Carbohydrate Polymers 88 (2012) 657–662

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

New pretreatment of wheat straw and bran in hexadecanol for the combined production of emulsifying base, glucose and lignin material

Aurélie Seguin, Sinisa Marinkovic, Boris Estrine*

Agro-Industrie Recherches et Développement "Green Chemistry Department", F-51110 Pomacle, France

ARTICLE INFO

Article history:
Received 8 July 2011
Received in revised form 4 January 2012
Accepted 5 January 2012
Available online 13 January 2012

Keywords:
Wheat straw
Wheat bran
Biomass
Glycosylation
Surfactants
Lignin
Hexadecyl pentosides

ABSTRACT

Wheat straw and bran are abundant residues of agriculture which are increasingly being considered as a feedstock for the production of fuels, energy and chemicals. The acidic hexadecanol based pretreatment of wheat straw and bran was investigated in this work. This pretreatment allowed the conversion in one step of hemicelluloses of wheat bran and straw into hexadecyl pentosides with yields up to 95%. Wheat-based surfactants thus obtained exhibited superior emulsifying properties compared to fossil-based polyethoxylated fatty alcohols that are largely used surfactants in cosmetics and pharmaceutics industries. The non-converted materials collected after the pre-treatment were characterized showing that cellulose, starch and lignin remain available for further processing. Enzymatic digestibility of the remaining cellulose contained in the hexadecanol pretreated straw was evaluated showing a glucose production of up to 63% compared to the 13% obtained with a non pretreated wheat straw. This result is of potential interest for ethanol fermentation. A lignin material was produced from the same hexadecanol pretreated straw residue. This material displayed a higher molecular weight than a lignin material obtained following an organosolv process, confirming its potential use in the field of thermosetting resin. In view of the growing importance of renewable resource-based molecules in the chemical industry, this approach may open a new avenue for the conversion of wheat straw and bran into various chemicals.

