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Effects of tribochemical treatments on the integrity of cellulose

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ABSTRACT

The effects of tribochemical impacts – sonochemical treatments and milling variants – on cellulose were studied, employing multi-detector size exclusion chromatography with group-selective fluorescence labelling ("CCOA method") and diagnostic beta-elimination as the analytical tools. Milling at different temperatures was compared to sonochemical degradation of cellulose by a 24 kHz ultrasound probe system in homogeneous solution.

Chain cleavage was generally accompanied by random oxidation of cellulose. The degree of oxidation increased with increasing temperature; at 77 K oxidation occurs only to a minor extent. Degradation proceeded towards a final value, the limiting molecular weight (Mw_{lim}), beneath which no further decrease of the chain length occurred even at prolonged treatment times. Regardless of the lignin or hemicellulose content of the pulps, the Mw_{lim} reached in a specific milling process was largely constant and showed little dependence on the substrate used.

The formation of radicals during tribochemical treatments under different conditions and with different substrates was discussed based on theoretical considerations and EPR data. The overall radical content increased with increasing time of milling until a plateau is reached. Here, the mechano-radical content largely depends on the lignin content in the pulp as anticipated. The formation of different radical species and their precursor structures were discussed.

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1. Introduction

The importance and the properties of cellulose are very well known. Still, the almost ubiquitous introductory remark about this compound – cellulose being the main reinforcing constituent for cotton and wood, the most abundant renewable and biodegradable compound on earth, and the carrier of extraordinary mechanical properties – might serve in this place to emphasize once more the prominence and extraordinary significance of that biopolymer. The positive cellulose characteristics and its good mechanical properties are the reasons why cellulose has been extensively utilized since the dawn of mankind. Today, paper and textile industries are main players in the economies of modern-day countries.

The molecular weight distribution of cellulose is the most characteristic analytical parameter, and is also closely linked to its properties. The same applies for the characteristic, complex network of hydrogen bonds which is mainly responsible for the cellulosesí insolubility in almost any solvents. Recently, the pharmaceutical, cosmetic, and food industries have considered utilization of plant derived cellulose but its high molecular weight and water-insoluble character have restricted direct usage in various applications (Wong, Kasapis, & Tan, 2009). A deliberate and controlled decrease of the molecular weight of celluloses on industrial scale has been performed for more than 120 years in the well-known viscose (rayon) production process, in which alkali cellulose is subject to an ageing or "ripening" step that is supposed to bring about favourable rheological properties of the spinning dope after xanthation and eventually beneficial fibre properties. Thus, controlled cellulose degradation plays an important role and it is an important general step in cellulose processing: for some applications, cellulose chains need to be shortened or – in other words – the molecular weight distribution must be transposed to lower values. In most cases, a simultaneous narrowing of the molecular weight distribution is intended. More recent applications, frequently covered by the somewhat ill-defined term "biorefinery approaches"

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even attempt to drive the degradation of cellulose as far as to the monomer or oligomer level in order to submit these low-molecular weight products to fermentation processes. Also here, the degradation is not to be seen as necessary evil, but as an integral and crucial step along the utilization line. Especially in such biorefinery steps the neatness of the degradation, the minimization of yield penalties and resulting yields as optimal as possible are paramount.

These examples are presented to demonstrate that cellulose degradation is not always the unwanted side process as which it is usually considered. Evidently, most processing steps in cellulose manufacture as well as ageing processes (ageing with time as well as thermal, radiation, light or chemical stress) will have a negative impact and will cause changes in the "natural" chain lengths and chain lengths distribution of the cellulose, and much research is done to suppress those side reactions or to revert their negative effects. But, as said, there are also several examples where the molecular weight reductions are well wanted and brought about intentionally.

Several methods, with various kinds of chemical and energy input, are available for preparation of low molecular weight polysaccharides, i.e. also for degradation of cellulose. They comprise chemical, thermal, or mechanical energy (Liu, Bao, Du, Zhou, & Kennedy, 2006). Regarding chemical methods for reduction of molar mass of polysaccharides, acid hydrolysis is the most common and a very efficient method (Kim, Lee, & Torget, 2001; Lenihan et al., 2010). There are several variants that apply different organic and inorganic acids, different reaction conditions or simultaneous derivatization such as methanolysis (Sundberg et al., 1996). Alkaline hydrolysis is less suitable as it requires higher temperatures and induces side reactions. Alkali-induced chain cleavage of oxidized celluloses, proceeding already at room temperature and at pH slightly above neutral, cannot be regarded as an alternative type of direct cellulose degradation since it implies prior oxidation of the polymer under introduction of carbonyl groups. This oxidation reaction is a fundamental chemical impact which changes mere hydrolysis to a beta-alkoxy elimination mechanism.

The pros and cons of acidic hydrolysis are well known: the latter are mainly connected with aspects of high costs, lower yields due to side reactions (mainly acidic catalyzed cyclizations and dehydrations to furanoid compounds which readily undergo further condensations and polymerizations to an intractably complex by-product mixture), low accessibility of the cellulose part, and discharge problems with the operating chemicals. Some of those disadvantages are usually tackled by enzymatic approaches, but the high costs and accessibility restrictions still remain important issues.

Mechanical degradation methods, although having been treated somewhat step-motherly in reports and research accounts, do offer an alternative. Two main variants, sonochemical and tribochemical (milling, grinding) paths, are counted among these degradations. It is certainly true that mechanical degradation had acquired a less favourable reputation of being somewhat "archaic" and hard to control and predict. But at the same time it is equally true that the tattered reputation of mechanical cellulose degradation is partly due to the fact that it has never been as meticulously scrutinized as the acidic and enzymatic counterparts, and that mechanic degradation has the obvious advantage of not using any chemicals or enzymes. The resulting benefit with regard to avoidance of side reactions, cost savings, and waste discharge requirements is evident. At the same time, the high energy costs and low energy efficiency of grinding treatments are known, although there are ways for their reduction (Brandt et al., 2012). Mechano-treatment of wood is the basis of mechanical and chemomechanical pulping.

The driving force of sonochemistry is cavitation. Ultrasound generates high- and low-pressure waves in an exposed liquid. Cavitation bubbles are produced during low pressure cycles, and they collapse violently during the high pressure cycles, generating high temperature and pressure in excess of one thousand atmospheres (Mason, 1997). Implosion of the cavitation micro bubbles causes strong hydrodynamic shear forces and/or extensional forces. Polymer chain fragments in the field near those strong forces move at a higher velocity than the polymer fragments further away. The difference in the velocity between chain fragments causes chain elongation, and the stress to the polymer chain might eventually result in chain scission, i.e. causing cleavage of glycosidic bonds in the case of the carbohydrate-based polymer cellulose. Generally, two reaction modes must be distinguished: in a heterogeneous system cellulose is not dissolved, but might be swollen or solvated in surface regions. In a homogeneous system, cellulose is the solute surrounded by a large excess of molecules of a suitable solvent (Stefanovic, Potthast, & Rosenau, 2013). For homogeneous solutions of cellulose, sonochemical degradation was shown to be not a purely hydrolytic process just causing cleavage of glycosidic bonds, but to be accompanied by an oxidative action. The location of the introduced carbonyl groups along the cellulose chain was determined to be at or very near to the cleaved glycosidic bond, using the approach of diagnostic beta-alkoxy elimination in combination with group-selective fluorescence labelling, the so-called CCOA-method (Ahn, Henniges, Banik, & Potthast, 2012; Potthast, Kostic, Schiehser, Kosma, & Rosenau, 2007; Röhrling, Potthast, Rosenau, Lange, Borgards, et al., 2002; Röhrling, Potthast, Rosenau, Lange, Ebner, et al., 2002).

