Dry and Wet Milling of Malt. A Preliminary Study Comparing Fermentable Sugar, Total Protein, Total Phenolics and the Ferulic Acid Content in Non-Hopped Worts

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ABSTRACT

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In this work, fermentable sugar, total protein, phenolics and ferulic acid content were estimated in sweet worts at different points of lautering. Transfer of these selected malt compounds into worts was analyzed in relation to the method of malt milling (wet milling of malt - the "test worts" or dry milling of malt - the "reference worts"). Glucose, maltose and maltotriose were more rapidly transferred into sweet worts at the early stages of lautering (40 hL and/or 80 hL of wort) after wet milling in comparison to dry milling. Total protein content in the test worts was significantly higher than in the corresponding reference worts at each stage of lautering. Transfer of phenolic compounds and ferulic acid (in the free as well as in the ester form) from the mash into sweet worts was significantly improved by dry milling, but not by wet milling. No difference in the total antioxidant activity was observed between the two types of worts. In conclusion, it can be stated that wet conditioning of malt before milling enhances the fast transfer of fermentable sugars and proteins from the mash into the sweet wort during lautering. Lautering is a time-consuming process, and time reduction without the loss of wort quality should be a priority. Therefore, wet milling can be of interest to professionals in the field as an interesting alternative method to improve the mashing process.

Key words: dry milling, fermentable sugars, ferulic acid (FA), lautering, mashing, wet milling, wort.

INTRODUCTION

Beer is the most popular low-alcohol beverage consumed in the world. Beer brewing, however, is a very complex process, which is made even more complicated by the fact that brewing raw materials can significantly differ from batch to batch, and the physiological properties of yeast can change during the process. Over the years, genetic, technological, chemical and microbiological inventions and improvements have been implemented to refine the various stages of the brewing process. Among these production stages, milling has received comparatively scant attention from scientists.

Malt is obligatorily milled prior to mashing in order to optimize the extraction of malt components. Milling enables efficient enzymatic hydrolysis of polymeric compounds in malt (mainly starch, proteins and non-starch polysaccharides) to low-molecular wort components. The fast action of hydrolytic enzymes is determined by the physical contact between the substrate and the enzyme molecule[21]. The most important enzymes in brewing include α- and β-amylase, proteases, peptidases, β-(1,3)(1,4)-glucanases and lipases[1,24]. From the technological and economic point of view, the content of fermentable sugars, proteins and phenolic compounds should be controlled as these wort components are considered to be crucial for the overall cost-effectiveness of beer production and beer quality. The content of fermentable sugars is positively correlated with alcohol production by yeast, whereas proteins/polypeptides and phenolic compounds can cause problems with the colloidal stability of beer if present at elevated concentrations[8,18,46,47]. On the other hand, polyphenols from beer are potent antioxidants capable of "scavenging" free radicals,[12] and it is generally assumed that consumption of polyphenols results in a decreased number of cases of coronary heart disease. It has previously been proven that, although the total phenolic content in beer is lower than in white wines, the total antioxidant activity of beer is higher due to a higher content of some phenolic compounds [proanthocyanidin, epicatechin and ferulic acid (FA)][13]. Barley, a cereal commonly used in the brewing industry, contains considerable amounts of phenolic antioxidants, mainly flavan-3-ol derivatives, phenolic acids and flavon glycosides. Among all classes of barley polyphenols, flavan-3-ol derivatives (anthocyanogens) are repeatedly mentioned in the literature due to their high biological activity and their presence in malts predominantly in free forms. On the other hand, these compounds are prone to transformation into dark-coloured anthocyanidins at elevated temperatures, at decreased pH and in the presence of oxygen[13,25,42]. The β-glucans and arabinoxylans, the main non-starch polysaccharides in malt (beer), are responsible for problems during wort filtration[14]. They form the dietary fibre fraction, present at considerable concentrations in final beers[15,45,55,56], which, among others, can lead to the formation of beer

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hazes. FA, the main phenolic acid in barley and wheat malts, is ester-bound to arabinoxylans (in a constant arabinose : FA ratio). Feruloylated arabinoxylans are present in all parts of the grain, mainly in the aleurone layer (75%) and the endosperm[38]. Only a minor part of FA (shown to be an effective antioxidant[7,19,20,44,50]) is present in malts in free forms[17,28,29]. Malting and mashing result in a limited release of free FA from arabinoxylans[55,56].