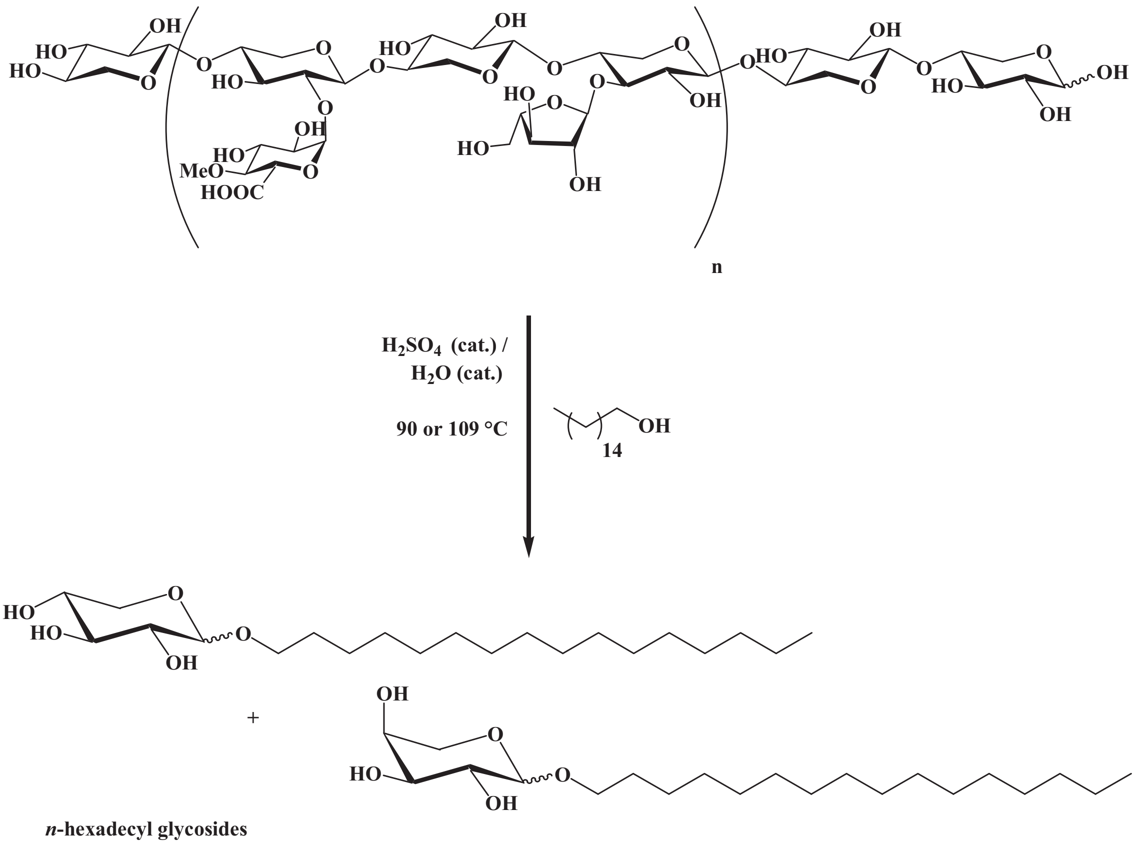
1. Introduction

Hemicelluloses, the second most abundant type of polysaccharides from biomass next to cellulose, are currently regarded as a promising alternative feedstock for chemicals. Their use as a raw material in the chemical industry, unlike starch, would not induce negative impact on food supplies (Klass, 1998). Wheat straw and bran represent a valuable source of hemicelluloses, cellulose and lignin; they are available co-products of wheat crop with strong potential tonnages. In the European Union, 1–5 million tons of wheat straw could be collected per year, and probably more than 100 million tons of bran are available (Martel, Estrine, Plantier-Royon, Hoffmann, & Portella, 2010). In a context where interest is constantly growing for integrated lignocellulosic biorefineries, there is a need for optimizing value of hemicelluloses (Kamm, Kamm, Schmidt, Hirth, & Schulze, 2006). On the one hand, when lignocellulosic ethanol is considered, hemicelluloses are potentially a bottleneck as the industrial fermentation of pentoses has not yet been achieved in a cost-efficient way (Ragauskas et al., 2006). On the other hand, the chemical industry is also looking for new feedstocks for their production and hemicelluloses could play a role on this field. As an example, the surfactant industry started proposing some green surfactant solutions in the last decades. However, most of them are based on food related carbohydrates such as glucose from starch and remain poorly used due to their price. A solution would be the manufacture of surfactant from pentoses extracted from agricultural wastes. These molecules have high detergent properties and a good ecological profile (Martel et al., 2010). In this area, we recently described the direct trans-glycosylation of xylan or wheat bran hemicelluloses in short tail alcohols such as butanol, octanol and decanol (Bouxin, Marinkovic, Le Bras, & Estrine, 2010; Marinkovic & Estrine, 2010). The work presented in this paper (Scheme 1) described an extended version of this pretreatment process of wheat straw and bran for producing mixtures of *n*-hexadecyl-glycosides with emulsifying properties. Indeed, the emulsifying bases thus obtained have strong volume potentials in cosmetics and pharmaceutical industry as substitutes of the million tons produced ethoxylated alcohols (von Rybinski & Hill, 1998). The method developed herein potentially contributes to broaden the use of pentose-based surfactants in cosmetics and pharmaceutical industry. It should allow a substantial cost saving as glycosylation of pentoses occurs during pretreatment of biomass and not after several fractionation steps for production of carbohydrates (Bertho, Mathaly, Dubois, & De Baynast, 1997). Furthermore,

---

* Corresponding author. Fax: +33 326 054 289.
E-mail address: b.estrine@a-r-d.fr (B. Estrine).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2012.01.018



Scheme 1. Transglycosylation of hemicelluloses into hexadecyl glycosides (glucosides and furanosides are omitted for clarity).

the wheat straw residue that remains rich in cellulose and lignin can be submitted to an enzymatic hydrolysis for glucose production and lignin materials.

2. Material and method

2.1. Raw material

The wheat straw (92% dry matter) was supplied by local farmers in Champagne Ardennes region (France). The wheat bran (90% dry matter) was supplied by Euromill (France). The polysaccharides content was determined by quantitative saccharification according to the National Renewable Energy Laboratory (NREL) analytical methods (Sluiter et al., 2007). The compositions are given in Table 1 and are mean values of three repeat trials. Following this protocol, we established a minimum accuracy of the results of 95% for the polysaccharides contents in the wheat bran and straw.

Table 1
Compositions of the wheat derived materials.

	Araban (wt.%)	Xylan (wt.%)	Glucan (wt.%)	Protein (wt.%)
Wheat straw	5.0 ± 0.3	24.0 ± 1.2	39.0 ± 2.0	3.0 ± 0.2
Wheat straw residue	0.0 ± 0.0	0.7 ± 0.0	47 ± 2.4	3.0 ± 0.2
Wheat bran	8.0 ± 0.4	13.5 ± 0.7	11.4 ± 0.6	15.3 ± 0.8
Wheat bran residue	0.2 ± 0.0	1.3 ± 0.1	5.0 ± 0.3	11.3 ± 0.6

Polysaccharides composition (araban, xylan, glucan) determined following quantitative saccharification procedure (Sluiter et al., 2007). Glucan is considering starch and cellulose for the wheat bran. Protein content is determined by Kjeldhal method. The rest of residue is non-quantified components such as extractives, ashes, lignin (see Table 2).

