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# The Cleaning Effect on Brewing Barley Using Vibrations during Wet Steeping

Nowadays, industrial malting plant process batches of up to 300 tons whereas achieving a homogenous malt quality within one batch is a major goal. Consequently, cytolytically badly modified or even ungerminated kernels may lead to lautering and filtration problems e.g. due to high remaining unhydrolysed  $\beta$ -glucan fractions. Hence, the homogeneity and cytotoxicity of a malt batch should be improved.

In this study, a cement vibrator was used to treat barley with vibrations during wet steeping at a frequency of 180–200 Hz in order to obtain the influence of sonic waves on the final malt quality. The input of sonic waves during steeping resulted in a faster water uptake and a considerably improved cleaning effect on barley. Higher steeping degrees and remarkably reduced microbial contaminations in turn led to faster and more homogenous germination by means of higher germination energies and malt homogeneities in comparison to a reference malt which was processed without sonic waves treatment. Furthermore, an improved lautering performance of the produced malts as evaluated by an in-house laboratory lautering test, was improved when treating with vibrations during wet steeping. Additionally, the cleaning effect of the vibrations resulted in remarkably lower iron content of the malts yielding an improved oxidative flavour stability of worts produced thereof as determined by electron spin resonance spectroscopy. Outcomes of these trials benefit the malting industry in terms of improving the malt homogeneity and quality.

Descriptors: sonic waves, vibrations, water uptake, microbiology, germination energy, homogeneity

## 1 Introduction

During steeping, which is the initial and key step in malt production, the grain's water content, the so-called steeping degree, is increased to induce the germination [3]. According to *Sims* [35], the grain kernels start germinating at water contents above 30 %. The steeping regime in most cases consists of 2–3 immersion phases (wet steps) and periodic dry rests. After steeping, the steeping degree normally ranges between 38 and 41 %. The germination performance is primarily influenced by the steeping regime, the temperatures and the rate of water uptake. Latter in turn is dependent on the cultivar, the crop year, the kernel size, the nitrogen content, the kernels' physiological status (dormancy, water sensitivity) [3, 7, 8] and a sufficient oxygen supply [11, 15, 41, 42]. An uneven oxygen supply within steeping systems can also yield an inhomogeneous batch [16]. However, the steeping temperature is probably the most important influencing factor on the water uptake of the grain [37].

Nowadays, very large malt batches are processed, which frequently leads to heterogeneities within the grain beds. As a result,  $\beta$ -glucanase activities vary within the batches, thus remaining high concentrations of viscosity increasing non-hydrolyzed  $\beta$ -glucan in parts of the batches. This in turn can lead to lautering and filtration problems during the brewing process [2, 9, 16, 26]. The kernels' enzyme activities mainly depend on their physiological status (germinative energy, water sensitivity) which can be influenced by the steeping parameters. According to *Aalbers* et al. [1], heterogeneities during steeping cannot be corrected during the later germination or kilning processes. Apart from inhomogeneities in a barley batch originated from the growth on the field, e.g. kernel size or physiological status, *Axcell* et al. [3] proposed that a homogenous water uptake of the kernels in one batch is very important for a simultaneous start of the germination and thus for the homogeneity of the resulting malt. For this reason, *Home* et al. [14] suggested the counting of chitted kernels during steeping and at the start of germination to evaluate the homogeneity of the germination ignition and progress.

During the last decades, several possibilities to improve the germination performance and homogeneity of brewing barley have been researched.

Probably the most investigated method to overcome the dormancy is the addition of gibberellic acid, a botanic hormone, which enhances the germination and the formation of amylases without affecting the homogeneity. For compensation of resulting loss increases, gibberellic acid often is combined with growth suppressing substances like bromate [6, 21, 27, 29]. However, adding gibberellic acid is not permitted in every country due to food laws like the German purity law.

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Müller et al. [23] found that applying steeping temperatures between 20 up to 30 °C leads to an improved homogeneity, enhanced cytolytic modification and improved lautering performance without negatively affecting the malt quality as compared to lower steeping temperatures from 12 to 17 °C which is widely suggested by the literature [24, 28]. In former investigations, done 30–40 years ago [4, 5, 30, 40], contradictory results were found. Here, steeping at higher temperatures also led to an enhanced growth but the malt quality was negatively influenced due to lautering problems and increased malting losses.