Tribochemical treatment is a mechanical treatment commonly used for processing a wide variety of polymers (Ago, Endo, & Hirotsu, 2004; Maier, Zipper, Stubicar, & Shurz, 2005; Ouajai & Shanks, 2006; Paes et al., 2010). The combination of shear forces and compression forces upon milling causes breakage of chemical bonds. The terms "ball milling" and "cryomilling" are usually used synonymously with such a tribochemical treatment, although strictly speaking this is not correct: tribochemistry – *per definitionem* – involves chemical changes of a material brought about by the impact of mechanical energy. Milling, although evidently effecting massive physical changes, does not necessarily cause chemical reactions. Actually, both "classical" ball milling and cryomilling are done with balls as grinding elements which result in optimum transfer of energy into the material to be ground. Cryomilling is just a variant that is done at lowered temperatures, typically in cryostated solvents or liquid nitrogen, in order to avoid material squeezing or crushing, and to reduce chemical side reactions. It is evident that different milling setups will have different impacts on the mill charge and will produce different results.

With regard to cellulose and cellulose-containing natural materials, milling is generally very efficient for lowering the molecular weight. The strong compression and shear forces are obviously very effective in crystal structure modification of polymers and in their mechanical destruction (Bonadies et al., 2012; Chen, Yi, Wu, & Guo, 2010). Tribochemical treatment is also valuable to isolate so-called lignin–carbohydrate complexes (LCCs) and for further analytical characterization of wood cell wall components (Karinkanta, Illikainen, & Niinimäki, 2013). At the same time, it must be understood that the molecular weight distributions of the polymeric components after tribochemical treatment cannot be those of the polymers in native state. It is the very purpose of the milling to reduce the molecular weight by chain scissions, and this is the very mode of action to bring about solubility of the treated material, e.g. extensively milled wood in some ionic liquids. But this must not spuriously lead to the conclusion that a merely physical separation of chemically unchanged individual components enabled the dissolution process as previously reported (Zoia, King, & Argyropoulos, 2011). It is rather a – sometime quite extensive – polymer degradation that caused this effect.

It has long been known that prolonged milling, beside polymer chain scission, causes production of radicals, so-called mechano-radicals, which are capable of undergoing or inducing further chemical reactions (Bonadies et al., 2012; Kuzuya, Yamauchi, & Kondo, 1999; Sakaguchi et al., 2010). Information about the type and stability of those mechano-radicals in cellulose or cellulose-containing systems are contradictory. Some authors reported that those radicals are unstable at room temperature and are immediately converted into other chemical species (Sakaguchi, Makino, Ohura, & Iwata, 2012), other studies demonstrate that those mechano-radicals may be quite stable for a long period of time at room temperature (Kuzuya et al., 1999; Solala et al., 2012). In the view of the authors, the debate on tribochemically induced radical formation might arise from the fact that the tribochemical systems, and first of all the cellulosic substrates, might have been different. The presence of solvents or swelling agents as well as the presence of other polymeric components, such as hemicelluloses or lignin might change the system fundamentally with regard to contained radical species. If one thinks about aromatic radicals, such as those derived from hemicelluloses-derived furanoid systems or lignin-derived phenoxyl systems, or about the strong temperature-dependence – and thus mobility dependence – of radical recombination reactions, it is evident that statements about radical species occurring during tribochemical reactions requires a most careful setting of the reaction conditions. The presence or absence of oxygen is an equally important aspect.

Both sonochemical and tribochemical degradation accompanied with mechano-radical formation are dependent on many factors, including the intensity and duration of the treatment (Chen et al., 2010; Wong, et al., 2009), temperature (Chen, Chang, & Shyur, 1997; Price & Smith, 1993; Sakaguchi et al., 2010; Schwanninger, Rodrigues, Pereira, & Hinterstoisser, 2004; Trzciński & Staszewska, 2004; Vijayalakshmi & Madras, 2006; Wu, Zivanovic, Hayes, & Weiss, 2008), concentration (Grönroos, Pirkonen, & Ruppert, 2004), type of solvent (Ago et al., 2004; Ago, Endo, & Okajima, 2007; Vijayalakshmi & Madras, 2006), type of polymer and chemical structure of the polymer, including branching (Sasai, Yamauchi, Kondo, & Kuzuya, 2004; Schittenhelm & Kulicke, 2000). Anomeric configuration of the monosaccharide units (β-configuration in cellulose vs. α-configuration in starch) plays an important role upon sonochemical degradation of polysaccharides (Striegel, 2007), and also upon mechano-radical formation upon milling (Kuzuya et al., 1999).

In this account we would like to add some insight into the mechanism of tribochemistry of celluloses, with regard to chemical changes – possible chain cleavage and oxidation – of the polymer. The degradation effects are also discussed in relation to the presence of lignin in the pulp: hence three different pulps with different lignin content were used (cotton, bleached kraft pulp and unbleached kraft pulp). Tribochemical degradation of cellulose is compared with ultrasonic degradation of cellulose. A possibly occurring oxidation of the pulp was monitored as the increase in the total number of carbonyl groups relative to the molecular weight distribution (MWD) according to the CCOA method (Röhrling, Potthast, Rosenau, Lange, Borgards, et al., 2002; Röhrling, Potthast, Rosenau, Lange, Ebner, et al., 2002). Diagnostic beta-elimination reactions were used as an additional way to identify the location of the oxidation along the cellulose molecule, i.e. at chain ends or rather in the middle of a chain (Stefanovic et al., 2013). In order to get some further insight on how the oxidation was brought about we have analyzed the pulp samples by electron paramagnetic resonance (EPR) spectroscopy. Semi-quantification of the radical content in the presence and absence of lignin and analysis of radical species present at 77 K for the cryomilling case and at room temperature using spin traps were performed. Based on the results, we are reasonably confident that mechanical treatments of polysaccharides – once an in-depth understanding of the degradation mechanisms and kinetics is established – will offer a viable way for controlled conversion of high-molecular weight species into polymers of lower molecular weight and hence a reliable means of tuning.

2. Experimental

2.1. Materials

Chemicals were obtained from commercial sources and were of the highest purity available. Whatman filter paper with and cotton linters (Buckeye, kappa number 0.11) were chosen to study the influence of mechanical degradation methods on samples that consist of almost 100% cellulose and only very minor amounts of hemicelluloses, extractives and lignin. To study the impact of lignin and hemicelluloses on degradation process, we introduced unbleached – kappa number 25.9 and bleached kraft pulps – kappa number 3.0, supplied by Södra Cell, Sweden. Prior to the actual milling process the pulp samples were washed and homogenized by mixing with a large excess of deionized water for 3×10 s, in a kitchen mixer, filtering and washing with acetone on a Buchner funnel and drying at ambient conditions. It should be noted here that extensive washing with ethanol will quench most radical species detectable in subsequent EPR analysis.

2.2. Methods

2.2.1. Ultrasonic treatment

The treatment was performed with a Hielscher UIS 250 ultrasonic probe system (24 kHz, 250 W) in a 100 ml beaker for different times (0–120 min) as described by Stefanovic et al. (2013).

2.2.2. Milling

The pulps (2.5 g), were milled for 0–120 min in a Retsch cryo mill using a steel grinding jar of 50 ml and three stainless steel balls with 9 mm diameter. In order to investigate the influence of water on the cellulose crystallinity 2.5 ml of water was added. Three different grinding modes were used, cryogenic, and dry milling at ambient temperature and milling at around 80 °C. Ball milling was performed on Retsch mixer mill for dry and wet milling with two grinding jars of 25 ml and three balls of 9 mm.

2.2.3. GPC and carbonyl group analysis

The labelling of carbonyl groups and analysis of carbonyl profiles were performed as described (Röhrling, Potthast, Rosenau, Lange, Borgards, et al., 2002; Röhrling, Potthast, Rosenau, Lange, Ebner, et al., 2002). The GPC setup used the following components: online degasser, Dionex DG-2410; Kontron 420 pump; pulse damper; auto sampler, HP1100; column oven, Gynkotek STH 585; refractive index (RI) detector, Shodex RI-71; fluorescence detector, TSP FL2000; and multi-angle laser light scattering (MALLS) detector, Wyatt Dawn DSP. Data evaluation was performed with standard ASTRA and GRAMS/32 software.

