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Kinetics of Enzyme-Catalyzed Cross-Linking of Feruloylated Arabinan from Sugar Beet

Dayang Norulfairuz Abang Zaidel,†§ Anis Arnous,§ Jesper Holck,§ and Anne S. Meyer†§

†Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 Skudai Johor, Malaysia
§Center for Bioprocess Engineering, Department of Chemical and Biochemical Engineering, The Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

ABSTRACT: Ferulic acid (FA) groups esterified to the arabinan side chains of pectic polysaccharides can be oxidatively cross-linked in vitro by horseradish peroxidase (HRP) catalysis in the presence of hydrogen peroxide (H₂O₂) to form ferulic acid dehydrodimers (diFAs). The present work investigated whether the kinetics of HRP catalyzed cross-linking of FA esterified to α-(1,5)-linked arabinans are affected by the length of the arabinan chains carrying the feruloyl substitutions. The kinetics of the HRP-catalyzed cross-linking of four sets of arabinan samples from sugar beet pulp, having different molecular weights and hence different degrees of polymerization, were monitored by the disappearance of FA absorbance at 316 nm. MALDI-TOF/TOF-MS analysis confirmed that the sugar beet arabinans were feruloylated, and HPLC analysis verified that the amounts of diFAs increased when FA levels decreased as a result of the enzymatic oxidation treatment with HRP and H₂O₂. At equimolar levels of FA (0.0025—0.05 mM) in the arabinan samples, the initial rates of the HRP-catalyzed cross-linking of the longer chain arabinans were slower than those of the shorter chain arabinans. The lower initial rates may be the result of the slower movement of larger molecules coupled with steric phenomena, making the required initial reaction of two FAs on longer chain arabinans slower than on shorter arabinans.

KEYWORDS: arabinan, dehydrodimers, ferulic acid, horseradish peroxidase kinetics

INTRODUCTION

Pectic polysaccharides extracted from sugar beet pulp mainly consist of homogalacturonan and rhamnogalacturon I (RGI), the latter notably being arabinan- and arabinogalactan-substituted RGI.1 As in other dicot plant cell walls the backbone of sugar beet RGI is made up of repeating units of (→2)-α-L-Rhap—(→4)-α-d-GalpA—(→1).2 The rhamnose moieties of the RGI in sugar beet pectin may be substituted at the O-4 position with α-(1,5)-linked arabinans having a high degree of α-(1,2)- and α-(1,3)-arabinofuranosyl (Araf) substitutions and with minor amounts of β-(1,4)-linked galactan.5,6 Some of the Araf moieties in the α-(1,5)-linked arabinans are substituted with feruloyl groups, as single ferulic acid (FA) moieties or in the form of ferulic acid dehydrodimers (diFAs) that may even cross-link two different arabinan chains.4,5 The FA moieties are mainly esterified to the O-2 position of the Araf residues in the α-(1,5)-linked arabinan backbone, but may also be bound to the O-5 on the terminal arabinose, or, to a much lesser extent, at the O-6 position of the galactopyranosyl (Galp) residues in the β-(1,4)-galactan chains.6

The FA bound to the side chains of pectic polysaccharides have long been known to have the ability to be oxidatively cross-linked via enzyme catalysis by oxidoreductases, that is, peroxidase or laccase activities.7,8 Previous work has shown that addition of, for example, peroxidase, together with H₂O₂ or laccase, results in a reduction of the amount of FA esterified to the arabinan chains while the levels of diFAs increase simultaneously.8,9 The enzyme-catalyzed cross-linking of two feruloylated arabinan oligosaccharides takes place via radical coupling of the FA resulting in the formation of different diFAs: 5,5’, 8-O-4’, 8,5’, and 8,8’.10 The formation of diFA cross-linking between feruloylated arabinans or feruloylated arabinino-oligosaccharides may result in gel formation.10,11 Although studies on the oxidative cross-linking of feruloyl substitutions in polysaccharides have been amply reported,7,8,9 there are no reports on the effect of the length of the backbone of the arabinans on the rate of the cross-linking of feruloylated arabinan. Currently, the arabinan fraction from sugar beet pulp is a byproduct, but the utilization of the arabinan as a food additive could help maintain the competitiveness of the sugar beet based industry. Thus, the provision of knowledge regarding the cross-linking of feruloylated arabinan would, for example, be important for the rational design and control of enzymatic processes for upgrading sugar beet pulp arabinans for use as viscosity-regulating or gelling agents in foods and beverages.

