LAS-AC) with DS of 0.48 presented greater hydroxyl radical and hydrogen peroxide scavenging activities, and higher reducing power compared to the native polysaccharide and other acetylated LAS samples. The results of this work suggest that acetylation of lasiodiplodan could contribute to enhance biological functionalities and make this biopolymer attractive for biomedical and industrial applications. It is important, however, to emphasize that it is still necessary to conduct studies related to cost evaluation and the scale-up of the process.

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