The following parameters were used in the GPC measurements: eluant flow = 1.00 ml min^{-1}; columns = four PL mixed A LS, 20 μm, 7.5 mm × 300 mm; fluorescence detection λ_{ex} = 290 nm, λ_{em} = 340 nm (CCOA); injection volume = 100 μl; run time = 45 min; DMAc/LiCl 0.9% (v/w), filtered through a 0.02-μm filter, used as a mobile phase.

2.2.4. Electron paramagnetic resonance (EPR) spectroscopy

Electron paramagnetic resonance (EPR) measurements were carried out on a Bruker EMX continuous wave (cw) spectrometer, operating at X-band (9 GHz) frequencies, equipped with a high

sensitivity resonator for RT measurements and a TM Cavity for 77 K measurements.

For EPR analysis the samples were either measured at room temperature after room temperature ball milling for 0, 15, 30, 45, and 60 min or at 77 K after cryomilling for 60 min without thawing. The frozen samples were also investigated at room temperature using the spin trap 5,5-dimethyl-pyrroline-*N*-oxide (DMPO), for determination of short-lived carbon- and oxygen-centred radicals.

Semiquantitative measurements were carried out on ball milled cotton, unbleached and bleached kraft pulp samples at RT. The milled samples were transferred in EPR quartz tubes (4 mm O.D.) and measurements were also carried out at RT.

In another set of experiments, samples of cotton, bleached and unbleached kraft pulp for EPR measurements were cryomilled and immediately frozen and stored in liquid nitrogen. One part of the frozen powder was transferred into EPR quartz tubes (4 mm O.D.) and placed in a finger dewar (Bruker) filled with liquid N_2 in the EPR cavity for measurements at 77 K. For comparison, samples of cotton, bleached and unbleached kraft pulp were milled at RT and then frozen, stored and measured at 77 K.

For spin trapping experiments at room temperature, two spoons of the frozen powder samples were added to 800 μL spin trap solution (100 mM DMPO) and the solution was shaken for 15 min and then centrifuged at 8000 rpm for 2 min. 500 μL of the supernatant were transferred into a flat cell for EPR measurement.

EPR spectra were recorded under non-saturating conditions using 1 mW microwave power (MWP), 100 kHz modulation frequency (MF), 0.5 mT modulation amplitude (MA), 41 ms conversion time (CT), 41 ms time constant (TC) and 1024 points for the 77 K-measurements. EPR measurements at RT were carried out using 0.32 mW MWP, 100 kHz MF, 4 G MA, 41 ms TC, 41 ms CT and 1024 points. For spin trapping experiments the parameters were chosen with 20 mW MWP, 100 kHz MF, 0.1 mT MA, 41 ms CT, 41 ms TC and 1024 points.

Simulations of EPR spectra were carried out using the software EasySpin (Stoll & Schweiger, 2006). The intensity of the EPR spectra of milled samples at RT (in arbitrary units) dependent on the milling time was determined by double integration of the whole EPR spectrum after baseline correction using the programme WinEPR (Bruker) and then normalized by the sample weight.

2.2.5. Beta-elimination reaction

Beta-elimination as a diagnostic tool to analyze the distribution of carbonyl groups along the cellulose chain was performed as described earlier (Ahn et al., 2012; Stefanovic et al., 2013). The milled pulp was treated with 0.1 M NaOH at 22 °C. After one hour, the liquid was filtered and the pulp was washed with water until neutral and was subsequently analyzed by GPC/fluorescence labelling.

2.2.6. Solid state NMR

All solid state NMR experiments were performed on a Bruker Avance III HD 400 spectrometer (resonance frequency of ^{1}H of 400.13 MHz, and ^{13}C of 100.61 MHz, respectively), equipped with a 4 mm dual broadband CP-MAS probe. The samples were swollen in deionized water over night before measurement. ^{13}C spectra were acquired by using the TOSS (total sideband suppression) sequence at ambient temperature with a spinning rate of 5 kHz, a cross-polarization (CP) contact time of 2 ms, a recycle delay of 2 s, SPINAL 64 ^{1}H decoupling and an acquisition time of 43 ms. Chemical shifts were referenced externally against the carbonyl signal of glycine with δ = 176.03 ppm. The acquired FIDs were apodized with an exponential function (lb = 1 Hz) prior to Fourier transformation. Peak fitting was performed with the Dmfit programme (Massiot et al., 2002).

3. Results and discussion

Several studies have addressed the impact of different milling conditions on polymer degradation (Agarwal, Zhu, & Ralph, 2012; Ago et al., 2007; Karinkanta et al., 2013; Kuzuya et al., 1999; Kwan, Ghadiri, Papadopoulos, & Bentham, 2003; Sakaguchi et al., 2010; Schwanninger et al., 2004). In order to investigate the influence of the temperature on the degradation process, three different milling conditions were used. Cryomilling was performed at a constant temperature of 77 K (liquid nitrogen conditions). Conventional milling was done at room temperature and without cooling, which produced temperatures around 80 °C for the ball milling system applied.

First, cotton linters was used as a model for pure, lignin-free and hemicelluloses-free cellulose. Two tribochemical treatments, sonication and milling, were compared. For all tribochemical treatments, also at cryomilling temperature, a reduction of the weight-average molecular weight (Mw) was observed (Fig. 1). The Mw decreased from 148 kg mol^{-1} to 123 kg mol^{-1} during the first 60 min of cryomilling (Fig. 1, left). The effect of further cryomilling (90 min) on cotton pulp was not very pronounced: the Mw levelled off at about 80% of the starting value. Mw did not decrease further at 77 K.

The reduction of Mw is accelerated at increasing temperature. For the non-cooled milling types the temperature is still lower than 130 °C, hence a sole temperature effect and thermal modification of cellulose and radical formation can be excluded (Schwanninger et al., 2004; Solala et al., 2012). Interestingly the first decrease in Mw is similar for the 77 K and for room temperature. This indicates that initially some glycosidic bonds are broken no matter what temperature is applied. After about 30 min the degradation at room temperature increases compared to the low temperature milling (Fig. 2, left).

Upon both sonochemical and tribochemical treatment degradation of cellulose until a so-called limiting molecular weight (M_{lim}) is reached – the molecular weight beneath which no further degradation occurs (cf. Fig. 1). For both mechano-treatments, i.e. sonochemistry and tribochemistry, this effect is explained by the cellulose chain units becoming too small to take up the mechanical energy so that it is dissipated into larger units still present. In sonication, the limiting molecular weight is mainly dependent on the solvent used for sonication. In case of sonication in cuprammonium hydroxide (cuam), M_{lim} of about 46 kDa is reached (Wong, Kasapis, & Huang, 2012). For milling, a recent study (Schwanninger et al., 2004) reported dependence of limiting molecular weight on the milling process and for ball milling a limiting DP of 430, which was not further decreased even after 7.5 h of further milling.

What is obvious from the molecular weight distributions (Fig. 1) is the different behaviour of the polydispersity index (PDI). While the sonication produces more narrow distributions with the PDI approaching 1.1, milling causes a broadening and hence a significantly larger PDI (PDI of 1.9; Fig. 1, middle).

Ball milling at about 80 °C reduces the DP to half within 60 min. An initial rapid decrease in Mw occurred during the first 45 min, and then the degradation levelled off. At extended times of milling (120 min) eventually the M_{lim} of around 26 kg mol^{-1} was reached, which corresponds to approximately 17% of starting Mw. Even after 3 h of further ball milling, further changes were only marginal. Generally, no further degradation was observed beyond 120 min of ball milling.