We hypothesized that the rate of cross-linking of feruloylated arabinans obtained from sugar beet pulp might be affected by the backbone length of the arabinan to which the FA moieties are attached. Hypothetically, the rate of cross-linking between FA on longer arabinan chains is expected to be slower than the rate of cross-linking between FA on shorter arabinan chains mainly because of the relatively slower movement (diffusion) of larger molecules. However, once some initial diFAs formation has taken place with the longer chains, hence cross-linking two arabinan chains, the rates of diFA formation of long and short arabinan chains may approach each other because the diFAs on the longer chains will be in closer proximity, resulting in a sort of...
“zipping” of the FA esterified to the arabinan chains (Figure 1). The purpose of this study was to test the hypothesis that the kinetics of the cross-linking of feruloylated arabinans is affected by the chain length of the arabinan.

Figure 1. (A) Schematic representative structures of feruloylated arabinans from sugar beet pectin illustrating the one-step enzyme-catalyzed oxidative cross-linking model of short-chain arabinans. (B) Two-step enzyme-catalyzed oxidative cross-linking or “zipping” model for long-chain feruloylated arabinans.
Materials and Methods

Enzyme and Chemicals. Horse radish peroxidase (HRP) (EC 1.11.1.7) type VI-A, 1000 U mg⁻¹, ferulic acid (4-hydroxy-3-methoxy cinnamic acid) 99%, isopropanol, sodium hydroxide (NaOH) 50% (w/w), ethyl acetate, acetonitrile, hydrogen peroxide (H₂O₂) 50% (w/w), sodium dihydrogen phosphate monohydrate (NaH₂PO₄·H₂O), trifluoroacetic acid (TFA) 98% (w/w), pullulan standard with molecular weight of 1.3 kDa, and monosaccharide standards including D-galactose, D-arabinose, D-xylose, L-rhamnose monohydrate, and p-galacturonic acid monohydrate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). D-Glucosamine hydrochloride, D-xylose, anhydrous sodium sulfate (Na₂SO₄), and disodium hydrogen phosphate (Na₂HPO₄) were from Merck (Darmstadt, Germany). trans-Cinnamic acid 99% was from Alfa Aesar GmbH & Co. (Karlsruhe, Germany). Dextran standards with average molecular weights of 10 and 40 kDa, respectively, were from Pharmacia (Uppsala, Sweden). Arabinino-oligosaccharide standards from DP2 to DP5 were purchased from Megazyme (Bray, Wicklow, Ireland).

Arabian Raw Material. Arabian samples were supplied by Danisco A/S (Næskov, Denmark). The two batches of samples, Ara1 and Ara2, respectively, were two different permeate streams obtained from the preparation of pectin from sugar beet pulp via nitric acid hydrolysis involving removal of insoluble cellulose, ultrafiltration, and diafiltration with a 50 kDa cutoff.

Monosaccharide Composition of the Raw Material. The monosaccharide compositions of these starting materials were determined by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on a Dionex BioLC system (Dionex Corp., Sunnyvale, CA), after acid hydrolysis (4 g L⁻¹ substrate, 2 M TFA, 2 h, 121 °C) as described previously.

Oligosaccharide Profiles of the Raw Materials. Separation and quantification of arabinino-oligosaccharides were done by HPAEC-PAD using a Dionex BioLC system equipped with a CarboPac PA1 analytical column (4 × 250 mm) controlled via Chromelon 6.80 Sp8 Build 2623 software (Dionex Corp.) as described in ref 13. Hydrophilic Interaction Chromatography (HILIC). Lyophilized (feruloylated) arabain samples were suspended in deionized water followed by cleanup using 70% (v/v) final concentration of acetonitrile. The acetonitrile cleanup step was included to eliminate the noise from the presence of peptides and polar compounds prior to chromatographic separation of the arabain samples. Acetonitrile (70% (v/v)) treated samples were immediately centrifuged for 3 min at 16000g, and each supernatant was then injected (10 µL) into the HILIC system. The HILIC separation of the feruloylated arabains according to hydrophilic interaction was performed using an ICS-3000 system coupled to a photodiode array detector (PDA-100) (Dionex Corp.) and equipped with a TSKgel Amide-80 column (250 × 4.6 mm i.d., 5 µm, Tosoh Biosciences) at 55 °C. The eluent system consisted of three components (A, acetonitrile; B, deionized water containing 1% (v/v) TFA; C, deionized water) that were delivered in a gradient at a flow rate of 1 mL min⁻¹. The gradient was run from 75% (v/v) of A and 25% (v/v) of B to 25% (v/v) of A and 75% (v/v) of B for 35 min and then at 100% of C for 2 min. The gradient was then returned to 75% (v/v) of A and 25% (v/v) of B for the final 2 min for reconditioning. Feruloylated compounds were detected and quantified by UV absorption at 316 nm using ferulic acid as external standard.

