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Improvement of enzymatic digestibility of wood by a sequence of optimized milling procedures with final vibratory tube mills for the amorphization of cellulose

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Abstract: A three-stage wood milling process was investigated leading to coarse, fine and amorphization of milled wood (MW) as a pretreatment for enzymatic wood hydrolysis. An eccentric vibratory tube mill (EVTM) and a spring suspended vibratory tube mill (SSVTM) were found to be suitable for wood cellulose amorphization. Both methods gave rise to highly digestible and amorphous wood powders amenable to enzymatic hydrolysis. The SSVTM had superior energy efficiency. The resulting MW afforded a 70% sugar yield via enzymatic hydrolysis and the total energy consumption was around 1.5 kWh kg⁻¹ oven-dried wood (odW) for all three milling stages. In contrast, EVTM consumed 17 kWh kg⁻¹ odW energy. Accordingly, SSVTM has a high potential for preparing wood for enzymatic hydrolysis.

Keywords: amorphization milling, energy consumption, particle size, pretreatment, sugar yield

Introduction

There has been much research effort to improve the enzymatic and acid hydrolysis of wood towards saccharification, which can be done using ozone pretreatment (Sugimoto et al. 2009), steam pretreatment (Schütt et al. 2011, 2013; Muzamal et al. 2015), organosolv pretreatment (Díaz et al. 2011), sulfite pretreatment (Zhou et al. 2016) and acid hydrolysis (Park and Um 2015), just to name just a few. These kinds of pretreatments are expensive and entail environmental problems including high chemical

and water consumption. In the case of a mechanical pretreatment (MP), the environmental burden is lower. MP via milling leads to milled wood (MW) with destroyed cell wall ultrastructures and reduced cellulose crystallinity, in which all components have an improved accessibility for chemical and enzymatic hydrolysis. The MW can be separated into lignin via dioxane/water extraction (leading to MW lignin, i.e. to MWL), and digestible carbohydrates by cellulolytic enzymes. This approach is well tested in laboratory experiments, but a cost-effective large scale technology is not available.

Up to 90% sugar yields can be obtained via enzymatic hydrolysis of MW (also called micronized wood powder) (Inoue et al. 2005; Jamal et al. 2011; Hoeger et al. 2013) because of the large accessible surfaces (Bychkov et al. 2012). Moreover, the crystallinity index (CI) of cellulose is diminished and the polysaccharides are depolymerized (Schwanninger et al. 2004; da Silva et al. 2010; Tunc et al. 2010; Avolio et al. 2012). However, the milling in small ball mills consumes a lot of energy (Agarwal et al. 2012).

Conventional milling operations, such as hammer milling, knife milling and disk milling, typically break down the particle size, but do not alter the CI of cellulose. Only high-energy milling is able to reduce the cellulose CI effectively (Bychkov et al. 2012; Jiang et al. 2017), which is needed for a better enzymatic digestibility (Leu and Zhu 2013; Jiang et al. 2016). Effective bench-scale milling techniques in this regard are rotary ball milling (Agarwal et al. 2012), oscillating ball milling (Karinkanta et al. 2013), vibratory ring milling (Takahashi et al. 2014b), converge ball milling (Fukumura et al. 2010), planetary ball milling (da Silva et al. 2010; Jamal et al. 2011), attrition ball milling (Kim et al. 2013) and roller compression milling (Ryu et al. 1982). The milling parameters influencing the size reduction and the CI are feedstock type, initial feedstock size, type of grinding media (balls, rods, cylinders, rings, etc.), mill rotation speed, vibration amplitude and milling duration (Karinkanta et al. 2013, 2014; Kim et al. 2013). The velocity of a rotary ball mill is limited by its critical speed, thus the size reduction rate is much lower than that of a planetary ball mill (Schwanninger et al. 2004; Zhao et al. 2006; Kim et al. 2013). In terms of hydrolysis sugar yield per unit of energy consumption, a vibratory ball mill performs better than a rotary ball mill, but less well than a planetary mill and a converge mill (Fukumura et al. 2010). The two

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latter mentioned mills have a low scaling up potential to industrial production size. A vibratory tube mill is based on the medium impact energy combined with a stable vibrating mechanism and it is effective to produce MW as a digestible powder. A vibratory “tandem-ring mill” with cog-rings or disks as grinding media, which are arranged in parallel inside a tube chamber, are versatile, as they also have an optimizable gap between the rings and the tube chamber (Takahashi et al. 2010, 2012). The tandem-ring mill shortens the pulverizing time by one-fifth compared to a conventional vibratory ball mill (Takahashi et al. 2010). Thinner tandem cog-disks perform better than the thicker ones with respect to enzymatic digestibility (Takahashi et al. 2010). Tandem cog-rings perform better than tandem cog-disks; a pulverization gap of 26-mm is better than that with a 33-mm gap (Takahashi et al. 2012). The energy efficiency of the continuous tandem ring mill for potential scale-up production was also investigated (Mori et al. 2012) but the design process is not yet optimized.