The milling, i.e. the type of mill, milling setup, and temperature, governed the results much more than the substrates used. This is shown in Fig. 2, right, by means of three rather different pulps with various hemicelluloses and lignin contents. The presence of hemicelluloses (bleached kraft pulp) or hemicelluloses plus

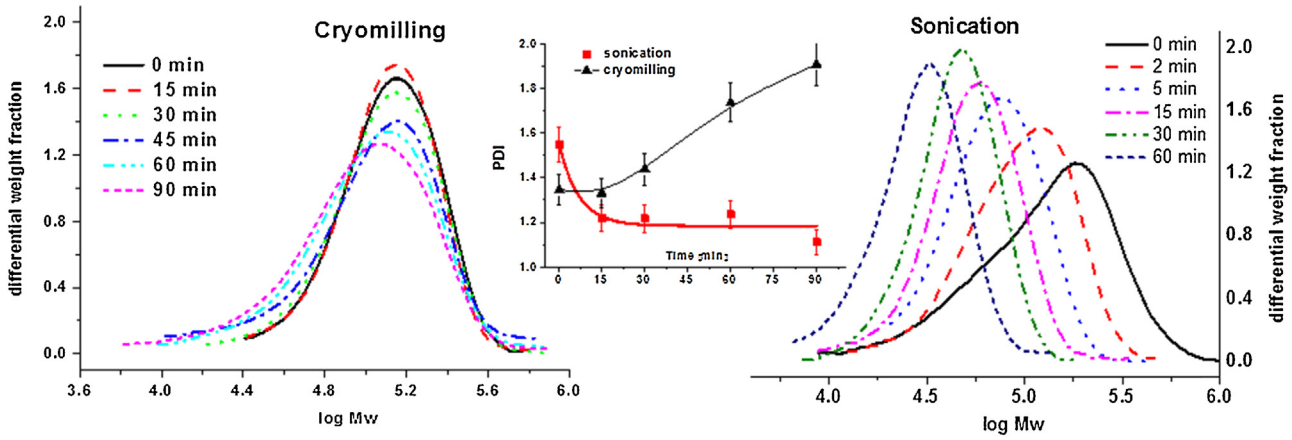

Fig. 1. Change of molecular weight distribution during cryomilling (left) and sonication in homogeneous solution (right) of cotton linters. The trend of the PDI is given in the middle.

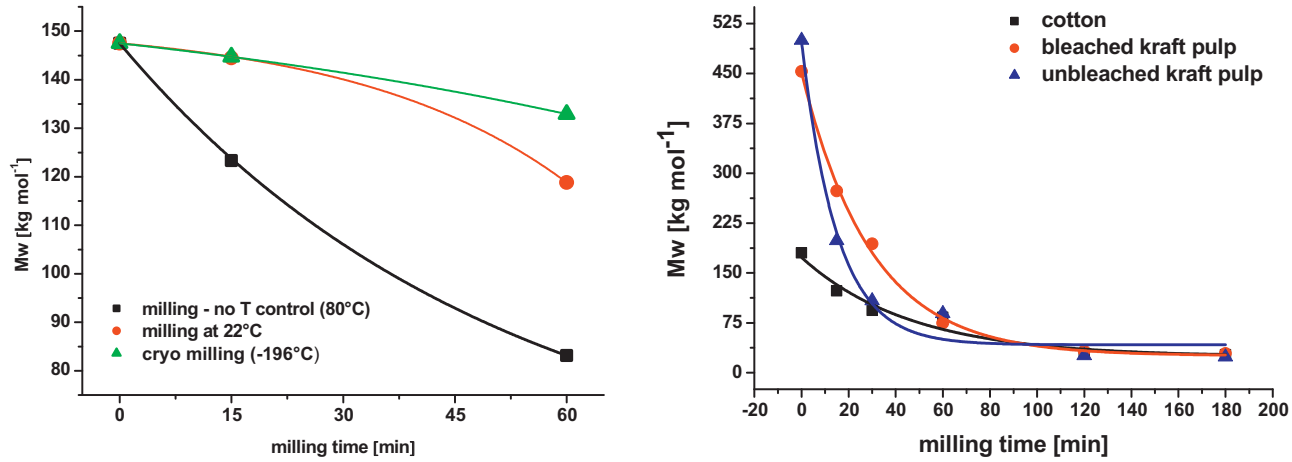

Fig. 2. Effect of different milling types on cotton linters (left) and different lignocellulosic substrates (right) on molecular weight (Mw) degradation (no control of temperature).

lignin (unbleached kraft pulp) had only very little effect on the final limiting Mw. Although the degradation kinetics of the different pulps is slightly different, the Mw_{lim} is quite comparable (25 kg mol^{-1}). Thus, it was once more confirmed that M_{lim} is defined by the size of the remaining cellulose chains – when they become sufficiently small they are no longer affected by the tribochemical shear, compression, or extension forces. Hence, the molecule is not further degraded despite further energy input – neither by sonication nor by milling.

In order to follow oxidative changes upon a tribochemical treatment, a topic which has not been addressed so far, the content of carbonyl groups – i.e. "oxidized hydroxyl groups" – was analyzed. As also reducing end groups (REGs) are reported as carbonyls, the REG content has to be separated from the overall carbonyl content to judge if an oxidative modification was brought about by the respective treatments. The REG value can be retrieved mathematically from the number-average molecular weight (Mn) value. Chain cleavage would just increase the REG number by the "new" reducing ends generated, but not increase the number of along-chain carbonyl groups. For the rather mild cryomilling at 77 K, the number of oxidized positions in cotton linters cellulose was only minor: 5.9 μmol g^{-1} for the untreated cotton linters and 17.3 μmol g^{-1} after 90 min of milling. For conventionally milling (without cooling) the situation changed: the same cotton linters milled up to 90 min, showed a significant carbonyl content increase from 5.9 μmol g^{-1} to 51.9 μmol g^{-1}. After subtracting the reducing end groups a carbonyl content of about 11 μmol g^{-1} remained, clearly indicative of an oxidation (Table 1). A possible source of oxidants is the formation of radicals. Many primary mechano-radicals will react with dioxygen (a biradical itself), this reaction being either direct (in the case of carbon-centred radicals) or indirect after beta-fragmentation (in the case of alkoxy radicals), see below. In any case, it will provide oxidized species, such as peroxyl radicals and peroxides, which eventually result in detectable carbonyl structures. Moreover, our spin trapping experiments (see Fig. 8) showed the presence of carbonate anion radicals which are known to oxidize cellulose (Carlsson, Lind, & Merényi, 2006; Stenman, Carlsson, Jonsson, & Reitberger, 2003). If the introduction of new carbonyl groups is

Table 1
Overall carbonyl, reducing end groups (REG), and keto/aldehyde content in pulp after milling and sonication.

	Carbonyl groups[a] 60 min milling [μmol g^{-1}]	REG[b] [μmol g^{-1}]	Keto (aldehyde) groups [μmol g^{-1}]
Ball milling	51.9	41.2	10.8
Cryo milling	17.3	15.5	1.8
Sonication	54.2	28.4	25.8

[a] Carbonyl content determined by the CCOA method.
[b] REG = 1/Mn (kg mol^{-1}) × 100.