MALDI-TOF/TOF Analysis. Mass spectrometric analyses of the arabain samples were performed on a 4800 Plus MALDI TOF/TOF (AB SCIEX) mass spectrometer. The sample (0.5 µL) was deposited onto a stainless steel MALDI target with 0.5 µL of the matrix (α-cyano-4-hydroxycinnamic acid from Sigma-Aldrich (St. Louis, MO)) and dried. The matrix was prepared at a concentration of 10 mg ml⁻¹ in a 70% ACN/0.1% TFA/water (v/v) solution. The instrument was operated in positive ion reflector mode, and the m/z range from 100 to 3500 was monitored. A total of 1000 laser shots were applied to each sample in MS mode. In MS/MS mode 2000 shots were obtained. The laser intensity was manually varied to obtain the best possible spectra (MS, from 4500 to 4800; MS/MS, from 4800 to 5100). MALDI-TOF-MS and MS/MS data were exported as text files using DataExplorer (version 4.0). Each spectrum were smoothed, labeled, and analyzed manually in M/Z (Genomic Solutions).

Cross-Linking Kinetics. Stock solutions of 10 mg L⁻¹ HRP and 85 µM H₂O₂, respectively, were prepared in 0.1 M sodium phosphate buffer, pH 6 (both were made fresh every day). Reaction mixtures for the cross-linking were prepared by adding H₂O₂ (14 µM) and HRP (0.2 mg L⁻¹ = 0.2 U mL⁻¹) to different defined FA concentrations (0.0025—0.05 mM) of the feruloylated arabain samples. After preliminary
Table 1. Total Ferulic Acid and Diferulates Content in Arabinan Samples before and after Cross-Linking Using HRP and H₂O₂ Analyzed Using RP-HPLC

<table>
<thead>
<tr>
<th>sample</th>
<th>FA (mg g⁻¹ dry matter) before cross-linking</th>
<th>after cross-linking</th>
<th>total diFAs (mg g⁻¹ dry matter) before cross-linking</th>
<th>after cross-linking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara1</td>
<td>7.0 ± 0.5</td>
<td>2.4 ± 0.2</td>
<td>2.4 ± 0.6</td>
<td>5.1 ± 1.3</td>
</tr>
<tr>
<td>Ara2</td>
<td>2.5 ± 0.6</td>
<td>1.4 ± 0.3</td>
<td>3.2 ± 0.03</td>
<td>2.82 ± 0.04</td>
</tr>
<tr>
<td>Ara1b</td>
<td>2.4 ± 0.5</td>
<td>0.29 ± 0.06</td>
<td>0.44 ± 0.04</td>
<td>1.01 ± 0.08</td>
</tr>
<tr>
<td>Ara2b</td>
<td>1.1 ± 0.2</td>
<td>0.1 ± 0.01</td>
<td>0.6 ± 0.3</td>
<td>0.30 ± 0.02</td>
</tr>
</tbody>
</table>

Results are shown as average data ± SD of duplicates. Total diFAs = 5,5-diFA + 8,5-diFA benzofuran + 8-O-4'-diFA.

Figure 2. Arabino-oligosaccharide profiles of the feruloylated sugar beet pulp arabian samples Ara1 and Ara2 as analyzed by HPAEC. The numbers over the peaks represent α-(1,5)-arabinose to α-(1,5)-arabinopentaose.

Figure 3. HILIC gradient chromatogram illustrating the presence of trans-feruloyl groups attached to sugar beet pulp derived arabinan oligosaccharides of different lengths eluting in the order of chain length, shorter first. The FA was detected at 316 nm, but the peak of the spectra reached the maximum at 326–328 nm.