Nevertheless, MP is energy intensive (Karinkanta et al. 2012). Probably, the energy efficiency can be improved by employing several operation units arranged in a sequence (Reineke 1966; Silva et al. 2012). A three-stage milling process was envisioned to obtain coarse, fine and amorphized powders, because each stage can be separately optimized. Size reduction can be achieved by a hammer mill and an air classifier mill (ACM) for the coarse and fine milling stages, respectively. The subsequent vibratory milling leads to CI decrement of cellulose (“amorphization”). The construction of modern hammer mills and ACM systems are designed to break down particles with minimal friction heating. ACM is more effective than a hammer mill for target sizes between 200 and 100 μm (Heimann 2014). A vibratory media mill induces breakage across the microfibril orientation due to the high amount of compressive forces generated by the media and makes the cellulose more susceptible to enzymatic digestion. The most challenging stage is amorphization because of its high electrical energy consumption.

In the present paper, a hammer mill was applied to grind wood chips to a geometric mean particle size between 7810 and 700 μm and then MW was produced in an ACM with a median particle size (D_{50}) below 114 μm (Liu et al. 2016a). The amorphization milling performed in an eccentric vibratory tube mill (EVTM) is compared with that obtained by a spring suspended vibratory tube mill (SSVTM). The specific energy consumption, MW characteristics and enzymatic hydrolysis yields are analyzed with the expectation that an improved process design facilitates their engineering integration into the MP for enzymatic hydrolysis.

Materials and methods

Materials: Douglas-fir forest harvest residual (FHR) chips were the fraction passing through the 45-mm screen and retained on the 3.35-mm screen. Two fine MW samples (powders) were prepared as feedstock for the amorphization milling. The first, MW_I , was prepared by grinding wood chips into particles with a geometric mean diameter of 669 μm using a Bliss Eliminator Fine Grind Hammer mill circuit (EMF-24115-TFA, Bliss Industries, Inc. Ponca City, OK, USA), which was consuming 0.066 kWh kg^{-1} oven-dried wood (odW). These particles were further ground by an air classifier mill (ACM, Mikro ACM® Model 15, Hosokama Micron Powder Systems, Summit, NJ, USA) with an energy consumption of 0.458 kWh kg^{-1} odW. MW_I had $D_{90,I}$ of 339 μm , a $D_{50,I}$ of 114 μm and a $D_{10,I}$ of 21 μm ; the wet basis moisture content (MC_{wet}) was 3.6% and a bulk density of 0.175 g cm^{-3} . The total energy consumption was 0.524 kWh kg^{-1} odW.

MW_{II} was prepared by grinding the wood chips with the same hammer mill as for MW_I to particles with a geometric mean diameter of 316 μm , while the energy consumption was 0.19 kWh kg^{-1} odW. The subsequent milling in the same ACM as for MW_I with an additional energy input of 0.699 kWh kg^{-1} odW. MW_{II} had a $D_{90,II}$ of 194 μm , a $D_{50,II}$ of 63 μm , a $D_{10,II}$ of 11 μm , a MC_{wet} of 3.6%. MW_{II} required a total of 0.899 kWh kg^{-1} odW for the first two stages of milling.

Energy consumption: The target for final particle size of amorphization milling D_{50} was around and below 30 μm . The measures for the milling performances of the two vibratory mills were the CI and the enzymatic digestibility. The milling time was recorded for each run. The energy consumptions were calculated by multiplying the total rated powers of each vibratory mill and the milling duration. The energy consumed was not corrected by that of an empty run.

Trials in EVTm: MW_I was sent to Siebtechnik GmbH, Germany to be ground with an EVTm (ESM 236-bs, Siebtechnik GmbH, Mülheim an der Ruhr, Germany) (Figure 1a). Grinding elements are comprised of a steel wear lining and steel balls with diameters of 15–20 mm. The rated power of the milling circuit was 2.5 kW. Milling was carried out in batches with 0.8 kg of MW (4.5 l) per test and the mill was operated at a rotation speed of 1000 rpm and vibrating amplitude of 6 mm. Seven individual batches of samples were generated, respectively, for milling durations of 15, 30, 45, 60, 90, 120 and 300 min. Final temperatures in the milling zone were recorded with an infrared thermometer.