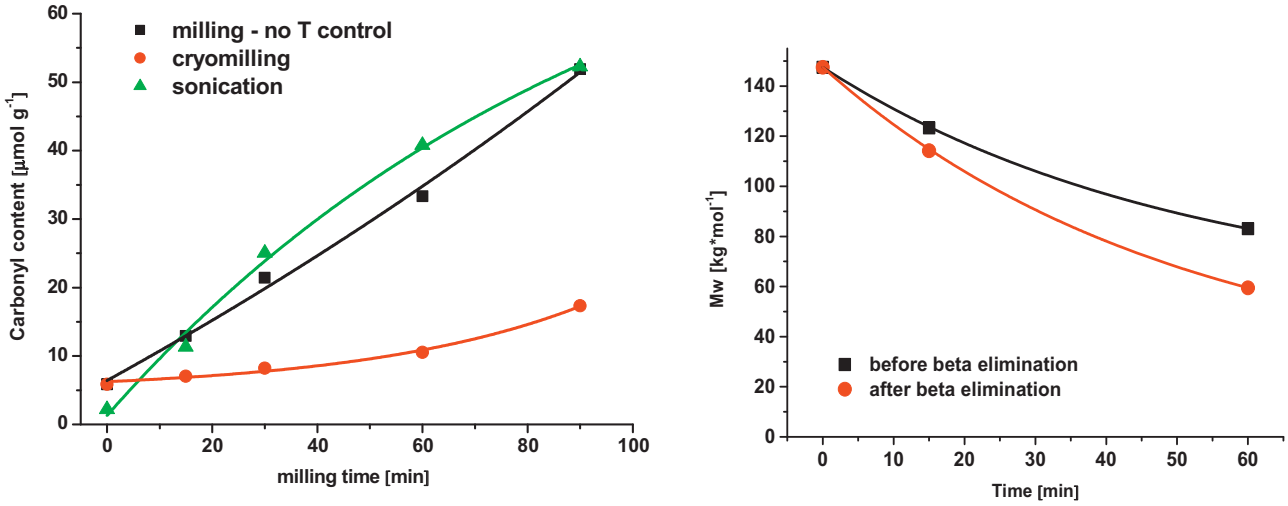

Fig. 3. Changes in the oxidation state (total carbonyl content) of cellulose upon milling over time (left) and change in Mw after beta-elimination (right).

compared to sonication in homogeneous solution a similar number of carbonyl groups is introduced (Fig. 3, left).

For the sonication case the introduction of novel carbonyls other than REGs is directly related to the cleavage process – the impacting energy will act at its spot of impact and break the bonds at this location. For milling the oxidation is more random and possibly connected to the generation of secondary radicals like carbonate anion radicals – see also the discussion in the radical reaction part. Applying diagnostic beta-elimination (Ahn et al., 2012) allows estimation whether the oxidation takes place in the middle of a cellulose chain or rather at chain end. In short, oxidation of a cellulose chain in a more central part will cause alkali-induced cleavage at this very point resulting in two large fragments and a significant shift of the molecular weight and a newly generated reducing end that is reported by the CCOA label. By contrast, alkali-induced cleavage from an oxidized spot close to the chain end will provide a chain of nearly unchanged Mw and a very small fragment that is not detectable by GPC. The CCOA value is just registered for the long, nearly non-shortened chain and thus remains unchanged.

The molecular weight distribution and the number of keto/aldehyde groups were analyzed directly after the tribochemical treatment. The samples were subject to an alkaline treatment (0.1 M NaOH, RT, 1 h) and the measurements (molecular weight distribution and carbonyl group content) were repeated. A clear reduction of Mw was observed. Fig. 3 right compares the weight average Mw of the milled sample (room temperature) before and after the beta-elimination reaction. The weight average Mw of the 60 min milled cotton sample decreased from 83 kg mol^{-1} to 59 kg mol^{-1} after beta elimination. The beta-elimination reaction causes chain fragmentation and both fragments (longer and shorter) are detected by GPC. Hence, the position of oxidation during milling is more central and occurs rather randomly along the cellulose chain. This is in stark contrast to sonochemical treatment which causes oxidation at or very close to the chain ends (Stefanovic et al., 2013). The number of carbonyls remained nearly constant, the 60 min milled cotton carbonyl group content changed from 52 µmol g^{-1} to 53 µmol g^{-1}.

The crystalline content of the untreated cotton linters based on CPMAS measurements and deconvolution of the C4 region is 65%. Milling without cooling produces fully amorphous cellulose as reported previously. Cryomilling reduced the crystallinity only to 43%. The presence of water during cryomilling (added before milling) did not change this to any significant extent. No conversion to cellulose II was observed.

It has long been known that prolonged milling will cause production of free radicals – mechano-radicals. As indicated in the introduction, this radical production was not of primary interest per se, but of secondary due to subsequent reaction modes that use the primary mechano-radicals as triggers of follow-up chemistry. In more recent attempts, for instance, these subsequent reactions are polymerizations which use milling to generate mechano-radicals that initiate a radical polymerization onto the cellulose chain (Sakaguchi et al., 2010; Solala et al., 2012).

It seems necessary to point out one issue which is not rigorously treated in several accounts discussing radical formation from pulps during milling: the question of the location of radical formation and the type of the formed radicals. Sakaguchi et al. (2010) have demonstrated that milling at temperatures of liquid nitrogen without oxygen causes breakage of bonds around the glycosidic linkage and formation of alkyl and alkoxyl radicals. Later on, this knowledge has been generalized somewhat too readily and the general conclusion appeared that milling always causes production of alkoxyl radicals which are the species observed by EPR and also the species initiating radical polymerizations. This general conclusion is not valid.

It is commonly discussed that input of mechanical energy into cellulose by milling will cause breakage of the weakest bond, with the bond strength being thermodynamically defined by the free bond dissociation energy between the two bound atoms. This energy always refers to the homolytic cleavage that leaves two radicals behind (and is thus different from the heterolytic bond dissociation energy that can be derived from the former value by thermodynamic transformations involving electron affinities). Comparing standard values of average bond energies it becomes clear that the glycosidic linkages – the bonds “left” and “right” of the glycosidic oxygen bridging two anhydroglucose units – are the weakest spots along the cellulose. However, that does not necessarily mean that they are the ones which are broken. The weakest bonds are then tribochemically cleaved when the incoming energy is equally affecting the whole molecule and all bonds are experiencing the same “stress”. This requires both time and sufficient mobility of molecular parts for the energy to dissipate uniformly. If time and/or mobility are missing, the impacting energy will act at its spot of impact and break the bonds at this location, independent of whether there are less stable bonds somewhere else in the molecule. Imagine a paper chain made of paper chain links.

When it is stretched slowly the weakest link along the chain will break. When it is beaten fast with a stick, it will break at the spot where the stick hits. The free cellulose chain behaves analogously upon mechanical impact. Thus, the results of mechanical impact on cellulose are primarily depending on the conditions, and this crucial dependence has been somewhat neglected so far. Under conditions where the incoming mechanical energy can freely dissipate, e.g. at room temperature with high thermal motion and in little ordered molecular structures, such as amorphous regions or solutions, the glycosidic linkages are most prone to bond cleavage and mechano-radical formation. When this mobility is restricted – either by restricting thermal motion at low temperatures or in regions of high molecular order, such as crystalline domains, a more random bond cleavage upon impact of mechanical energy will result.

First, dissipation of impacting mechanical energy implies molecular motion, and the mobility is much larger in amorphous regions of cellulose than in crystalline ones. Thus, mainly the amorphous regions that take up the incoming energy will be affected at room temperature. At very low temperatures (liquid nitrogen), the molecular motion is generally restricted and also the amorphous regions are frozen in a quasi crystalline state. Now, the impact of mechano-energy will be generally smaller as the energy cannot dissipate as efficiently (see Fig. 2), but it will affect both regions more equally, and bond cleavage/mechano-radical formation will also involve crystalline areas.

Second, the issue of (thermal) mobility does not only determine the location where the bond is broken – mainly in amorphous regions at room temperature vs. more uniform at low temperature or mainly around the glycosidic bond at room temperature vs. more uniformly along anhydroglucose units at low temperature – it will also govern the fate of the resulting mechano-radicals. Any further reaction of the primary radicals – if we disregard redox processes involving electron tunnelling – will require molecular motion and proximity of molecular parts to pass on the single-electron character. This is even true for fragmentation reactions and concerted mechanisms that require the molecular part to adapt a special geometry for the reaction to proceed.

At room temperature and especially in solution, the primary mechano-radicals have a rather unrestricted mobility and thus encounter manifold reaction partners and experience multiple pathways. An observation of the radicals by EPR in this case will never report the primary mechano-radicals, but the subsequently formed, more stable radicals. The primary radicals will find ample opportunities to undergo subsequent reactions, to recombine or to form more stable, secondary radical species. Also a reaction of the radicals with spin traps is possible. These spin traps are diamagnetic compounds that are added to form an adduct with the primary radical species which is persistent and observable by EPR. Also a reaction within other moieties in the cellulosic material itself is possible, such as structures being able to stabilize free electrons by steric or electronic (resonance) effects. Aromatic systems, such as furanoids formed from hemicelluloses or phenolic structures contained in lignin, would represent such moieties that would convert unstable primary radicals into more stable secondary ones.