2.2. Pretreatment procedure

In a typical pretreatment procedure, 700 g of hexadecanol containing 0.7 wt.% of sulfuric acid, 0.7 wt.% of deionized water, and 50 g of bran or straw are loaded in a reaction vessel. The medium is warmed up to the reaction temperature (90 or 109 °C) and then maintained under stirring for 5 h. Glycosides yields are followed by gas chromatography (GC) analysis of the filtrates. At the end of the pretreatment, the reaction medium is allowed to cool down to 65 °C and the biomass residue is filtered off on a paper filter and the crude filtrate containing hexadecyl-glycosides analyzed after neutralization with 8 g of a 30.5 wt.% NaOH solution. The filtrate is then used as such for emulsions (see Section 3.4). The residual biomass material is washed with acetone, dried at 90 °C under vacuum and weighed. It is then used as such for enzymatic digestion of cellulose (see Section 3.2) or for lignin material production (see Section 3.3).

Hexadecyl arabinosides, xylosides and glucosides are known products. Standards for GC calibration were prepared by Fisher's glycosylation of D-glucose, D-xylose, and L-arabinose in hexadecanol excess and were purified using column chromatography (Bertho, Mathaly, De Baynast, & Dubois, 1999; Bouxin et al., 2010; Ferlin, Duchet, Kovensky, & Grand, 2008). The wt.% of each hexadecyl glycoside is expressed based on arabinose, xylose and glucose concentration available in the biomass raw material (see Section 2.1). The glycosides yields indicated are the mean values obtained from 2 repeat trials. Following this protocol, we established a minimum accuracy of the results of 97% (Gorius, Bertho, & Nuzillard, 2001).

2.3. Enzymatic digestibility of cellulose

Production of glucose by enzymatic hydrolysis of cellulose contained in the straw residue was carried out following literature procedure with an accuracy of 95% (Persson, Ren, Joelsson, & Jönsson, 2009).

2.4. *Lignin material production and analysis*

Lignin was prepared from the straw residue by chemical hydrolysis with concentrated sulfuric acid following literature procedure (Liu, 2004). Molecular weight analyses were carried out following literature method (Tejado, Pena, Labidi, Echeverria, & Mondragon, 2007).

2.5. *Production of emulsions*

The emulsifying properties of the filtrates compositions containing glycosides were assessed using the method described in standard NF T 73409 (surfactants–emulsions production–emulsifying power determination). Series of emulsions were produced with a growing concentration of filtrate at 70 °C by mixing vigorously the ingredients (8000 rpm) for 1 min using a mechanical agitator. Compositions of the emulsions were: 1–10 wt.% of the filtrate composition, 50 wt.% of isopropyl myristate and 40–49 wt.% of demineralized water. Emulsions were then allowed to rest for 15 h at 20 °C. All emulsions were produced in duplicate. The emulsions were considered as stable if a phase separation of less than 5 vol.% was observed relative to the total volume of the emulsion after centrifuging the emulsion at 4000 × *g* for 30 min (corresponding to a minimum volume of residual emulsion of 95%). Both emulsions of each trial needed to be stable for considering the test as positive.

The emulsifying power is the minimum filtrate concentration necessary to obtain a stable emulsion following this protocol.