Trials in SSVTM: The mill VKE 1040 (Micro Grinding Systems, Inc., Little Rock, AR, USA) was available to produce MW_I and MW_{II} . Figure 1b illustrates the mill, consisting of a milling chamber with a hardened steel inner wall, mixed diameter rods as grinding media, an exciter with a 2-kW motor and 12 support springs, split evenly and radially configured on both ends of a frame. The mill chamber has an outer case and an interchangeable 9-mm-thick inner liner, each made with different materials. The mill chamber is shaken by rotary vibrations generated from the motor rotations combined with an unbalanced weight distribution. The circular movement of the mill chamber moves the grinding media in a rolling motion, which generates a centrifugal force for pulverization. Vibrational energy is stored in the springs and fed back into the milling action, which reduces the required energy.

Two grinding media were tested. The first was 135 kg, 990 mm long, 4140 steel rods with the mass divided evenly between three different diameters: 19, 12.7 and 9.5 mm. The second grinding media tested

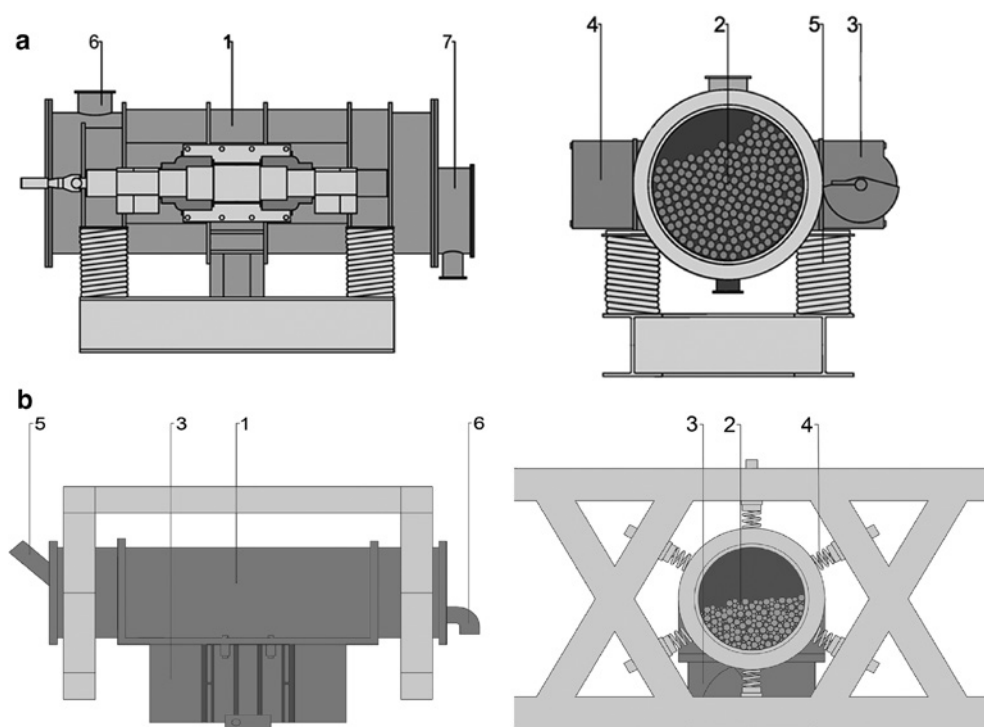


Figure 1: Two mills for the amorphization of cellulose.

(a) Diagram of the eccentric vibratory tube mill (ESM 236-bs, Siebtechnik GmbH, Germany) consisting of (1) cylindrical grinding vessel, $D \times L = 235 \text{ mm} \times 450 \text{ mm}$, 19 l volume, (2) grinding steel balls, $\varnothing 15\text{--}20 \text{ mm}$, (3) exciter, (4) counter weight, (5) helical springs, (6) inlet and (7) outlet. (b) Diagram of Model VKE 1040 ($D \times L: 10 \text{ in.} \times 40 \text{ in.}$, 51.5 l in volume) spring suspended vibratory mill showing the exciter (3) attached along the bottom of the tube (1), the hardened steel grinding rods (2) inside the tube (1), and six springs (4) on each end suspending the mill tube onto the frame. The material feeds in the tube from the inlet (5) and discharges from the outlet (6).