With respect to the overall cost-effectiveness of beer production, starch can be considered to be the most important malt component. Starch hydrolysis yields fermentable sugars (mainly maltose, glucose and fructose) as well as non-fermentable compounds (dextrins and limit dextrins)[24]. Hydrolysis of starch depends on the physical state and the particle size distribution of the starch granules[22,26]. It has previously been shown that starch granules in barley can be divided into two[19,27,54] or even three fractions[49], with average particle sizes of 20 μm (large), 7.5 μm (medium) and 2.3 μm (small). These types of barley starch granules are characterized by different amylose: amylopectin ratios, as well as different structures and physicochemical properties. Once the malt has been properly milled, starch granules are exposed to water for hydration followed by gelatinisation and hydrolysis[21]. Starch granules should be effectively extracted from the endosperm and damaged for fast and efficient water absorption and starch hydration. Pulverizing of the whole malt into flour should be avoided. The husk should be left intact in order to maximize the brewhouse yield and to reduce the time of lautering and sparging. Dry husk can easily be cracked by mill rollers yielding small husk particles. The fraction of small husk particles forms a rigid filtration layer resulting in a rapid elevation of back-pressure during lautering (stuck sparge). The filtration rate is low and the cost of the process increases due to the additional pumping and heating of the wort, regeneration of the filtration bed, etc. Moreover, tearing the husks leads to the leaching of harsh-tasting tannins into the wort.

Wet milling or wet conditioning of malt (described in detail and patented in the 1960s[23]) presents some advantages over dry milling. The husk with an elevated moisture content is pliable and the husk particles are bigger, which significantly improves the filtration process. At the same time, the endosperm remains dry allowing milling to proceed correctly[21,24]. Additionally, while dry milling is usually carried out in six-roll mills, wet milling can be successfully performed using two-roll mills. The only problems presented by wet milling occur during the sparging of the malt with water. Overall, however, wet milling has more advantages than disadvantages in comparison to dry milling[21].

In the present paper, the transfer of selected wort components (fermentable sugars, total protein, total phenolics and ferulic acid) from the mash into the sweet wort, as well as the antioxidant activity of intermediate sweet worts, was studied. Worts were produced during a short time interval using the same malt, equipment and production protocol, with the only difference being the method of malt milling (dry milling versus wet milling after malt conditioning). The goal of this study was to provide useful data to professionals in the field seeking the most suitable industrial-scale method of malt milling.

MATERIALS AND METHODS

Reagents

Glucose, fructose, maltose, maltotriose, bovine albumin, PVPP (no. P-6755), ABTS, DPPH and HPLC standards of phenolic acids (purity 99% or higher) were purchased from Sigma-Aldrich (St. Louis, MO, USA). A beta-glucan (K-BGLU, Mixed Linkage) assay kit was purchased from Megazyme, Wicklow, Ireland. For HPLC-UV and HPLC-RI, HPLC grade reagents were used (P.O.Ch. Gliwice, Poland). The Folin–Ciocalteau reagent, the Bradford reagent and other reagents of analytical grade were from P.O.Ch. Gliwice, Poland.

Worts

Sweet non-hopped worts were produced in an industrial plant (Perła Browary Lubelskie S.A., Lublin, Poland) using lager malt (for lager pale, bottom-fermented pilsner-style beer). Selected parameters of the malt, as declared by the producer, were as follows: moisture content 6.0%, total protein content 10.7%, Kolbach index 40.0%, malt colour ≤3.5 EBC, colour after boiling ≤4.8 EBC, extractability 80.0%, Hartong Index (45°C) 40 and congress wort viscosity < 1.45 mPas. The malt-to-water ratio during wort production was 18 kg: 1 hL for the production of a 12.2% (w/w) wort. The worts were produced either in December 2008 ("reference worts" produced using a six-roll mill for dry milling) or in January 2009 ("test worts" produced using a two-roll mill after wet conditioning of malt). The six-roll mill (6,000 kg/h) had three pairs of rolls (upper, middle and bottom) with respective gaps of 1.40 ± 0.15 mm, 0.80 ± 0.15 mm and 0.30 ± 0.15 mm. The diameter and length of the rolls were 250 mm and 1,250 mm, respectively. The rolls in the two-roll mill for wet milling (10,000 kg/h) were similar in diameter and length to the rolls in the six-roll mill. The gap between the rolls for wet milling was 0.3 ± 0.10 mm. A more detailed description of the construction of the rolls in the two mills was not possible. Directly before wet milling, husk water content was elevated to 12–15% using hot water (approx. 50°C). The enzyme preparations Viscozyme and Celluclast (both at 0.5 g/kg of malt) were added to all mashes at mashing-in (52°C). Two production lines [later referred to as line 1 (L1) and line 2 (L2)] were simultaneously used for mashing, and the only minor difference between L1 and L2 was the diameter of the mashing vessel. All the worts were produced using the infusion method including the following production stages: mashing-in at 52°C (protein pause, 20 min), 63°C (β-amylase pause, 30 min), 72°C (α-amylase pause, 30 min) and 76–78°C (mash-out, pumping into the lauter tun, lautering and sparging). Lactic acid was added at the beginning of the mashing, in order to adjust the pH to approximately 5.20–5.25. Sweet worts were lautered using the grain bed as a filtration medium (filter bed) followed by sparging of the grains with water (76–78°C). Two samples of test worts and one sample of reference worts were taken from each lauter tun at each lautering point (40 hL, 80 hL, 130 hL and 180 hL, n = 2). In this way, the results concerning the reference worts (dry milling) produced in L1 and L2 were calculated on the basis of two repeats (n = 2) and the results for the test worts on the basis of four repeats (n = 4). The