3. Results and discussion

3.1. *Pretreatment of wheat straw and bran in hexadecanol*

The goal of the pretreatment study was to maximize conversion of wheat straw and bran into hexadecyl glycosides, thus adding optimal value to under-utilized carbohydrates, and to assess the impact of the other components on the pretreatment. Firstly, the reaction was carried out at 90 °C during 5 h for wheat straw (Fig. 1a) and wheat bran (Fig. 1b) and the glycosides formation monitored by GC. Although previous reports from the literature showed that these pretreatment conditions allowed the preparation of short tailed glycosides (up to decyl alkyl chain length) in high yields from xylan or wheat bran (Bouxin et al., 2010; Marinkovic & Estrine, 2010), when applied to hexadecanol, the transglycosylation of hemicelluloses provided much lower yields. Indeed, the hexadecyl pentosides yield was measured in the range of 30–40% after 5 h of reaction (Fig. 1a and b). For both raw materials, the hexadecyl arabinosides were produced quickly (1 h for wheat straw, 3 h for wheat bran). The quicker formation of arabinosides is in accordance with the literature, and can be explained by the availability of arabinose moieties along the arabinoxylosides chains (Bouxin et al., 2010; Marinkovic & Estrine, 2010). The difference of time required for reaching a maximum yield for hexadecyl arabinosides between the two raw materials can be explained by the lower concentration of arabinose in the wheat straw (see Table 2: only 5 wt.%). Hexadecyl xylosides formation occurred slowly and reached a maximum yield of 31% in the case of wheat bran (Fig. 1b). In these conditions, 6% of hexadecyl glucosides were produced starting from wheat bran and only 1.8% from wheat straw. This is probably due to the nature of glucan (starch mainly) contained in wheat bran that is more accessible than the cellulose of wheat straw during the transglycosylation reaction.

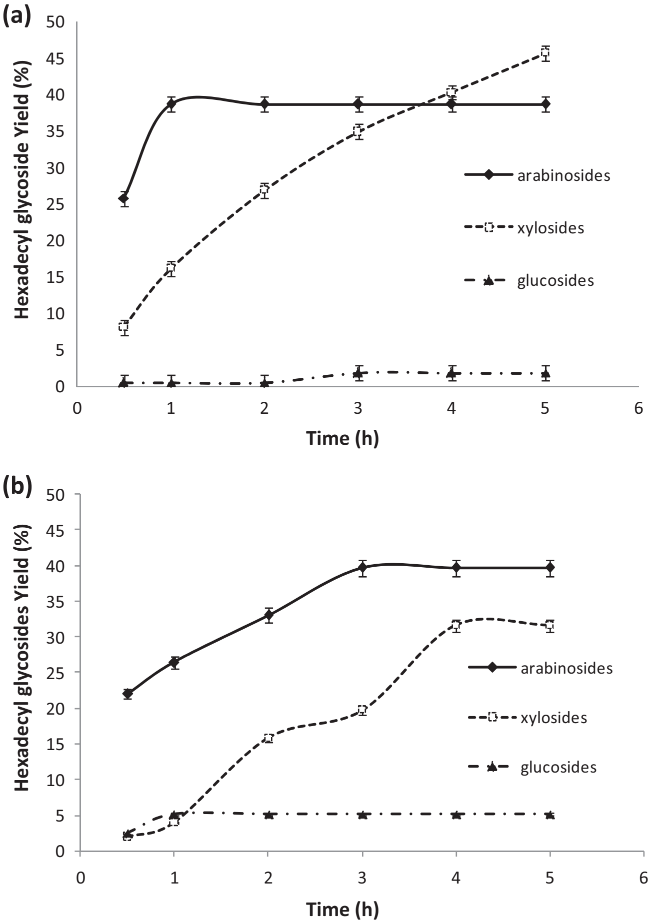


Fig. 1. Hexadecyl glycosides yields during pretreatment of wheat straw (a) and bran (b) at 90 °C. In a typical pretreatment procedure, 700 g of hexadecanol containing 0.7 wt.% of sulfuric acid, 0.7 wt.% of deionized water, and 50 g of bran or straw are loaded in a reaction vessel. The medium is warmed up to 90 °C and then maintained under stirring (600 rpm) for 5 h. Glycosides yields are followed by gas chromatography (GC) analysis of the filtrate.

Table 2
Molecular weight of the lignin materials produced from wheat straw.

	M_n (g/mol)	M_w (g/mol)	IP = M_w/M_n (g/mol)
Reference lignin	909 ± 27	2001 ± 60	2.2 ± 0.1
Wheat straw lignin	1007 ± 30	2992 ± 90	3.0 ± 0.2

Molecular weight measured by gel permeation chromatography following literature procedure (Tejado et al., 2007). Reference lignin was prepared by organosolv pretreatment (solubilization in formic/acetic acid mixture and precipitation at 90 °C) of wheat straw following literature procedure (Avignon & Delmas, 2008).

Same experiments were carried out at 109 °C. In the case of wheat straw (Fig. 2a), a substantial improvement was observed with a maximum yield of 95% reached at 4 h for hexadecyl arabinosides and at 5 h for hexadecyl-xylosides. A maximum hexadecyl-glucosides yield of 11% was achieved at 3 h, and remained constant till 5 h of reaction. This showed the good stability of the glucosides at 109 °C. In the case of wheat bran (Fig. 2b), a similar behavior was observed for hexadecyl pentosides although a slightly lower yield of xylosides was reached at 5 h (91%). A yield of 67% of hexadecyl glucosides is obtained, confirming the good reactivity of the starch contained in the wheat bran.