was 204 kg of tungsten carbide cylpebs with a diameter of 19 mm and a length of 31.8 mm. The cylpebs had a flat shoulder on one end and were rounded on the other end. The motor was set to 1882 kg of force at 1800 rpm with a vibrating amplitude of 2.38 mm. The MWs were processed in 1.91 kg (10.9 l) batches. Grinding durations for the hardened steel media were 45 and 75 min. Grinding durations for the tungsten carbide media were 20, 45 and 75 min. In addition to the tests for each chosen media, two runs were completed with hardened steel rods with the additions of 5% quartz powder (230 mesh particle size) boW to evaluate a performance improvement via hard mineral particles.

Characterization of micronized wood: Particle sizes were analyzed by a laser scattering particle size analyzer (Malvern Mastersizer 3000, Malvern Instruments Limited, Malvern, UK). Bulk density of the MWs was measured with a graduated cylinder and balance. The diffractograms were obtained with a powder X-ray diffractometer (Miniflex 600, Rigaku Corporation, Tokyo, Japan) with Cu K α ($\lambda = 0.154 \text{ nm}$) radiation generated at 40 kV and 15 mA. The Cls were calculated from the diffractograms according to Segal et al. (1959).

Hydrolysis conditions: Five percent solid slurry was prepared with 1 g of micronized wood in 20 g total of aqueous solution (including sodium citrate buffer at pH 4.8, water, sodium azide, enzyme and micronized wood) according to Selig et al. (2008). Enzymatic hydrolysis was conducted under 5.0% solid loading, 5.0% Cellic CTec2 and 0.5% HTec2 (Novozymes North America, Franklinton, NC, USA), while the enzyme dosages were based on odW mass; incubation time

72 h in a shaking table at 50°C. Sugar yields are reported as the average of two replicates.

Wood composition and sugar analysis: The usual two-step acid hydrolysis procedure was performed according to the NREL analytical procedure “determination of structural carbohydrates and lignin in biomass” (Sluiter et al. 2012). First, a 300-mg odW sample was hydrolyzed with 3 ml of 72% H_2SO_4 for 1 h at 30°C. Then, 84 ml of water was added into the mixture and autoclaved for 1 h at 121°C (Liu et al. 2016b). Sugar yields after enzymatic hydrolysis were analyzed by HPAEC (ICS-3000, Dionex, Sunnyvale, CA, USA) equipped with ED 50 electrochemical detector (Dionex Corp., Bannockburn, IL, USA). Sugars were separated on CarboPac PA 20 Guard ($4 \times 50 \text{ mm}$) and analytical columns ($4 \times 250 \text{ mm}$) at 25°C. Due to co-elution of xylose and mannose, the yield of these sugars are reported as xyl/mannan.

Results and discussion

Chemical composition of wood

The chemical composition of the original wood sample is 40.9% glucan, 14.8% xyl/mannan, 3.3% arabinan, 2.7% galactan and 29.9% total lignin. Total sugar yield was

calculated as the percentage of recovered glucan and xyl/mannan from the starting material.

Milling in EVT

The physical characteristics and enzymatic hydrolysis performance of the MW obtained by EVT is summarized in Figures 2 and 3. The milling generated heat was 75°C after 120 min (Figure 2). With water cooling, the temperature was held at 72°C for longer than 300 min milling time. The bulk density of MW increased with the milling time because the lowered aspect ratio of the particles permitted a higher packing density. The decreasing particle size leveled off to around 20 μm after certain grinding times. A milling time around 90 min can be considered as an optimum. Longer milling times led to agglomeration and the particles became coarser. After 300 min, the material was not finer than after 90 min of milling. The crystallinity decreased quickly with grinding time in the early grinding period and then the CI decrement was slow (Figure 3).

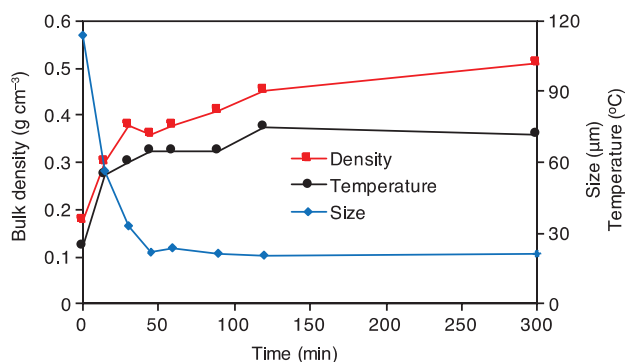


Figure 2: Bulk density, particle size and temperature in the milling zone for multiple milling durations (as a function of milling time).