worts were promptly centrifuged (8°C, 12,000 × *g,* 20 min) and analyzed within 1–2 days without freezing (cooled, with the addition of 0.01% w/v of sodium azide).

HPLC-RI determination of fermentable sugars

Wort samples were prepared for HPLC-RI analysis as described by Nogueira et al.[37] by dilution with acetonitrile [1:1 (v/v)] and centrifugation (4°C, 14,000 × *g,* 15 min). The analysis was performed in isocratic conditions (deionised water at 80°C) using an Aminex Carbohydrate HPX-42C column (calcium form, 300 mm × 7.8 mm, 25 µm particle size, Biorad, USA) with an Aminex precolumn (25 mm × 7.8 mm, 25 µm particle size, Biorad, USA) and a Micro HPLC pump, an RI detector and a 0.02 mL loop (Knauer, Germany). The identification of sugars was performed using standard solutions.

Determination of total protein content

Protein determination was performed as described by Bradford[4]. Phenolic compounds were removed from wort samples using PVPP (10 h, 0°C, 5 g/100 mL of wort) as described by McMurrough[30]. The samples were then centrifuged (4°C, 10,000 × *g,* 30 min), and the removal of phenolic compounds with PVPP was repeated in the same way. After centrifugation (4°C, 10,000 × *g,* 30 min), the samples were diluted with deionised water, and 0.05 mL of the sample was mixed with 2.5 mL of the Bradford reagent followed by a 60 min incubation at room temperature. The absorbance was read at 595 nm against a blank sample (containing 0.9% NaCl and the Bradford reagent). The protein concentration was calculated from a calibration curve prepared using bovine albumin. The analysis was performed in triplicate.

Determination of total phenolics content

Proteins were removed from the wort samples by mixing with acetonitrile [1:1 (v/v)] and centrifugation (4°C, 16,000 × *g,* 30 min) followed by mixing with trichloroacetic acid (1:1 v/v, centrifugation at 4°C, 16,000 × *g,* 30 min). The analysis was performed using the Folin–Ciocalteau reagent as described by Folin and Ciocalteu[11] with slight modifications. The pH was adjusted to 5.2, and 0.1 mL of the sample was mixed with deionised water (0.9 mL), 5 mL of the Folin–Ciocalteau reagent (0.2 mol/L) and 4 mL of a saturated sodium carbonate solution (75 g/L). The mixture was left for 2 h, and the absorbance was measured at 765 nm against a blank sample. The calibration curve was prepared using gallic acid standard solutions.

Determination of β-glucan content

The analysis of β-glucan content was performed using an enzymatic beta-glucan (Mixed Linkage) assay kit according to the method specified in the producer's brochure.

Extraction of free FA from worts

The worts were centrifuged (6°C, 12,000 × *g,* 20 min), and phenolic acids were extracted as described by Nardini and Ghiselli[35]. After a pH adjustment to 1.0 (2 mol/L HCl solution), 0.5 g of KCl was added to each sample. Phenolic acids were then extracted three times by vortexing (BioVortex V1 Plus, Biosan, Latvia), each time using a new portion (10 mL) of ethyl acetate. After each extraction, the wort samples were centrifuged (6°C, 12,000 × *g,* 20 min), ethyl acetate phase was transferred into a separate tube and another portion of ethyl acetate was added. The entire ethyl acetate phase was then evaporated to dryness (35°C, 0.01 MPa). The samples were dissolved in methanol (10 mL), filtered (Millipore 0.45 µm PTFE filters) and analyzed using a HPLC-UV system. Extraction was performed in triplicate and mean values were calculated.

Mild alkaline hydrolysis and extraction of total (free and alkali-extractable) FA from worts

The hydrolysis was performed according to Nardini et al.[34] A 30 mL aliquot of wort was mixed with 15 mL of NaOH solution (2 mol/L) containing ethylenediaminetetraacetic acid (EDTA, 10 mmol/L) and 1% (w/w) of ascorbic acid and hydrolyzed in darkness for 30 min, followed by adjustment of the pH to 1.0 using HCl (2 mol/L) and addition of KCl (0.5 g). Phenolic acids were then extracted using ethyl acetate and dissolved in methanol (for HPLC-UV) as described above. The recoveries of the phenolic acid standards (1 mmol/L, detailed data not shown) were used during recalculation of the HPLC-UV results.