The chemical composition of the straw and bran residues collected in the best pre-treatment trials (Fig. 2a and b, 109 °C, 5 h) were established and compared to those of wheat bran and straw (Table 1). These analyses allowed us to complete the carbohydrate

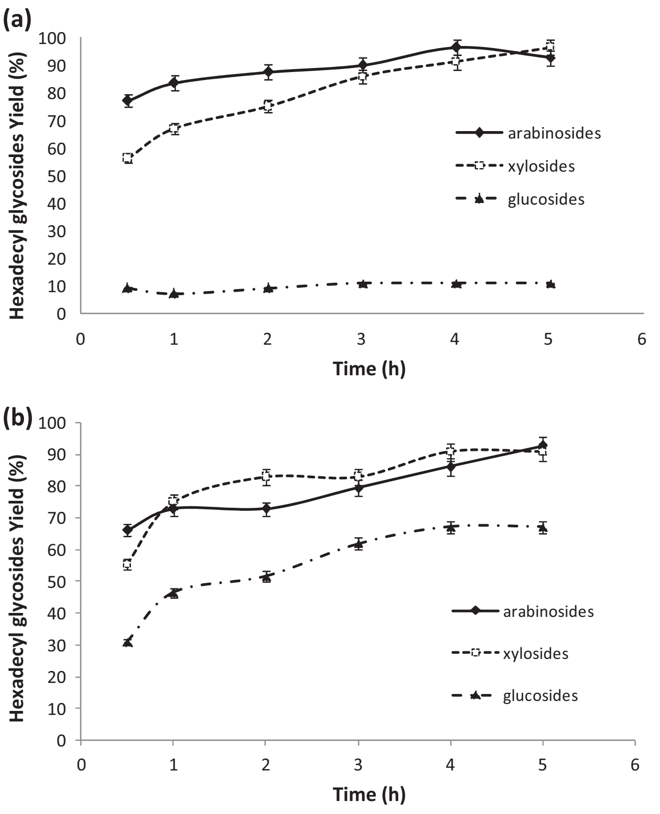


Fig. 2. Hexadecyl glycosides yields during pretreatment of wheat straw (a) and bran (b) at 109 °C. In a typical pretreatment procedure, 700 g of hexadecanol containing 0.7 wt.% of sulfuric acid, 0.7 wt.% of deionized water, and 50 g of bran or straw are loaded in a reaction vessel. The medium is warmed up to 109 °C and then maintained under stirring (600 rpm) for 5 h. Glycosides yields are followed by gas chromatography (GC) analysis of the filtrate.

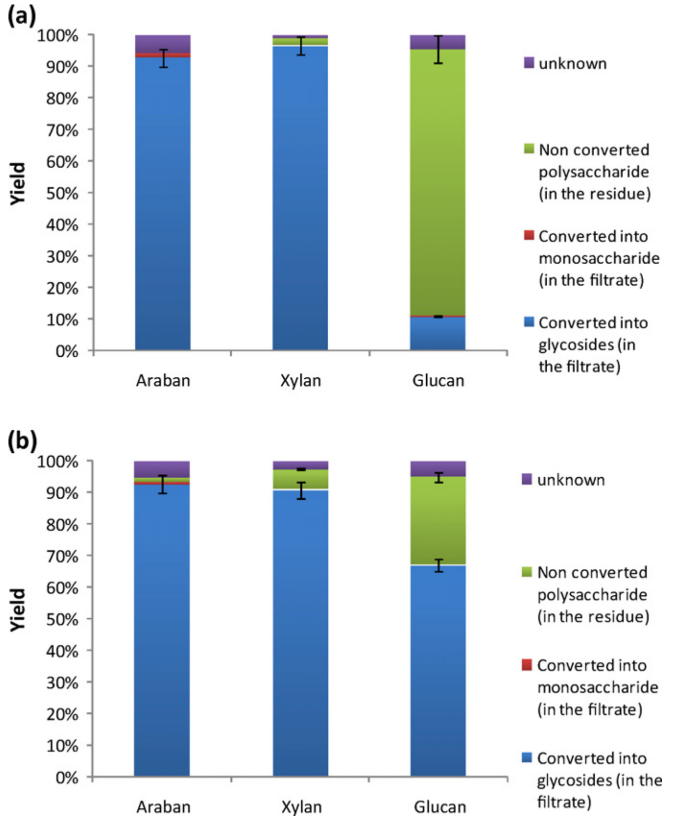


Fig. 3. Polysaccharides mass balance for wheat straw (a) and wheat bran (b) pretreatment at 109 °C for 5 h. Pretreatment procedure: 700 g of hexadecanol containing 0.7 wt.% of sulfuric acid, 0.7 wt.% of deionized water, and 50 g of bran or straw are loaded in a reaction vessel. The medium is warmed up to 109 °C and then maintained under stirring (600 rpm) for 5 h.