The application of ultrasonic waves (15–30 kHz), as a method for priming, was investigated in the agricultural sector [13, 34, 43]. Improved percentages of total seeds that germinated and an increased germination rate even at lower temperatures were found. It was assumed that this was caused by overcoming the dormancy due to a significantly higher water uptake and oxygen availability resulting from mechanical effects of the ultrasonication. The protective outer layers of the kernels, especially the pericarp, were thought to be perforated whose effect could be intensified by fumigation with different gases and the resulting cavitation of the gas bubbles. Other authors [10, 33, 36] did malting trials and applied an ultrasonication (35–1000 KHz) during wet steeping. In addition to the higher water uptake and the earlier germination start, as already mentioned before, a faster formation of  $\alpha$ - and  $\beta$ -amylases, higher extracts in the final malts and a washing effect, resulting in a reduced microflora of the malt, could be observed.

Own unpublished investigations could not confirm the found advantages applying ultrasonication using ultrasonic baths and sonotrodes during wet steeping, but the results showed neither an improved water uptake nor an enhanced germination performance of the barley.

The already introduced investigations about ultrasonication were done applying very old fashioned long wet steeping phases. It can be assumed that the effects of ultrasonication on the water uptake and germination performance are only effective when applying extended wet steep phases or steeping without air rests which are rarely used by the malting industry nowadays.

Although the influences of ultrasonic waves for the barley treatment has been investigated very well, as yet, based on our knowledge there are no investigations about the application of audible vibrations during wet steeping for improving the malt quality and homogeneity.

Schneid[32] investigated the influence of vibrations during mashing on the brewing process. Due to a faster deaeration of the mash and in particular the removal of air bubbles from the particles in the mash, the enzymatic degradation of higher molecular substances like starch and protein was improved resulting in a higher brewhouse yield. Nevertheless, it was also found that the vibrations of a specific frequency range lead to an improved particle permeation for a better accessibility of enzymes and to a faster moisture penetration of the husks because the vibrations intensify the natural resonance of the particles. Furthermore, the encounter of enzymes and their substrate might be simplified due to another movement dimension caused by the vibrations. In the case of malting process and in particular an unaerated wet steeping, a deaeration of the

steeping water is not advantageous [41]. Nevertheless, due to the mentioned mechanisms during mashing, it seemed worthwhile to investigate the application of audible sonic waves by means of vibrations during malting. Therefore, barley was treated with a cement vibrator (180–200 Hz) during the wet phases of steeping in order to observe the influence on the germination performance of barley as well as on the final malt quality in comparison to not-treated reference samples. All other parameters of steeping, germination and kilning were kept constant to assess the impact of the sonic wave treatment, exclusively.

## 2 Materials and methods

### 2.1 Small-scale malting trials

For investigating the impact of sonic wave treatment on the malt quality at different process conditions, in total, four trials were conducted where the frequency and length of sonication, the steeping temperature and the aeration were varied. For each of the individual trials (A–D), one reference malt was produced where the sonication treatment was omitted but all other process parameters were kept the same.

Two commercial two-row barley varieties were used for the trials: Marthe (water content: 13.9%, protein content: 10.5%, germinative energy: 98 %, water sensitivity: 35 %) and Tipple (water content: 13.5 %, protein content: 9.2 %, germinative energy: 96 %, water sensitivity: 26 %). An overview of the barley varieties used for each trial, the experimental design and the process parameters are depicted in table 1.

For all trials, steeping was carried out by mixing aliquots of 3200 g of barley with 9.2 L tempered tap water in 15 L containers. The sonic wave treatment (180–200 kHz) was achieved by dousing a cement vibrator (type IREN 30 combined with a frequency changer FUE 6/042/200W SC; Wacker-Neuson, Munich, Germany) in the containers and setting it for the desired level of vibration and desired length. To prevent a heating-up of the samples by the sonic wave treatment, the containers were temperature-controlled and cooled to the desired temperature with a cooling coil when needed. To aerate the barley during steeping, a flexible perforated plastic tube was installed at the bottom of the steeping containers and air was bubbled through the tube.