HPLC-UV determination of FA

The HPLC system consisted of a Waters Symmetry C18 column (USA, 250 mm, 4.6 mm i.d., 5 mm), a Waters Symmetry® C_{18} pre-column (5 µm, 8 × 20 mm), 306 Separation Module Piston Pumps, a PhotoDiode Array 170 Detector, a loop (0.02 mL), an 805 Manometric Module, a 234 Autoinjector and an 811C Dynamic Mixer (Gilson, USA). Two eluents were used: eluent A, 1% (v/v) acetic acid solution in deionised water, and eluent B, 50% (v/v) HPLC-grade acetonitrile in deionised water. The gradient program (0.8 mL/min) was as follows: start 92% A, 8% B 0–10 min; 70% A, 30% B 10–40 min; 60% A, 40% B 40–55 min; and 92% A, 8% B 55–70 min. The coefficient of variation for FA HPLC standard in within-one-day repeatability tests was 3.5%. In the analysis of reproducibility of the HPLC method, the coefficient of variation was 4.0–5.4%. Both coefficients were acceptable for the method used.

Total antioxidant activity

Total antioxidant activity was measured using ABTS and DPPH according to the modified methods of Miller et al.[31] and Brand-Williams et al.[5], respectively. In both methods, a 96-well microplate reader (Tecan Sunrise, Austria) was used for the measurements. In the first method, ABTS solution (7 mmol/L) was mixed with potassium persulphate (2.45 mmol/L) and left for 24 h at ambient temperature. Directly before the analysis, the absorbance of the ABTS solution (700 nm) was adjusted to 0.70 ± 0.02. A wort sample (0.01 mL) was mixed with 0.3 mL of ABTS solution, and the absorbance (700 nm, 20°C) was read every 30 sec until it reached a plateau. In the method with DPPH, a wort sample (0.01 mL) was mixed with 0.3 mL of DPPH solution (0.06 mmol/L in 100% methanol), and the absorbance (515 nm, 20°C) was read every 30 sec until it reached a plateau. In both meth-

ods, the final absorbance was taken for the calculation of the antioxidant activity. Total antioxidant activities were expressed in Trolox Equivalent Antioxidant Capacity units (TEAC) using calibration curves prepared from a series of Trolox solutions (synthetic vitamin E analogue). For each sample, measurements were repeated eight times.

Statistical analysis

Statistical analysis was performed using STATISTICA 8.0 (StatSoft, Poland). The routine statistical tests were used (mean values, standard deviations, Tukey's HSD test, one-dimensional and multidimensional analysis of variance). Results were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

In the present work, worts were produced using dry milling (reference worts) and wet milling of malt (test worts). The glucose content in all of the test worts (Fig. 1) was significantly higher at the beginning of lautering ("vorlauf", 40 hL and 80 hL) and lower at the end of the process (sparging with hot water, 130 hL and 180 hL) in comparison to the corresponding reference worts. The use of wet milling can, therefore, be beneficial for the fast transfer of glucose from the grain bed into the sweet wort.

No significant differences in fructose content between the two types of experimental worts were observed (Fig. 2).

Maltose was the major fermentable sugar in all of the worts. At the beginning of lautering ("vorlauf", 40 hL, Fig. 3), no significant differences in maltose content were observed between the worts from L1 and L2. Then, at a filtration point of 80 hL, the maltose content was significantly higher in both worts produced using wet milling in comparison to the corresponding worts produced using dry milling. At 130 hL, the content of maltose in the test worts was lower than in the reference worts, which suggests that the wet method of milling is more useful for the fast transfer of maltose from the mash into the sweet wort. In the last step of sparging (180 hL), no significant difference in maltose content was recorded between the test and reference worts. It can be concluded that the lack of differences between the worts at the late stage of lautering could be caused by the continuous sparging of the grain with water. Taking into account the low content of maltose at this stage, the structure of the filtration layer was of minor importance for the extraction of maltose into the wort.

The maltotriose content in the test worts (Fig. 4) was significantly higher than in the corresponding reference worts at the lautering point of 80 hL. At 130 hL, the content of maltotriose in the test worts was either lower (L1) or equal (L2) to the content of this sugar in the reference worts. Thus, as previously observed for glucose and maltose, the use of wet milling can be beneficial for the earlier transfer of maltotriose from the filtration bed into the sweet wort.