(arabinose, xylose and glucose) mass balance for the pretreatment trials made on wheat straw (Fig. 3a) and bran (Fig. 3b).

In the case of the pretreated wheat straw residue, cellulose was still present in high quantity and this material would merit further processing such as enzymatic hydrolysis into glucose to optimize the value of the co-products. The low amount of residual xylan and the absence of araban in the pretreated residues confirmed the good reactivity of hemicelluloses and their solubilization in the filtrate. In the case of the pretreated wheat bran, the residue contained a much lower concentration of glucan (only 5 wt.%) due to the good conversion of starch in glycosides. At this level, it is important to point out that similar yields of alkyl glucosides were obtained on transglycosylation of pure starch with butanol (Eskuchen & Nitsche, 1997) but using more drastic conditions (165 °C, 20 bar).

As bran is also known to contain a substantial amount of protein, we also analyzed the nitrogen content by Kjeldhal method and confirmed that the pretreated wheat bran was rich in protein. As lignin–protein complexes could be interestingly studied without further conversion in some resin application (Doherty, Mousavioun, & Fellows, 2011), we decided to focus on the straw residue for lignin material production as lignin–cellulose complexes require further processing for this application.

3.2. Enzymatic hydrolysis of cellulose contained in the hexadecanol pretreated wheat straw

Due to its concentration of cellulose, the 109 °C pretreated straw was then submitted to enzymatic hydrolysis. Results are presented in Fig. 4. Cellulose digestibility of the residue was 63% after 72 h, based on the initial cellulose content. This value is typically in the range of cellulose digestibility of a conventional dilute acid pretreated wheat straw (47%, Benkun, Xiangrong, & Yinhua, 2010) and substantially higher than a non pre-treated wheat straw (16%; Fig. 4) confirming the interest of the hexadecanol pretreatment in the context of ethanol fermentation.

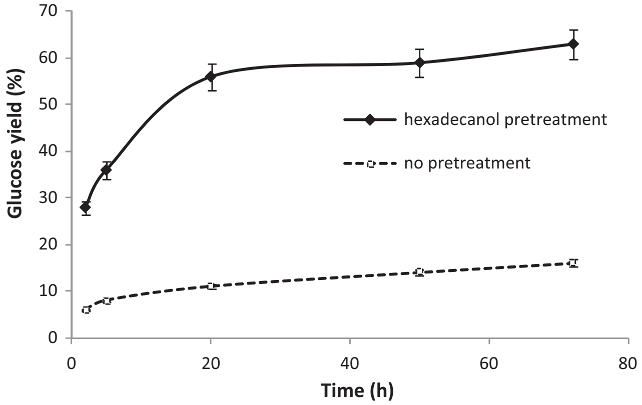


Fig. 4. Enzymatic hydrolysis of cellulose for wheat straw residue from hexadecanol pretreatment at 109 °C (non-pretreated wheat straw used as a reference). Hydrolysis conditions: 16 g of wheat straw or wheat straw residue is loaded in a reaction vessel containing enzymes (total volume of 800 mL). Each reaction vessel was sterilized at 121 °C for 20 min prior to the addition of the enzymes and straw or straw residue. The enzymes were added at the following amounts: 3.7 g Celluclast 1.5 L (60 FPU/g, 30 β-glucosidase IU/g) (Novozymes A/S), 0.8 g Novozyme 188 (500 β-glucosidase IU/g) (Novozymes A/S) and 0.8 g Multifect Xylanase (43 g protein/mL) (Genencor International Inc.). Enzymatic hydrolysis was performed at 40 °C for 72 h. The sample is immediately filtered through a 0.2 μm sterile filter and frozen to prevent further hydrolysis. Duplicate batches were run to verify the results.