In the case of limited molecular mobility, at very low temperatures and especially in crystalline regions of cellulose, the primary mechano-radicals can neither recombine nor be approached by coreactants that assume the radical character themselves. The enforced (limited) persistence in this case allows EPR to observe largely the primary radical species formed before being converted into more stable secondary products. The use of spin traps in this case is not constructive since the traps could not approach the mechano-radicals to convert them into more stable adducts.

The primarily formed mechano-radicals will be very unstable species. Although Sakaguchi described only the formation of alkyl

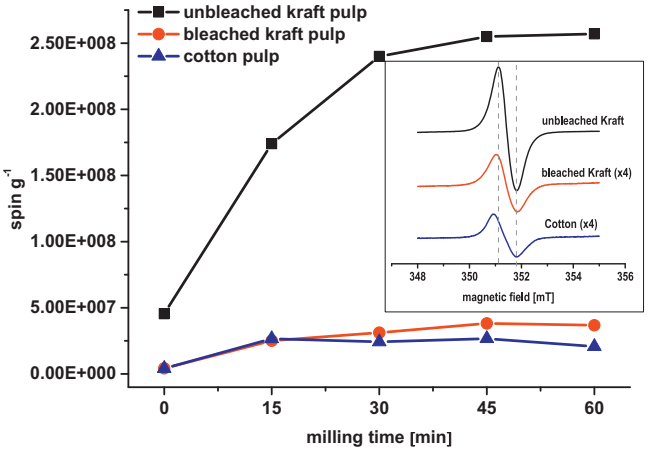

Fig. 4. Intensity of EPR spectra of secondary radicals in pulps after 60 min of ball milling at room temperature. Inset: respective EPR spectra at room temperature (the spectra of bleached kraft pulp and cotton is multiplied by 4 in order to see the change in the linewidth).

and alkoxyl radicals, it must be kept in mind, that homolytic bond cleavage around the glycosidic linkage will produce one carbon-centred radical per alkoxyl radical formed, even though it might not be observable by EPR. A cleavage of a carbon–carbon bond within the anhydroglucose unit will even produce two C-centred radicals. The most prominent reaction of the C-centred radicals will be the reaction with dioxygen – a biradical itself – to peroxyl radicals. This brings about a considerable resonance stabilization of the unpaired electron and much higher stability of the radical. The alkoxyl radicals do not consume dioxygen, but will primarily react according to fragmentation pathways, the beta-fragmentation being most common. The driving force is the formation of a stable carbonyl species with a thermodynamically favourable C=O double bond. The simultaneously formed C-centred radical will once more form a peroxyl radical with dioxygen. In many cases, peroxyl radicals falsely pretend to be the primary radical species as beta-fragmentation of the actual primary alkoxyl radical and subsequent reaction with dioxygen can be very fast processes. The peroxyl radicals can undergo H-abstraction under formation of organic peroxides which, in turn, due to the weakness of the O–O bond, might fragment into alkoxyl and hydroxyl radicals, thus initiating a whole cascade of homolytic follow-up processes.

In the present study we have observed the radical formation upon mechano-stress for three different cellulosic substrates, a cotton linters, a bleached pulp with a residual content of oxidizable structures (kappa number around 3), and an unbleached kraft pulp with typical residual lignin content (kappa number 26). The mechanical energy was introduced by ball milling at room temperature, and EPR spectra were measured afterwards. Based on the above, it is evident, that no primary radicals can be observed by EPR, but only those secondary radicals that are persistent enough to overcome the period between formation and measurement are detected, and can prevail up to several hours and days. All systems and reactions were performed under ambient conditions; oxygen was freely available to react. The EPR spectra of the three different samples were rather similar, but had slightly different line widths (ΔB_{PP}) (Fig. 4, Table 2). They are characterized by a broad single peak which are coming from stable radicals with an R–O· structure as indicated by *g*-values. While Solala et al. assign these to stable alkoxyl radicals which are formed by the cleavage of the glycosidic bond (Solala et al., 2012), we exclude this explanation based on the above general considerations, and propose a mixture of different peroxyl and to some extent phenoxyl radical species instead. In

Table 2
Dependence of mechano-radicals on lignin content in pulps.

Sample type	Kappa number	g value	ΔB_{PP} (mT)
Unbleached	25.9	2.0042	0.7
Bleached	3.0	2.0045	0.8
Cotton	0.11	2.0049	0.9

general, the overall radical content increased with increasing time of milling, and the mechano-radical content was largely dependent on the lignin content in the pulp, which would corroborate the phenoxyl radical hypothesis. Phenoxyl radicals can also be generated from pure celluloses or sugar model compounds by ageing processes (stress under alkaline, acidic or thermal conditions), which would explain its presence in low concentrations also in the lignin-free cotton.

In a set of consecutive experiments, the milling was conducted at low temperatures (77 K), and was followed by EPR at the same temperature. Thus the system had no opportunity to "equilibrate", and the primary radicals were supposed to be still observable. The EPR spectrum from cryomilled cotton (Fig. 5, left) had two contributions, a main one from peroxyl radicals and a minor from phenoxyl radicals. The EPR spectrum of the peroxyl radical (Fig. 5, left) was simulated with $g_{II} = 2.006$ and $g_{\perp} = 2.034$. This is similar to the parameters for peroxyl radicals reported by Hon (1979). This main observable radical species, contributing to about 74% of the total spectral intensity, is evidently not the primary mechano-radical. It can be formed from a primary carbon-centred radical by dioxygenation or from a primary alkoxyl radical according to a beta-fragmentation/dioxygenation sequence. The reaction with dioxygen was evidently so fast that the actual primary radical species (carbon-centred or alkoxyl radicals) were not detected.

The minor contribution to the total EPR intensity arose from phenoxyl radicals, characterized by $g = 2.0039$ (Kuzina, Brezgunov, Dubinskii, & Mikhailov, 2004). The underlying phenolic unit is evidently not originating from lignin (cotton linters), but is formed from carbohydrate degradation products in condensation processes. The term "phenoxyl" is somewhat misleading as the species can also be of similar types that cannot be distinguished solely by EPR, such as furanoxyl or benzofuranoxyl structures (Lagercrantz, 1964).

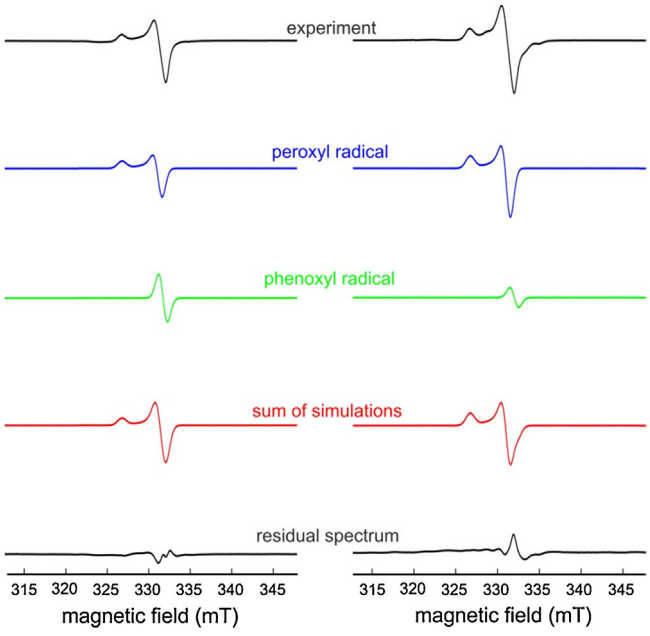

Fig. 5. Experimental EPR spectrum and spectral simulations from individual spectra of cryomilled cotton (left) and bleached kraft pulp (right) measured at 77 K. The residual spectrum is the experimental spectrum after subtraction of the simulations, indicating the contribution of additional radical species.