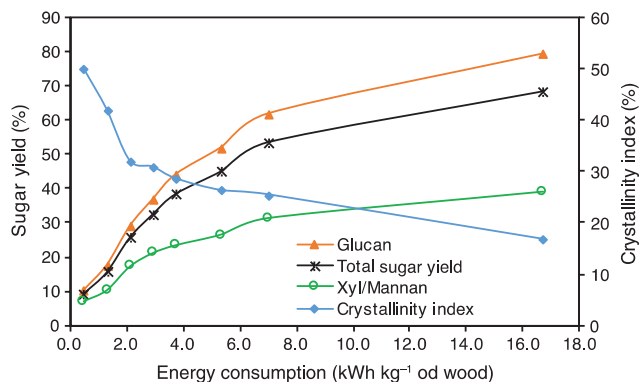


Figure 3: Enzymatic hydrolysis sugar yields and crystallinity index of the micronized wood powders as a function of the total energy consumption.

Takahashi et al. (2014a,c) made a similar observation concerning the agglomeration after an extended milling time and found that the attainable particle size is typically around 15–30 μm , though the results are dependent of species and milling parameters. The obtained lamellar and scaly aggregates were highly digestible. The milling can also be considered as a mechanical amorphizing, analogous to mechanical alloying (Watanabe et al. 1995), which is a repeated micro-forging and kneading process leading to decrystallizing the cellulose moiety, even if the particle size is not changing.

Figure 3 shows the increasing sugar yields as a function of milling time. However, the EVT did not meet the chosen target of 60% glucan yield at the energy input of 1.5 kWh kg odW higher energy input is economically not competitive with other pretreatment methods.

Milling in SSVTM

This approach was effective and the particle size of MW had a D_{50} less than 30 μm after 75 min milling time. Figure 4a shows that the tungsten carbide cylpebs are more energy-efficient than the hardened steel rods concerning the amorphization of cellulose. The addition of 5% quartz fine powder into the material enhanced the performance of the hardened steel rods. Figure 4b–d illustrate the sugar yields from enzymatic hydrolysis, which was consistently increased with increasing energy input. As expected from the CI trends, the tungsten carbide media rendered higher sugar yields than other grinding media. Quartz powder together with hardened steel rods led to similar sugar yields as the tungsten carbide media.

The energy input of 1.5 kWh kg⁻¹ odW generated almost 70% total sugar yield (Figure 4d), which is promising and indicates that this type of MP might be economically feasible. This sugar yield was also achieved with the tungsten carbide cylpeb grinding media and the hardened steel inner chamber wall. It can be assumed that a tungsten carbide inner wall would further increase the efficiency. Future work should optimize the grinding conditions concerning the media geometry, weight and charges. It is probable that a smaller size of grinding media could be more effective. SSVTM is more efficient than EVT.

Process design

Continuous milling can be realized with a closed circuit consisting of a bin, screw feeder, the SSVTM, blower, cyclone separator, air lock discharger and dust collector by recirculating the material through the milling zone continuously