**Table 1 Steeping parameters of all trials – control malting without treatment with vibrations**

Barley	Marthe Trial A	Tipple Trial B	Tipple Trial C	Tipple Trial D
1 <sup>st</sup> wet steep	19 °C; 3 h		16 °C; 3 h	
Treatment	half-hourly (180 Hz)		10 min / 30 min (200 Hz)	
Aeration	no	no	no	yes
air rest	aerated, 16 °C, 23 / 20 h		unaerated, 14 °C, 18.5 h	
2 <sup>nd</sup> wet steep	19 °C; 3 h		16 °C; 2.75 h	
Treatment	no	after 1.5 h, half-hourly (180 Hz)	after 1 h, 10 min / 30 min (200 Hz)	10 min / 30 min (200 Hz)
Aeration	no	no	no	yes

After steeping was finished, aliquots of 800 g of the malts were transferred to germination boxes and germination was then carried out using the small-scale malting plant (system model A1-2008, no. 176 / 1, Schmidt-Seeger, Beilngries, Germany). The automated plant consists of a combined steeping-/germination unit with a maximum load of 8 baskets á 800 g each and an accordingly adjusted kilning unit. The steeping-/germination unit is equipped with a box which contains the 8 baskets and is housed in an insulated floodable chamber. The box can be ventilated with tempered and humidified air and can be periodically rotated to avoid the formation of clusters.

Germination was kept constant for all trials. During germination (5 days at 16 °C), the water content of the samples was checked (in duplicate) daily by weighing the germination baskets and calculating the steeping degree with reference to the dry matter. Afterwards, the amount of water for adjusting the steeping degree to 45 % was calculated by the actual steeping degree and initial barley weight. It was sprayed onto the boxes on the first and second germination day.

Germination was controlled and germination performance and homogeneity were monitored by counting and evaluating the percentages of chitted and forked kernels after the dry rest, 3 hours after the second wet steep and 48 hours after steeping in.

Kilning was also done under equal conditions for all trials. After 5 germination days, the baskets were transferred to the kilning unit and were withered at 50 °C for 18 hours and cured at 80 °C for 5 hours.

## 2.2 Malt Analyses

The malt analyses extract, filtration time, turbidity, colour of boiled wort, Kolbach index, free amino nitrogen (FAN), modification and homogeneity (Carlsberg method),  $\beta$ -glucan content, friability and acrospire length were performed according to MEBAK [22]. The enzyme activities of  $\alpha$ - and  $\beta$ -amylases,  $\beta$ -glucanases and proteases were determined with commercial assay kits (Megazyme, Bray, Ireland). All analyses were done in duplicate.

## 2.3 Determination of the microbial contamination

For an evaluation of the microbial contamination of the final malts, the microbial count was determined. Therefore, 10 g malt was agitated in 100 mL 0.1 % TWEEN® solution at 170 rpm for 10 minutes. 0.1 mL of a serial dilution ( $10^{-1}$ – $10^{-6}$ ) was plate poured on ST1- and wort (overall microbial counts) as well as Chromocult agar (coliforms). The plates were aerobically incubated for 24 h at 36 °C and 48 h at 28 °C for the overall microbial counts and coliforms, respectively.

## 2.4 Determination of chemical oxygen demand

The chemical oxygen demand (COD) of the steeping waters was analysed using the commercial assay kits LCK 514 and LCK 014 (Hach-Lange, Berlin, Germany).

## 2.5 Determination of oxidative stability

The influence of the treatment with sonic waves on the oxidative wort stability was investigated by the EAP (Endogenous Antioxidant Potential) determination and  $T_{400}$ -value (radical generation) evaluation using electron spin resonance (ESR) spectroscopy according to MEBAK [17, 22]. Therefore, worts were produced in a congress mash bath (Bender & Hobein, Bruchsal, Germany) (75 g fine grist and 200 mL bidest. H<sub>2</sub>O mashed in at 55 °C, rest 10 min, rest at 62 °C for 60 min, rest at 72 °C for 20 min, heating to the filtration temperature of 78 °C, cooled to 20 °C, counterbalanced to 450 g, filtrated with a folded filter, atmospherically boiled for 60 min, extract adjusted to 12.1 %).

## 2.6 Determination of iron concentration

Worts were produced as described above and the iron content of the produced worts and of steeping waters were analysed by atomic-absorption spectrometry (ASS) according to MEBAK [22].