In the low-lignin, bleached kraft pulp, cryomilling induced formation of similar radical species, although the ratio was different with less phenoxyl radicals contributing to the overall radical amount (Fig. 5, right). The g-value of the phenoxyl radical is 2.0017, so it is most likely not the same radical source as for the phenoxyl free radical detected in the cotton sample or unbleached sample. In addition, at least one other radical species was detected which could not be identified yet (Fig. 5, right, residual spectrum). It should be noted that it is unlikely that the phenoxyl radicals have been formed by reaction of lignoid phenol units with the primary radicals in an antioxidant-type action mode. This would require large mobility of the systems with the phenols being able to closely approach the locations of mechano-radical formation. However, such mobility is not given under the prevailing conditions. It must be reasoned instead, that the phenoxyl radicals have been formed directly from the residual by tribochemical stress, not indirectly by the antioxidative action of the lignin towards carbohydrate-derived primary radicals. This is an interesting conclusion that complements – if not predicts – the usually proposed antioxidative action mode of residual lignins in pulps. The direct tribochemical formation of the phenoxyl radicals in the lignin part cannot occur from phenol moieties, i.e. by H-atom abstraction. There are two reasons for that: first, the phenolic hydroxyl cannot absorb sufficient mechanic energy to bring about cleavage of the O–H bond, and second, release of an H-atom would be thermodynamically extremely unfavourable. The phenoxyl radicals can thus only be formed by internal cleavage of interphenolic linkages in lignin, i.e. O-4-structures in beta (and some alpha) position. Generation of such a phenoxyl radical would be accompanied by concomitant formation of a C-centred radical at position beta (alpha), which would immediately react with dioxygen and contribute to the peroxyl radical part observed.

In cryomilled pulp with higher lignin content, also peroxyl radicals and phenoxyl radicals were detected in addition to at least one other radical species which could not be identified solely based on the EPR spectra (Fig. 6). With this sample, the ratio between peroxyl and phenoxyl radical species was shifted in favour of the latter, comparable to the ratio in the cotton sample. The lignin increasingly acted as sacrificial substrate – not in the sense of a traditional antioxidant by converting reactive primary radicals into less reactive secondary ones, but by taking up the impact of the mechanical energy and producing phenoxyl radicals and peroxyl radicals directly in addition to other paramagnetic reaction products (Fig. 6, residual spectrum). It was evident that the increased concentration of the lignin made reaction modes and lignin-derived radicals visible that remained unidentified for the bleached and unbleached pulp samples discussed above. The g-value of the "phenoxyl" contributor was in between the g-values from the corresponding free radicals from cotton and bleached kraft pulp with $g = 2.0029$. It is reported that the g-values of the phenoxyl radicals vary between 2.003 and 2.004 (Kuzina et al., 2004). This indicates that there are slightly different sources of radicals for the present free radical sample and hence different radical species contributing as well. These species will probably not be just "proper" phenoxyl radicals, but also similar species as indicated above.

In a subsequent experiment, the cryomilled cellulose were mixed with a spin trap (DMPO) and brought to room temperature, to detect reactive short-lived (primary and secondary) radical species and possible retrieve additional information about structures involved. Phenoxyl radicals are generally not detected by

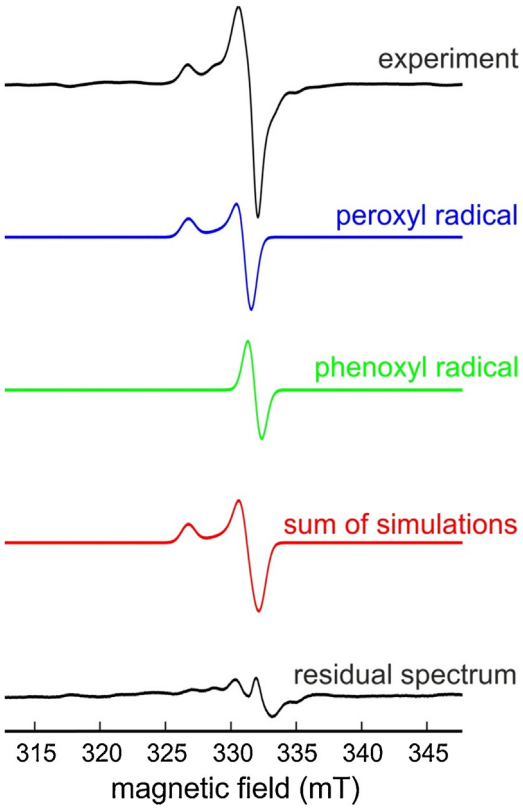

Fig. 6. EPR spectrum and simulations from individual spectra of cryomilled unbleached kraft pulp, measured at 77 K. The residual spectrum is the experimental spectrum after subtraction of the simulations, indicating the contribution of other radical species.

DMPO as they are too unreactive towards the spin trap, so that those radicals are "blinded out" and only the follow-up chemistry of the peroxyl radicals as the second main contributor is observed.

For the low-lignin and cotton sample two other radical species next to the hydroxyl radical adduct were trapped: the carbon dioxide anion radical ($a(^1H) = 1.88$ mT, $a(^{14}N) = 1.57$ mT (Buettner, 1987) and C-centred radicals ($a(^1H) = 2.30$ mT, $a(^{14}N) = 1.59$ mT (Buettner, 1987)) (Fig. 7).

A possible pathway for the formation is from phenoxyl radicals being generated tribochemically directly from residual lignin fragments with O-4 cleavages causing the actual phenoxyl radical, or from chromophoric condensation products of carbohydrate fragments. If such a reaction releases the beta-substituent as phenoxyl radical, the second resulting carbon-centred radical could be trapped directly with DMPO and account for the C-centred adducts. Another prominent reaction path would be dioxygenation, followed by beta-fragmentation and rearrangement of the peroxyl group to a hydrogen carbonate radical, which is relatively stable and has even been discussed as species in bleaching scenarios (Carlsson et al., 2006; Stenman et al., 2003). This radical is trapped as spin adduct by DMPO, after deprotonation. This radical may also be involved in the actual oxidation reaction of the pulp demonstrated in this study.

In the case of unbleached kraft pulp only a signal from the hydroxyl radical adduct ($a(^1H) = a(^{14}N) = 1.49$ mT; Buettner, 1987) was found (Fig. S1). The hydroxyl radicals are formed by homolytic O–O cleavage of peroxides R-OOH, which in turn are direct H-abstraction products of the peroxyl radicals. The simultaneously generated alkoxyl radicals evidently react faster by beta-fragmentation and with dioxygen than with the spin trap as no alkoxyl adducts were found. The ubiquitous and higher amount of lignin present in this sample does obviously not allow for an extraction of other radicals as they are fixed in the lignin structure. This is in agreement with the overall higher radical concentration (Fig. 4) and also with the higher concentration of stable phenoxyl radicals (Fig. 6).

In summary, under the conditions used, we were unable to detect the primary tribochemically generated radical species, but the peroxyl radicals resulting from them by reaction with dioxygen. Under room temperature conditions phenoxyl-type radicals dominate (cf. Fig. S2). They are even contained in cotton where they can be generated by condensation reactions from carbohydrate fragments. In low-lignin and especially high-lignin pulp they are derived from the naturally present lignin, of which the phenol units act in an antioxidative mode with the reactive primary radicals. Under cryomilling conditions, such a typical antioxidative action is not possible due to restricted molecular mobility. Since still phenoxyl radicals are detected, they must have formed tribochemically in a direct way from the lignin. The detection of other secondary radical species (C-centred radicals and carbon dioxide anion radicals) by spin trapping with DMPO, corroborate a formation by cleavage of alpha- and beta-O-4 structures, rather than a direct formation from the phenols. A schematic illustration of formation of different radicals is given in Fig. 8.