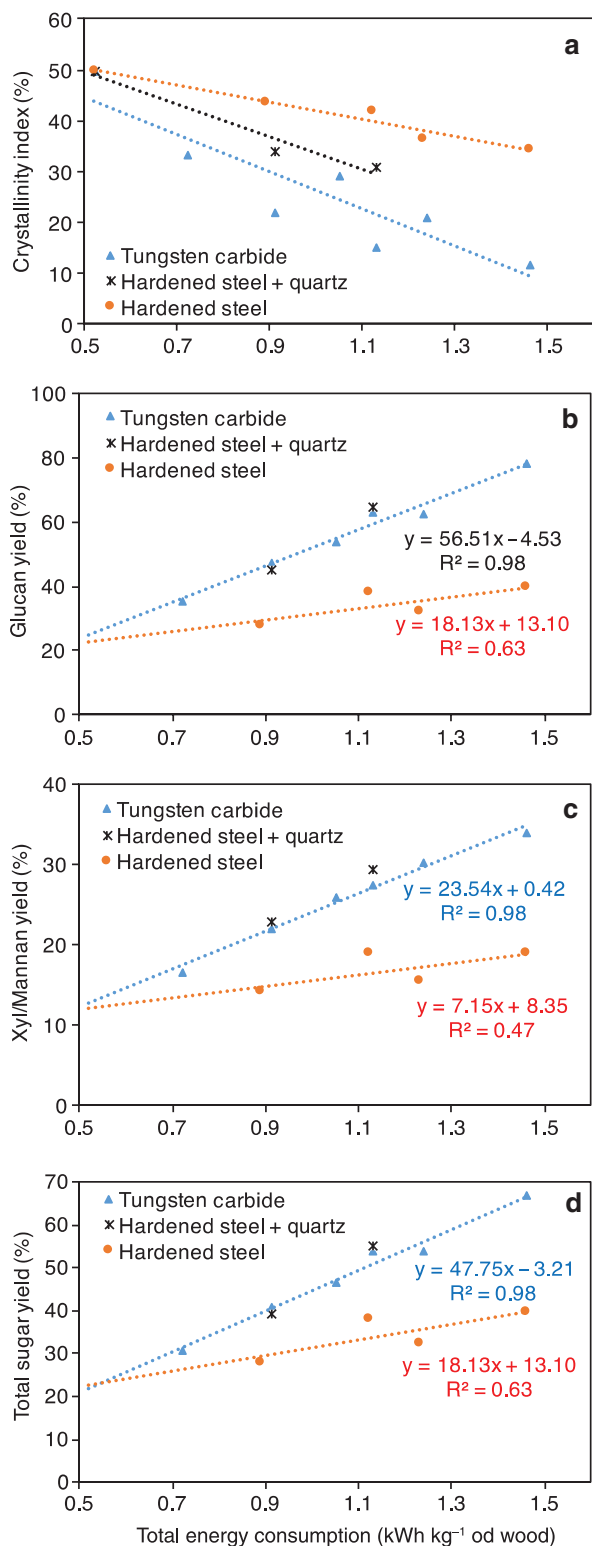


Figure 4: The results obtained by three grinding media in the spring suspended vibratory tube mill as a function of the total energy consumption concerning: changes of cellulose crystallinity index (a), glucan yield (b), xylan and mannan yield (c) and total sugar yield (d). The total energy is the sum of those consumed in three milling stages. Some data points came from Powder I and some from Powder II.

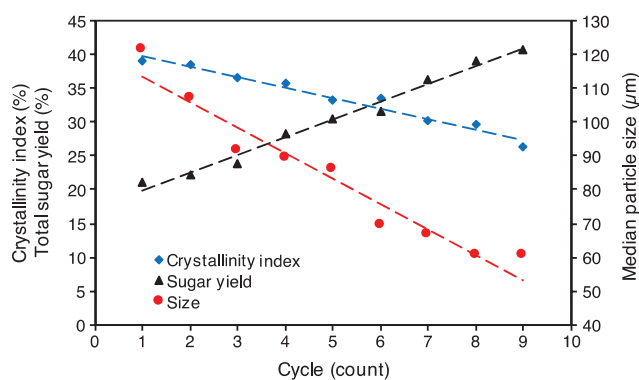


Figure 5: Results obtained in a continuous recirculating milling circuit via combination of a spring suspended vibratory tube mill, feeding unit, pneumatic conveying and dust collector. The physical characteristics and enzymatic performance changes with each pass of the milling zone.

until the best particle size and CI is obtained. Operational parameters for a target CI can also be preselected. To test this concept, MW_I went through nine milling cycles with the hardened steel rods and the hardened steel inner wall. The high-speed vibration kept the feed going through the mill as fast as it was fed. The residence time in the milling chamber was around 10–15 s with a feed rate of 7.6–11.5 kg min⁻¹. The physical characteristics and enzymatic performance are summarized as in Figure 5. As visible, the particle size and CI decreased linearly with the number of cycles, while the total sugar yield was increasing. In practice, the milling parameters – with a first rod milling and succeeding mills equipped with balls, or rings – can easily be adjusted to obtain a highly digestible powder with a high throughput rate (Takahashi et al. 2014a).

Conclusions

Milling trials were carried out by a combination of a hammer mill and an air classified mill down to D₅₀, which leads to particle sizes less than 30 μm either by milling in an eccentric tube mill or in a SSVTM. The milling performances were evaluated by particle size reduction, CI and enzymatically obtained sugar yields. The data correlated well with the energy input. The spring suspended vibratory mill is more energy-efficient and can be considered as an approach with a high industrial application potential.

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