The separation of oligosaccharides (degree of polymerization >4) in the HPLC column was not sufficient for a quantitative analysis. In the present work, dextrins were detected in each wort sample, but poor separations of the

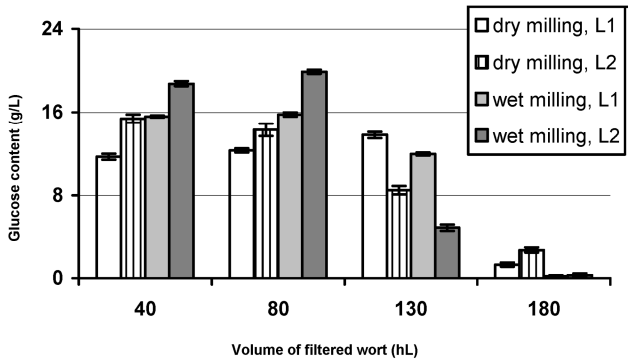

Fig. 1. Glucose content in filtered worts.

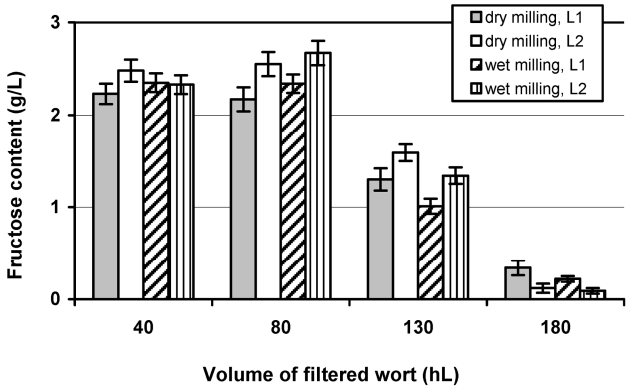

Fig. 2. Fructose content in filtered worts.

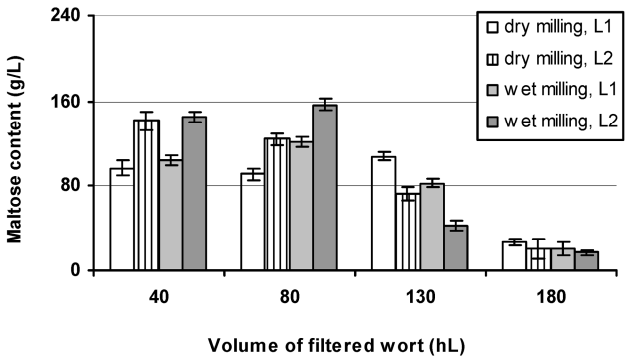

Fig. 3. Maltose content in filtered worts.

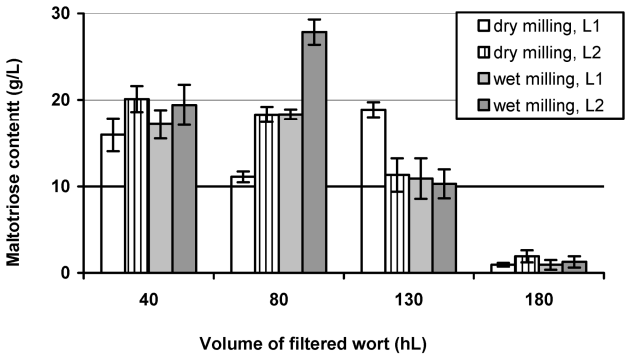

Fig. 4. Maltotriose content in filtered worts.

peaks only enabled a qualitative analysis. The complete degradation of dextrins can be an interesting point for future study because it leads to the formation of fermentable sugars from non-fermentable sugars.

The total protein content in the test worts from L1 (at filtration stages 40 hL, 80 hL and 130 hL) was significantly higher than in the corresponding reference worts (Fig. 5). In the case of L2, no significant differences between the two types of worts were observed except at the filtration stage of 130 hL. It can be seen from Fig. 5, that generally the total protein content was higher in the worts produced using wet milling. The differences between the results obtained for the two lines were not studied further in detail. However, the results suggest that most probably the protein content, in the worts produced using wet milling, was higher due to a more efficient extraction from the malt grist (during mashing) or from the filtration bed. The negative role of elevated protein levels during beer production is very well known. Therefore, control of protein content in malt and its degradation during mashing should be taken into consideration during the implementation of wet milling in the mashing process.

The total content of phenolic compounds in the test worts (Fig. 6) was significantly lower at the beginning of lautering (40 hL, L1), as well as at 40 hL–130 hL (L2), in comparison to the corresponding content in the reference worts. At the end of lautering (sparging with water), no differences between the two types of worts were seen. It must be noted that these results should be verified in the future because significant differences between test worts produced by wet milling in L1 and L2 were observed. All worts were produced within 2–3 days, and the slight difference in the diameter of the two mashing vessels used can be ignored. Other factors that influenced the observed results should, however, be taken into consideration.