RESULTS AND DISCUSSION

Composition of Sugar Beet Arabinan Substrates. The monosaccharide profiles of the starting materials, Ara1 and Ara2, confirmed that the substrates could be classified as arabinans, and Ara1 has also been characterized previously. Their compositions (in mmol g⁻¹ dry matter) were as follows: for Ara1, arabinose, 3.4 (equivalent to 88 mol %); glucose, 0.27 (equivalent to 7 mol %), with traces of <1 mol % of rhamnose, galactose, and galacturonic acid; for Ara2, arabinose, 2.9

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Arabino-oligomer Profile of the Arabinan Substrates.

Analysis by HPAEC indicated that Ara1 and Ara2 mainly consisted of short DP arabino-oligomers, including mainly monomers (notably arabinose) and α-(1,5)-arabino-oligosaccharides up to DP5 (arabinopentaose), with the Ara2 being relatively richer in all of the detected short oligomers, including the monosaccharide peak, when evaluated at the same dry matter concentration (Figure 2). The peaks of Ara1b and Ara2b eluted similarly to Ara1 and Ara2 but at much lower levels (approximately 7–15% of Ara1 and Ara2) when analyzed at 1 g L\(^{-1}\) for DP2–DP4, but the large MW molecules escaped this analysis (data not shown). The HPAEC spectra also revealed triple peaks, of which the latest eluted peak in each set was

Figure 2.

Figure 4. Mass spectra from MALDI-TOF analysis of (A) Ara1 and (B) Ara2.

Figure 5. MS/MS spectra for arabinan oligosaccharides for ions at m/z (A) 309, (B) 441, (C) 573, and (D) 705 of Ara1.
Different glycosyl-linkage positions of the arabino-(arabinose).

Figure 6. HPSEC chromatogram for Ara1, Ara2, Ara1b, and Ara2b. Standards (○), from left to right: 40 kDa, 10 kDa, 1.3 kDa, and 150 Da (arabinose).

Table 2. Ferulic Acid (FA) Content per Amount of Dry Matter (DM) Arabinan in the Samples Used for Cross-Linking of (Feruloylated) Arabinan Samples with HRP and H2O2 Treatmenta

<table>
<thead>
<tr>
<th>sample</th>
<th>FA (mM)b</th>
<th>dry matter (g L−1)</th>
<th>FAa (μM)</th>
<th>FA/DM (mg g−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara1</td>
<td>0.010</td>
<td>0.19</td>
<td>9.87 ± 0.01</td>
<td>9.87 ± 0.01</td>
</tr>
<tr>
<td>Ara2</td>
<td>0.0025</td>
<td>0.08</td>
<td>5.81 ± 0.02</td>
<td>15.1 ± 0.1</td>
</tr>
<tr>
<td>Ara1b</td>
<td>0.010</td>
<td>0.20</td>
<td>14.0 ± 0.3</td>
<td>13.6 ± 0.3</td>
</tr>
<tr>
<td>Ara2b</td>
<td>0.0025</td>
<td>0.04</td>
<td>0.27 ± 0.03</td>
<td>12.7 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>0.06</td>
<td>2.11 ± 0.03</td>
<td>7.20 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>0.010</td>
<td>0.16</td>
<td>11.2 ± 0.7</td>
<td>13.4 ± 0.9</td>
</tr>
</tbody>
</table>

a Results are shown as mean ± SD for samples analyzed as duplicates.
b Initial FA value. c FA measured by absorbance measurement at 316 nm.

determination of molecular weight. HPSEC analysis showed that Ara1 and Ara2 gave dual peaks with one peak at ~24.5 min corresponding to monomers and an earlier peak at ~23.5 min (Figure 6). These peaks were shaped differently for Ara1 and Ara2, corresponding to molecular weights somewhat higher than 1.3 kDa for Ara1 and ~1.3 kDa for Ara2 (Figure 6). The SEC profile of Ara1b showed that this sample contained
larger molecular weight polysaccharides eluting broadly from 22 to 24 min with a shoulder on the peak with a maximum at ~23.5 min indicating molecular weights around and above 1.3 kDa. The Ara2b contained relatively more of the larger molecular weight polysaccharides than Ara1b with a broad peak eluting mainly around 22 min (Figure 6). Hence, the SEC profile confirmed that larger molecules, that is, longer chain arabinans, were extracted from the samples by the isopropanol precipitation and also revealed that Ara1 and Ara2 contained slightly differently sized arabinan and arabino-oligosaccharides around 1.3 kDa and above.