3.3. *Production of lignin material from wheat straw residue*

Lignin material was produced and analyzed following a literature procedure starting from the 109 °C hexadecanol pretreated straw material (Tejado et al., 2007). A reference lignin material (reference lignin in Table 2) obtained from wheat straw by an organosolv pretreatment process (acetic and formic acid solubilization and precipitation procedure from Avignon and Delmas (2008)) was also analyzed for comparison of the impact of the pretreatment conditions and solvent used. The lignin material from the hexadecanol pretreated straw was obviously different as an organic-insoluble material. This difference was clearly displayed by weight-average molecular mass (M_w) measured for the lignin material produced from the hexadecanol pretreated straw that was higher than the M_w of the organosolv lignin (Table 2). The research works found in literature support the utilization of high M_w lignin for the synthesis of modified phenolic resins (Tejado et al., 2007). The higher M_w could be potentially explained by structure differences (more guaiacyl units; Boeriu, Bravo, Gosselink, & van Dam, 2004). The hexadecanol pretreatment used a different type of solvent and a lower concentration of acids. This could explain the lower M_w degradation than the one observed with the organosolv process (Stewart, Kadla, & Mansfield, 2006). A complementary study by NMR technique would help to reach a conclusion on that point and will be envisaged by authors in future works.

3.4. *Emulsifying properties of the hexadecanol filtrates from wheat straw and bran*

The filtrates recovered at the end of the 109 °C hexadecanol pretreatment of wheat straw and bran were used without further purification as emulsifying bases for the production of emulsions following a standard technique (NFT 73409). The hexadecanol contents of the filtrates compositions were analyzed by GC (Table 3). For comparison, emulsions were also produced with a broadly used commercial emulsifying base (GALENOL® 16/18 AE, Sasol). This product is based on alcohols polyethoxylated but is also composed of a high amount of fatty alcohols (hexadecanol and octadecanol; Table 3). The comparison of emulsifying base with the same range of alcohol concentration is important as fatty alcohols are known to greatly influence the texture and the stability of emulsions.

The emulsions of isopropyl myristate were produced with an increasing concentration of emulsifying base. The emulsifying power is the minimum filtrate concentration necessary to obtain a stable emulsion (Table 3). Isopropyl myristate is widely used in cosmetics for ointments, hair conditioners or creams formulations. The results displayed in Table 3 showed an equal emulsifying power for the commercial ethoxylates and the wheat straw filtrate (8%). The wheat bran derived emulsifying base possessed superior emulsifying properties (emulsifying power = 4%). Synergistic surface properties have already been observed for mixtures of alkyl xylosides and glucosides (Marinkovic & Estrine, 2010) that could explain a better emulsifying power for the wheat bran filtrate which contained a higher amount of glucosides.

Table 3
Fatty alcohol concentration and emulsifying power of the wheat derived filtrates.

	Fatty alcohol (wt.%)[a]	Emulsifying power (wt.%)[b]
Wheat straw filtrate[c]	83.6 ± 2.5	8
Wheat bran filtrate[c]	82.6 ± 2.5	4
GALENOL® 16–18 AE[d]	81.5 ± 2.4	8

[a] Fatty alcohol % determined by GC in the filtrate.

[b] Minimum filtrate concentration necessary to obtain a stable emulsion.

[c] Wheat straw/bran filtrate: filtrate from the hexadecanol pretreatment of wheat straw/bran at 109 °C for 5 h.

[d] GALENOL® 16–18 AE: commercial self emulsifying base from Sasol company.

4. Conclusions

The acidic hexadecanol based pretreatment of wheat straw and bran was investigated in this work. This pretreatment allowed the conversion in one step of hemicelluloses of wheat bran and straw into hexadecyl pentosides with yields up to 95%. Wheat-based surfactants thus obtained were used without further purification for the production of emulsions. They exhibited equal to superior emulsifying properties compared to fossil-based polyethoxylated fatty alcohols that are largely used surfactants in cosmetics and pharmaceutics industries. The non-converted materials collected after the pre-treatment were characterized showing that cellulose, starch and lignin remained available for further processing. For example, the enzymatic digestibility of the cellulose contained in the 109 °C hexadecanol pretreated straw was evaluated showing a glucose production of up to 63% compared to the 13% obtained with a non pretreated wheat straw. This result is of potential interest for ethanol fermentation. A lignin material was produced from the same hexadecanol pretreated straw residue. This material displayed a higher molecular weight than a lignin material obtained following an organosolv process, confirming its potential use in the field of modified phenolic resins. In view of the growing importance of renewable resource-based molecules in the chemical industry, this approach may open a new avenue for the conversion of wheat straw into various chemicals.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2012.01.018.