The content of phenolic compounds is usually decreased after each stage of beer production due to the precipitation of tannins and non-tannin flavonoids, with the loss of phenolic acids being the slightest amongst all of the phenolic compounds[10]. In the present study, the loss of phenolic compounds during the whole brewing process reached 28%, and the highest phenolic content, as well as antioxidant activity, was demonstrated for wort after boiling. Polyphenols in hopped wort originate from malt and it is estimated that as much as 80% of all phenolic compounds in beer after pasteurisation are phenolic acids[10]. These results have been confirmed by other authors[42] who have noted a positive correlation between the content of phenolic compounds and the total antioxidant activity of worts and beers at different stages of beer production. In the present paper, the total antioxidant activity of all worts was evaluated using methods with ABTS and DPPH free radicals. No significant differences in total antioxidant activities between the two types of worts were observed (Fig. 7 and Fig. 8), with some minor exceptions in the case of individual worts at certain points of lautering (e.g., Fig. 7, 130 hL, or Fig. 8, 130 and 180 hL). However, these differences in the TEAC values between the worts can be

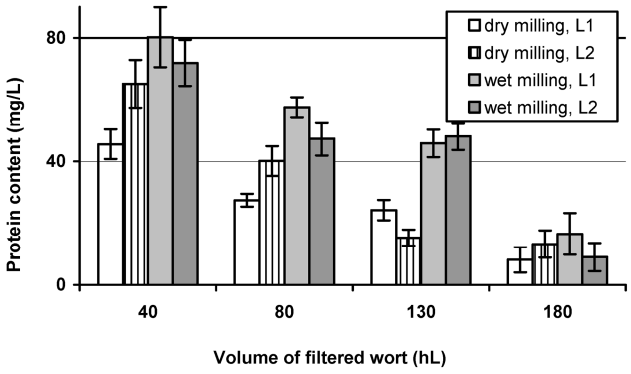

Fig. 5. Total protein content in filtered worts.

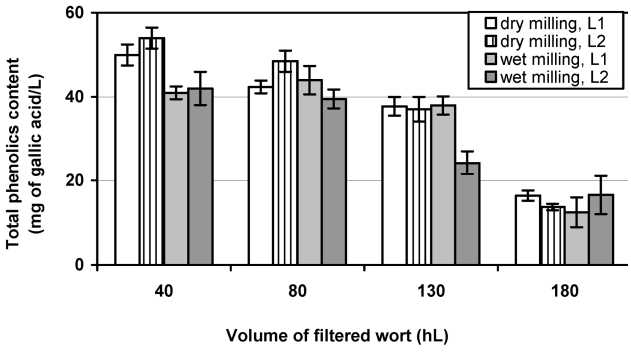

Fig. 6. Total phenolics content in filtered worts.

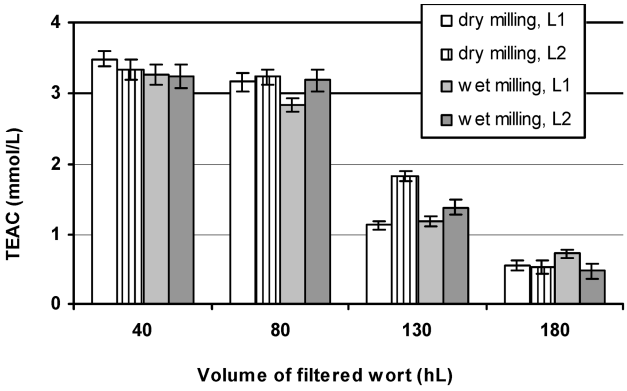

Fig. 7. Antioxidant activity of filtered wort samples measured using ABTS.

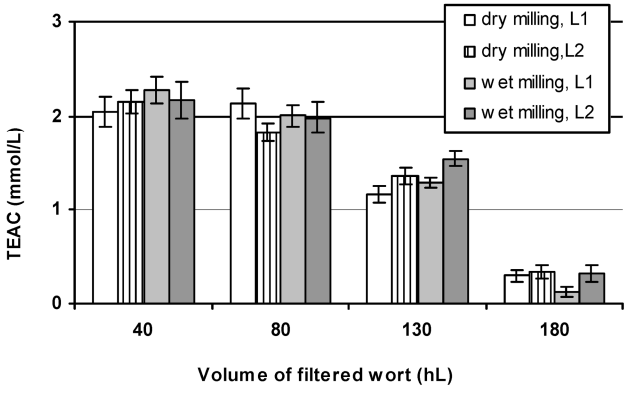

Fig. 8. Antioxidant activity of filtered wort samples measured using DPPH.

attributed to the imperfections of the methods used, rather than to real differences in antioxidant activity. It is of note that the TEAC values measured using ABTS were higher than the corresponding TEAC values obtained using DPPH free radicals.