Cross-Linking Kinetics of Feruloylated Arabinan. To ensure that the rate of cross-linking was not affected by the different levels of FA in the different arabinan samples, the cross-linking experiments were compared at equimolar FA levels (0.0025–0.050 mM), but with slightly different levels in the different samples designed to achieve significant differences in the reaction rates (Table 2). The enzyme-catalyzed oxidative cross-linking experiments were done at 25°C and pH 6 with 14 μM H₂O₂ and 0.2 mg L⁻¹ HRP (equivalent to 0.2 U mL⁻¹) as defined from preliminary experiments as being the optimal conditions of reaction for monitoring the rate of the HRP-catalyzed reaction (Table 3). At this concentration the changes in absorbance took place at moderate rates, allowing for precise absorbance measurement after the addition of the HRP into the reaction mixture. The level of H₂O₂ was sufficient for the cross-linking as a higher concentration of H₂O₂ did not increase the rate (data not shown). Rather, a higher concentration of H₂O₂ may lead to partial inactivation of the enzyme.20 In general, for the Ara1 and Ara2, the relative absorbance at 316 nm decreased steeply at the beginning of the reaction (from 0 to 1 min) and then decreased more gradually until it reached a pseudosteady state (Figure 7A,B). Presumably, at this point there were still some FA groups left that were unable to react to form the diFAs. The corresponding slopes of the reactions of the longer chain arabinan samples, Ara1b and Ara2b, were much lower, indicating relatively slower consumption of the FA as compared to the consumption in the shorter arabinans at the same FA concentrations (Figure 7C,D).

Rates of Cross-Linking of Different Arabinan Sizes. Due to the different initial absorbances of the different substrate concentrations,
the data for the cross-linking reactions at different substrate concentrations were plotted as a graph of normalized absorbance ($A(t)/A_0$) (Figure 7). When the reaction rates were assessed as the amount of FA consumed per minute ($\mu\text{M min}^{-1}$), it became evident that the initial reaction rates increased with increased FA substrate concentration and, moreover, that the reaction rates of Ara1 and Ara2 in general were several-fold higher than the reaction rates of samples Ara1b and Ara2b (Figure 8). Hence, at similar FA concentrations of, for example, 20 $\mu$M, the rates of cross-linking for Ara1 and Ara2 were 5.6 and 4.1 $\mu$M min$^{-1}$, respectively, and thus statistically significantly higher ($P < 0.05$) than the rates of cross-linking for the longer chain arabinans, which were approximately 5 times slower at 0.94 and 0.78 $\mu$M min$^{-1}$ for Ara1b and Ara2b, respectively (Figure 8). Due to the fast reaction rates for Ara1 and Ara2, rates were calculated from the slope of the decreasing absorbance for the first 10 s, whereas for Ara1b and Ara2b the rates were calculated for the first minute of the cross-linking reaction due to absorbance fluctuation in the first few minutes of the longer chain cross-linking reactions (Figure 7C,D). At the relatively low FA levels, from 2.5 to 10 $\mu$M, the reaction rates of Ara1 and Ara2 were similar and ranged from ~1.5 to 3.0 $\mu$M min$^{-1}$. The reaction rates of the Ara1b and Ara2b substrates were also similar, even from 2.5 to 20 $\mu$M, and ranged from 0 to 0.4 $\mu$M min$^{-1}$ (Figure 8). At higher substrate concentration, the reaction rates of the pairs of Ara1 and Ara1b were significantly higher ($P < 0.05$) than those of Ara2 and Ara2b. Apart from Ara1b having a higher content of the relatively shorter arabinans, of molecular weight 1.3 kDa, than the Ara2b sample (Figure 6), it was not possible to relate the differences in the rates directly to the subtle differences in the molecular weight profiles among the pairs of shorter arabinans Ara1 and Ara2 and among the longer chain arabinan samples Ara1b and Ara2b, respectively. The data did indicate, however, that the “zipping” between FAs in the arabinan to form diFAs was very slow and increased less with increased FA concentration in the longer chain arabinans than in the shorter, probably due to the limited access of FA for the cross-linking to occur. It appeared to be more difficult to initiate the reaction between the FA on the longer chains than on the shorter arabinan chains, corroborating the interpretation that longer chains react more slowly as illustrated in Figure 1. Hence, the overall result that the cross-linking rates of the longer chain (feruloylated) arabinans were much slower than the corresponding rates of the shorter (feruloylated) arabinans when reacted at the same FA concentration supported the hypothesis that the rate of cross-linking is affected by the extent of the backbone length of the arabinan.