References

Avignon, G., & Delmas, M. (2008). Method for producing paper pulp, lignins, carbohydrates and acetic acid by fractionation of lignocellulosic vegetable material in formic/acetic acid medium. *US Patent No. US 7402224*.

Benkun, Q., Xiangrong, C., & Yinhua, W. (2010). Pretreatment of wheat straw by nonionic surfactant-assisted dilute acid for enhancing enzymatic hydrolysis and ethanol production. *Bioresource Technology*, *101*, 4875–4883.

Bertho, J. N., Mathaly, P., De Baynast, R., & Dubois, V. (1999). Emulsifying composition for cosmetics containing polyglycosides and fatty alcohol. *European Patent No. EP 895805*.

Bertho, J. N., Mathaly, P., Dubois, V., & De Baynast, R. (1997). Process for the preparation of surface active agents using wheat by-products and their applications. *US Patent No. US 5688930*.

Boeriu, C. G., Bravo, D., Gosselink, R. J. A., & van Dam, J. E. G. (2004). Characterisation of structure-dependent functional properties of lignin with infrared spectroscopy. *Industrial Crops and Products*, *20*, 205–218.

Bouxin, F., Marinkovic, S., Le Bras, J., & Estrine, B. (2010). Direct conversion of xylan into alkyl pentosides. *Carbohydrate Research*, *17*, 2469–2473.

Doherty, W. O. S., Mousavioun, P., & Fellows, C. M. (2011). Value-adding to cellulosic ethanol: Lignin polymers. *Industrial Crops and Products*, *33*, 259–276.

Eskuchen, R., & Nitsche, M. (1997). Technology and production of alkyl polyglycosides. In K. Hill, W. von Rybinski, & G. Stoll (Eds.), *Alkyl polyglycosides: Technology, properties and applications*. Weinheim: VCH.

Ferlin, N., Duchet, L., Kovensky, J., & Grand, E. (2008). Microwave-assisted synthesis of long-chain alkyl glucopyranosides. *Carbohydrate Research*, *343*, 2819–2821.

Gorius, O., Bertho, J. N., & Nuzillard, J. M. (2001). Determination and prediction of the average polymerization degree of alkyl polyglucosides. *Analytica Chimica Acta*, *440*, 231–237.

Liu, S. C. (2004). *Analysis and measurement in papermaking industry*. Beijing: Chemical Industry Press.

Kamm, B., Kamm, M., Schmidt, M., Hirth, T., & Schulze, M. (2006). B. Kamm, P. R. Gruber, & M. Kamm (Eds.), *Biorefineries – Industrial processes and products*. Weinheim: Wiley-VCH.

Klass, D. L. (1998). *Biomass for renewable energy, fuels, and chemicals*. San Diego: Academic Press.

Marinkovic, S., & Estrine, B. (2010). Direct conversion of wheat bran hemicelluloses into n-decyl-pentosides. *Green Chemistry*, *12*, 1929–1932.

Martel, F., Estrine, B., Plantier-Royon, R., Hoffmann, N., & Portella, C. (2010). Development of agriculture left-overs: Fine organic chemicals from wheat hemicellulose-derived pentoses. *Topics in Current Chemistry*, *294*, 79–115.

Persson, T., Ren, J. L., Joelsson, E., & Jönsson, A. S. (2009). Fractionation of wheat and barley straw to access high-molecular-mass hemicelluloses prior to ethanol production. *Bioresource Technology*, *100*, 3906–3913.

Ragauskas, A. J., Williams, C. K., Davison, B. H., Britovsek, G., Cairney, J., Eckert, C. A., et al. (2006). The path forward for biofuels and biomaterials. *Science*, *311*, 484–489.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., et al. (2007). *Determination of structural carbohydrates and lignin in biomass, laboratory analytical procedure-002, 003, 017, 019*. USA: National Renewable Energy Laboratory, U.S. Department of Energy.

Stewart, J. J., Kadla, J. F., & Mansfield, S. D. (2006). The influence of lignin chemistry and ultrastructure on the pulping efficiency of clonal aspen. *Holzforschung*, *60*, 111–122.

Tejado, A., Pena, C., Labidi, J., Echeverria, J. M., & Mondragon, I. (2007). Physicochemical characterization of lignins from different sources for use in phenol–formaldehyde resin synthesis. *Bioresource Technology*, *98*, 1655–1663.

von Rybinski, W., & Hill, K. (1998). Alkyl polyglycosides: Properties and applications of a new class of surfactants. *Angewandte Chemie International Edition*, *37*, 1328–1345.