As mentioned earlier, FA is the main phenolic acid in wort and beer. The content of free FA (Fig. 9) and esterified FA (Fig. 10) in all the test worts at lautering points 40–130 hL was significantly lower than in the corresponding reference worts, probably due to the less efficient extraction from husk[21]. Therefore, wet milling can be successfully used in order to decrease the content of free as well as esterified FA in worts. However, health-related reasons might dictate increasing the content of phenolic acids in wort and beer as these strong antioxidants are very effectively absorbed after consumption[3,6,36,41,43].

Last but not least, it should be noted that β-D-(1,3)(1,4)-glucans were not detected in either the test or the reference worts probably due to the use of Viscozyme and Celluclast, multi-enzyme preparations containing hydrolases such as cellulase, β-glucanase, xylanase (Celluclast, Viscozyme) and, additionally, ferulic acid esterase activity (Viscozyme)[48]. The β-D-(1,3)(1,4)-glucan and arabinoxylans are the main non-starch polysaccharides of dietary fibre. The amount of dietary fibre is not negligible in beers and ranges from 183 mg/L to 3,534 mg/L (Doppelbock and Rauchbier)[15]. Schwarz and Han[45] reported that the average content of β-glucan in the fifteen commercial beers that they studied (with different extract, alcohol and sugar contents) was 247.7 mg/L of beer. No information on the use of enzyme preparations during wort production was provided in the cited works.

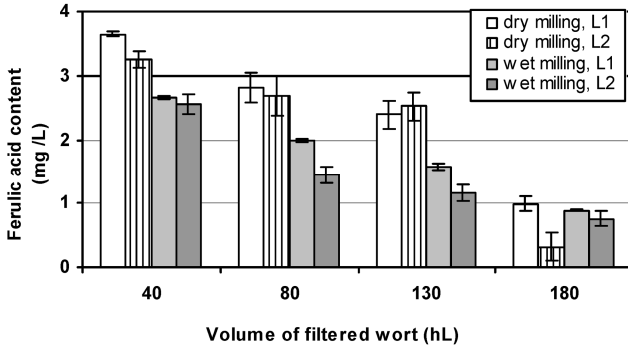

Fig. 9. Free ferulic acid content in filtered worts.

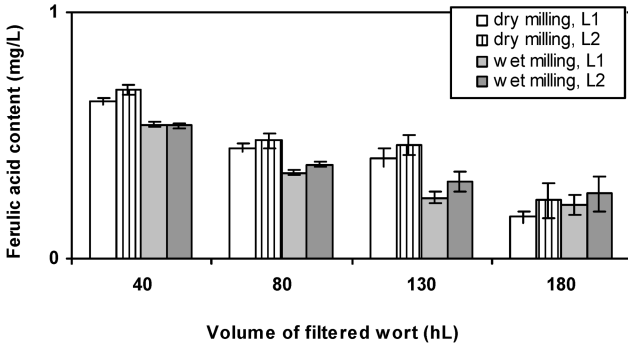

Fig. 10. Ester-bound ferulic acid in filtered worts.

Mashing is a multi-stage, time-consuming process, and efforts are concentrated on shortening the time of hydrolysis of malt components within one mashing procedure. Prior to mashing, milling is performed for maximal exposure of the kernel endosperm, which allows proper extraction and hydrolysis of malt components (mainly starch and proteins/polypeptides). Other kernel polymers (β-glucans, arabinoxylans, cellulose, hemicelluloses, etc.) should be degraded, as their hydrolysis facilitates the extraction and hydrolysis of starch. Starch hydration is effective only when the mashing water is effectively absorbed by the starch granules. The key advantage of the wet milling of malt is that it permits maximal pulverization of the malt endosperm (reduction of the size of flour particles), while the husk can be left nearly intact. The integrity of the husk is maintained by moderate elevation of the husk water content (typically 18–22% by the addition of approximately 15 L of water/100 kg of malt[21]). The fraction of big husk particles form an excellent filtration bed during lautering. Indeed, in the course of this study it was shown that the transfer of glucose, maltose, maltotriose and total protein from the mash into the wort during lautering was significantly improved during the early stages of the process under wet milling. This indicates that in wet milling a higher fermentable extract yield can be obtained after a reduced lautering time, as an alternative to investing in additional equipment in the mashing house. On the other hand, the transfer of total phenolics and FA (in free as well as in the ester form) from the mash into sweet worts was significantly decreased during the early stages of lautering. The observed results can be explained by the less efficient extraction of phenolic compounds from the larger husk particles. It is known that different classes of polyphenols are located mainly in barley husk[28,29,38] and extraction from the husk after wet conditioning of malt is limited.