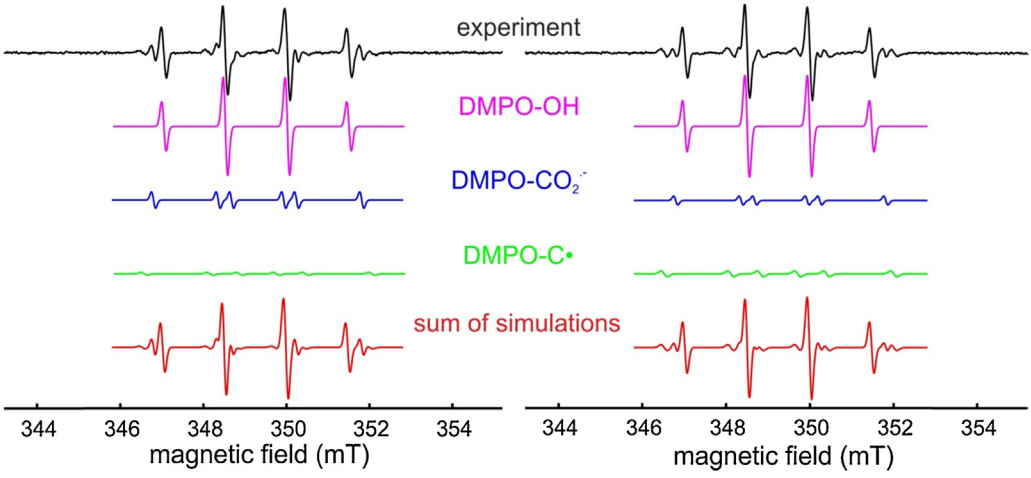

Fig. 7. EPR spectrum of the DMPO-extract from cotton (left) and bleached kraft pulp (right). Simulations of the individual DMPO-adducts have been included.

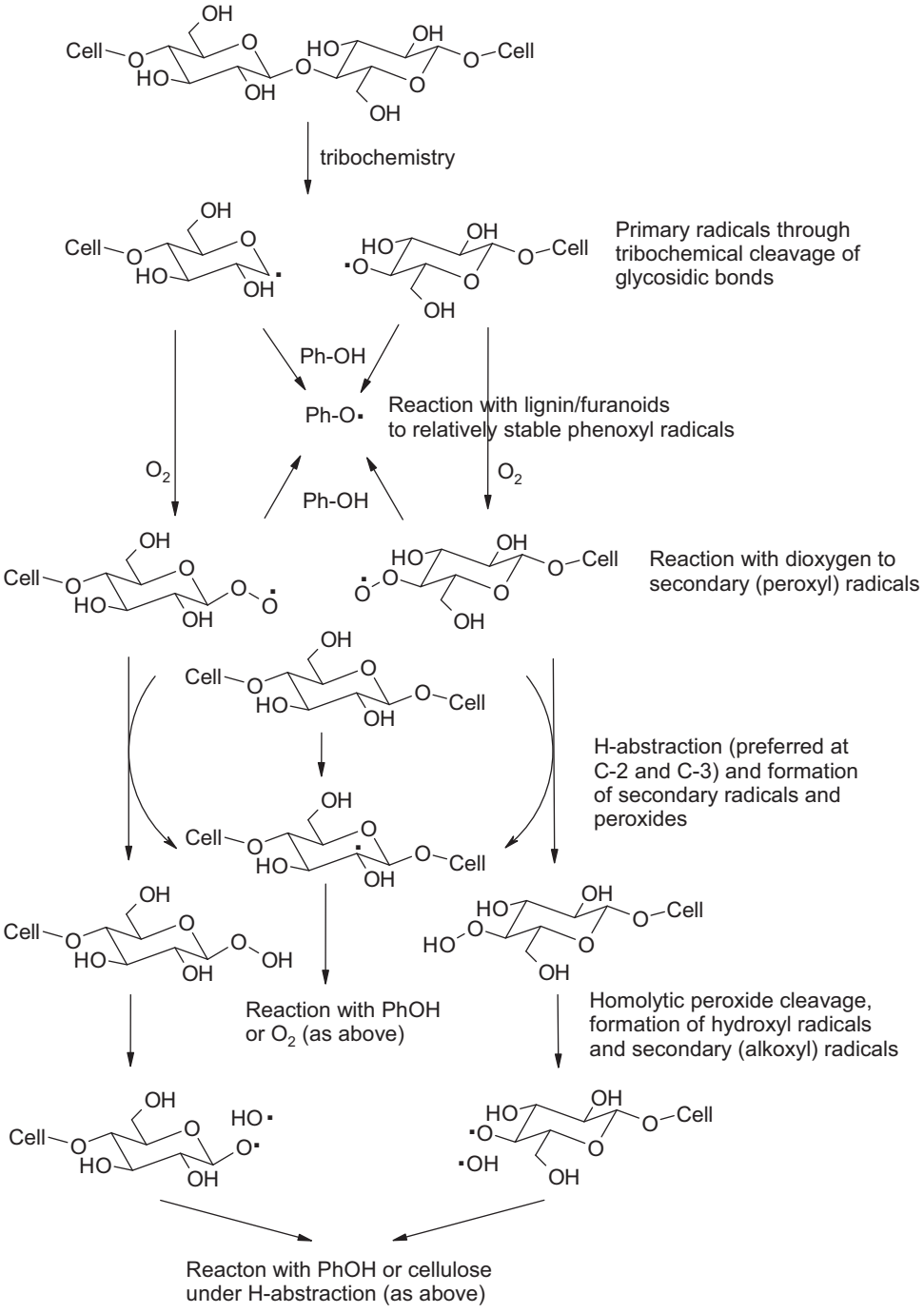

Fig. 8. Schematic illustration of different primary and secondary radicals formed.

4. Summary and conclusion

Mechanical treatments are very effective in order to degrade cellulose pulp and lignin containing pulps in a defined way. Both sonochemical treatment and milling degrade the cellulose towards a limiting molecular weight which is not further diminished even by prolonged further tribochemical treatment.

Cryomilling (77 K) caused less pronounced cellulose degradation, reaching a Mw level of about 80% of the starting material after 60 min of milling. The chemical changes observed are just cleavage of glycosidic bonds, no significant degree of additional oxidation was observed. Also the crystallinity index was only slightly affected. Hence, if one aims at slightly reducing the molecular weight of celluloses without additional changes, milling at 77 K is the method of choice.

Increasing the milling temperature to ambient conditions changed the situation and caused a more intense degradation, which shifted the Mw_{lim} to significantly lower values. A similar value is obtained upon sonication of cellulose in solution. However, the shape of the molecular weight distribution, reflected in the polydispersity index (PDI) is completely different in the two systems – for sonication it moved towards monodispersity whereas milling definitely increased the PDI. The dissipation of energy during milling effects different chain lengths and leads to cleavage of different chain lengths, whereas sonication preferentially cleaves longer chains. The M_{lim} was largely independent of the presence

of lignin and hemicelluloses, but evidently dependent on the conditions (time, temperature, solvent, etc.). During ball milling at ambient temperature and above, also oxidation occurred as a side reaction. The formed carbonyl groups are located rather randomly along the cellulose polymer chain.

The presence of secondary radicals was shown by EPR also in combination with spin trapping. A major radical type is peroxyl radicals, formed directly from C-centred radicals by reaction with dioxygen, or indirectly from alkoxyl radicals after beta-fragmentation and dioxygenation. In particular the carbonate anion radical, detected as minor contributor to the overall radical content, may serve as a possible oxidant. In general, elevated temperatures clearly favour oxidative stress at cellulose during milling. In addition to peroxyl radicals, also a minor contribution of phenoxyl-type radicals was found, which originate from residual lignin or chromophores which are ubiquitous in cellulosic materials, also in cotton linters and fully bleached pulps. Stable alkyl or alkoxyl radicals were not detected.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.05.011.

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