**Formation of Ferulic Acid Dehydrodimers.** Enzymatic cross-linking of FA was an oxidative reaction that produced feruloyl dehydrodimers (5,5$^0$,8-4$^0$, and 8,5$^0$ benzofuran-diFA). Disappearance of FA and formation of diFAs from the cross-linking reaction was further confirmed by HPLC analysis (Table 1 and Figure 9), which is in complete agreement with previous studies. Of the diFAs identified, the 8,5$^0$-diFA and 8-O-4$'$-diFA forms predominated, with traces of 5,5$^0$-diFA detected (Figure 9). A minimal peak was observed at the retention time equivalent to 8,8$'$-diFA, but the level was too low to be quantified. It has been reported that 8,8$'$-diFA is one of the most favorable forms resulting from the oxidative cross-linking.
of FA with different peroxidases and laccases in arabinoxylan, \(^{21}\) whereas in pectic polysaccharides, 8-O-4' and 8,5'-diFA appear to dominate.\(^ {9}\) As suggested in the previous study, \(^ {21}\) one of the possibilities that the 8,8'-diFA form was not detected in the analysis of the cross-linked arabinan might be due to its transient properties and involvement in further polymerization to form more complex dehydropolymers. However, it has previously been reported that 8,8'-diFA can be found in pectic polysaccharides with a small decrease after the addition of HRP and \(\text{H}_2\text{O}_2.\)^{9}

Among the different types of substrate samples, the amount of 8,5-diFA increased significantly in Ara1 and with a lower relative increase in the 5,5'-diFA form by cross-linking (35%) and no change in the 8-O-4'-diFA (Figure 9A). A similar pattern was evident for Ara2 and Ara1b, whereas Ara2b deviated somewhat from this trend as the levels of all three types of diFAs in fact decreased after cross-linking (Figure 9D). We interpret the decrease in diFAs in Ara2b as being due to the oxidation by excess \(\text{H}_2\text{O}_2\) because the \(\text{H}_2\text{O}_2:\text{FA}\) level was relatively higher for Ara2b due to the low FA levels in this sample (Figure 9D). Reaction with a further excess amount of \(\text{H}_2\text{O}_2\) caused a significantly decreased level of FA but no increase in diFAs (data not shown). This is because a higher ratio of \(\text{H}_2\text{O}_2:\text{FA}\) in the reaction caused a higher extent of oxidation of FA and diFAs and, hence, reduced the amount of diFAs as well. A previous study also reported a decrease in oligomerization with higher ratios of \(\text{H}_2\text{O}_2:\text{FA}\), possibly be-

diFAs as well. A previous study also reported a decrease in polymerization; \(\text{FA},\) ferulic acid groups; \(\text{HRP},\) horseradish peroxidase; \(\text{H}_2\text{O}_2,\) hydrogen peroxide; \(\text{NaOH},\) sodium hydroxide; \(\text{Na}_2\text{HPO}_4,\) disodium hydrogen phosphate; \(\text{Na}_2\text{SO}_4,\) anhydrous sodium sulfate.

## ACKNOWLEDGMENT

We acknowledge Danisco A/S for supplying the materials for this study and Southern Danish University for performing the MALDI-TOF/TOF analysis.

## ABBREVIATIONS USED

\(\text{ARA},\) arabinan; \(\text{diFAs},\) ferulic acid dehydrodimers; \(\text{DP},\) degree of polymerization; \(\text{FA},\) ferulic acid groups; \(\text{HRP},\) horseradish peroxidase; \(\text{H}_2\text{O}_2,\) hydrogen peroxide; \(\text{NaOH},\) sodium hydroxide; \(\text{Na}_2\text{HPO}_4,\) disodium hydrogen phosphate; \(\text{Na}_2\text{SO}_4,\) anhydrous sodium sulfate.

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**AUTHOR INFORMATION**

**Corresponding Author**

*Phone: +45 4525 2800. E-mail: am@kt.dtu.dk.*

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