The number of papers providing a direct comparison of dry milling and wet milling of brewery malt is limited. Eneje et al.[9] studied dry and wet milling of pre-soaked millet malt followed by mashing (infusion, double decoction and decantation mashing). For dry milling, the malt water content was 4%, which was lower than in the present study (6%). During wet milling, the authors adjusted the moisture content of the millet malt to 7%. In a third method, they studied dry milling with steep conditioning of malt to final water contents of 6% and 8.5% (pre-soaking of malt in water for 1 h and drying at 50°C for 1 h to remove surface moisture). Eneje and colleagues[9] found that the worts produced using wet milling were characterized by a significantly higher extract yield and a less intense colour in comparison to worts produced using dry milling of malt. Moreover, milling preceded by steeping of millet malt produced the highest overall extract yield and a hot water extract with the lowest filtration times among the three methods of malt milling. Milling of the millet malt to a fine particle size gave a higher extract yield than milling to coarse particles. No difference in soluble nitrogen and free α-amino nitrogen contents was seen between worts produced from malt milled using dry versus wet milling. The wetting of malt prior to milling resulted in an increase in the hot water extract yield of the worts.

It should be underlined that in the present work the gap between the third pair of rolls for dry milling (0.30 ± 0.15 mm) and between the rolls for wet milling (0.3 ± 0.10 mm) was similar. Previous studies reported that the gap size influenced the mechanical damage of starch, which could affect the rate of starch hydrolysis[58,59]. Mousia et al.[33] showed that the dry milling method could be optimized by modifying the number of rolls, roll gaps, roll speeds, differential roll speed, roll feed rate (milling capacity), etc. The authors milled the malt in two different ways: using a two-roll mill with fluted rolls (the actual gap was 0.1, 0.2, 0.4, 0.6 or 0.8 mm) and using a hammer mill (1 mm sieve). The content of glucose and maltose was higher for hammer milling than for roll milling throughout the mash. Also, the total content of the two sugars in the final worts was higher for hammer milling. The contents of glucose and maltose in the wort were higher for roll-milling at a gap setting of 0.1 mm in comparison to the 0.8 mm gap. It was concluded that the narrowing of the roll gap resulted in an increment in starch damage and the susceptibility of malt polymers to enzymatic hydrolysis[33,58]. Mousia et al.[33] pointed out that the time of mashing could be reduced, without the loss of the content of fermentable sugars, by proper adjustment of the roll gap. Those authors also simulated four-roll milling by a second milling of the same sample after initial milling and screening. The six-roll mill was found to be a superior milling tool to a four-roll mill for dry milling of malt[33]. The results cited above should be taken into consideration during evaluation of the results obtained in the present work. In contrast to the Mousia et al.[33] study, in these experiments, the difference in the gap width between the third pair of rolls of the dry mill and the rolls of the wet mill was too small to affect the results. There was no difference in gelatinisation between the two mashes, which in accordance with the suggestions of other authors[33] could reflect differences in the gap size. Presumably, the only factor influencing the results of this study was the difference in the water content of the malt.

It should be noted that in this study FA was present in all six worts predominantly in the free form (Fig. 9 and Fig. 10), contrary to a number of other works that showed that it was present in barley and malt mainly in the bound (esterified) form[17,28,29]. The FA, as well as other phenolic acids, have repeatedly been shown to be present in different beers in the free form[2,32,36], but some studies suggest that beer contains considerable amounts of esterified FA[35,48]. In the present work, the presence of FA in the free rather than in the esterified form could be attributed to the use of the enzyme preparation Viscozyme, possessing accessory ferulic acid esterase activity[48]. In order to avoid excess levels of free FA in the wort, inactivation of ferulic acid esterase in commercial enzyme preparations is recommended. The esters of FA obtained after enzymic degradation of arabinoxylans (FA-Araf and FA-Araf-Xylp-Xylp) exhibit a higher antioxidant activity against low density lipoproteins (LDL) than free FA[39,40]. The form of FA in beer (free or bound) also affects the flavour stability of this beverage. Free FA is prone to decarboxylation (at elevated temperature or due to bacterial activity) leading to the formation of 4-vinylguaiacol. This vinyl compound is responsible for an undesirable off-flavour in beers[57]. However, the access of oxygen and microorganisms to wort and beer in modern production lines is limited and the risk of decarboxylation of FA appears to be low[16,57,51,52,53].

In conclusion, it can be stated that the method of malt milling affects the content of wort components responsible for the quality of the beer and the overall cost-effectiveness of beer production. In this work, it was shown that wet milling of malt, compared to dry milling, improves the rate of migration of maltose, glucose and maltotriose from the filtration bed into the sweet wort, in this way reducing lautering time. The wet method also prevents extraction of phenolic compounds from the barley husk. On the negative side, wet milling promotes better extraction of proteins from the grain and thus the protein content should be more thoroughly monitored when using this method. Ultimately, the choice of which method to use should be made after a thorough analysis of malt composition.

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