## 2.7 Laboratory lautering tests

To get further information about the processability of the produced malt, an in-house-method of a laboratory lautering test was used to determine the lautering properties of the mashes produced from the respective malt samples. The lautering test was performed in triplicate at 20 °C using a Filtercheck®-apparatus (Stabifix, Gräfelting, Germany). 50 g malt were ground with a DLFÜ-mill (Bühler, Uzwil, Switzerland) at a disk gap of 0.8 mm and mashed in with 180 mL bi-distilled water of 45 °C. For mashing, the congress mashing apparatus (Bender & Holbein, Bruchsal, Germany) and regime according to MEBAK [22] were used. After cooling the mash to 20 °C, bi-distilled water was added to the mashes to adjust the beaker contents to 200 g. After filling the mash onto a steel mesh with a gap size of 0.25 mm placed on the bottom of the Filtercheck®-apparatus, followed by a lautering rest of 2 minutes, the filtrate was collected on a scale and the filtrate volume was recorded during the test and afterwards plotted against the lautering time. Comparing the curves and the filtrate volume after a certain time of 225 seconds provided information about the malt's lautering properties.

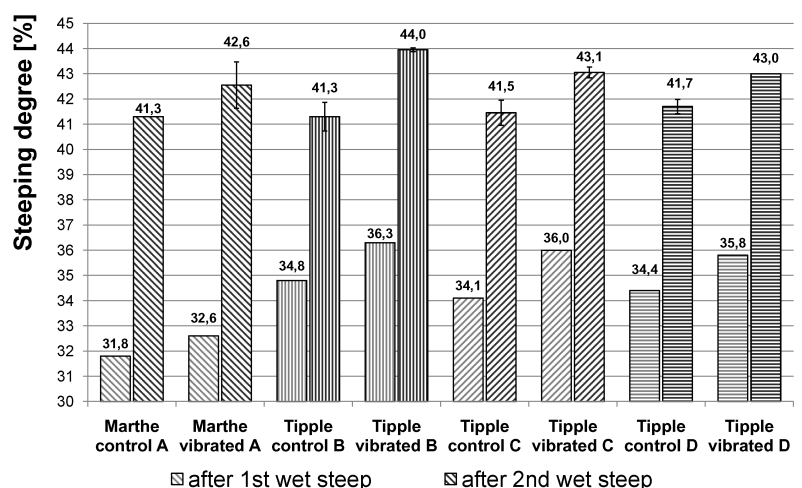
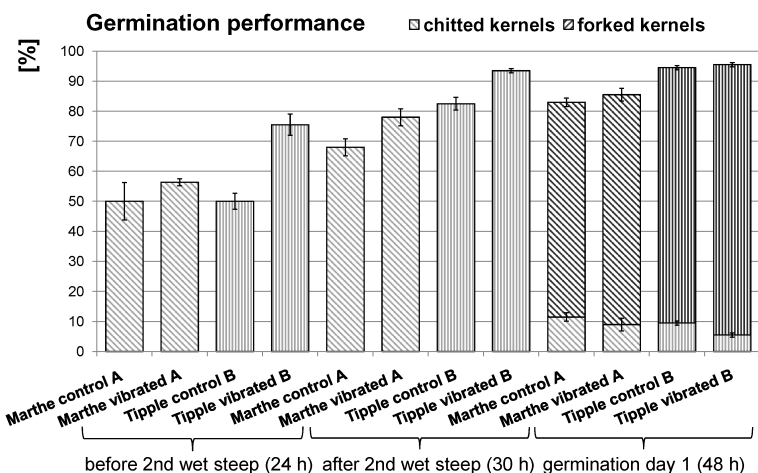


Fig. 1 Reached steeping degrees, 2 hours after the first and second wet steep



**Fig. 2** Evaluation of the germination performance by counting the percentages of chitted and forked kernels before and after second wet steep and on the first germination day, 24, 30, 48 hours after steeping in, respectively. (mean of duplicate analysis; standard deviation of forked and germinated kernels included) – Trial A–B

### 3 Results and Discussion

The reached steeping degree during and after steeping is a crucial steeping parameter as it significantly influences the germination performance and also the resulting malt quality [25]. In general, treating the barley with specific sonic waves (180–200 Hz) during wet steeping resulted in an increased water uptake (Fig. 1). The

steeping degrees were 0.8–1.9 % higher after the first wet steep and increased further up to 2.3 % after the second wet steep. In all trials made with the barley Tipple (B–D), which were vibrated during both immersion phases, a higher increase of the steeping degree could be observed.

The germination performance was monitored 24, 30 and 48 hours after steeping in by counting and determining the percentage of chitted and forked kernels in duplicate. In figure 2, only the results of trial A and B are displayed because for the second series of trials no significant differences could be observed due to the applied unaerated air rest in trial C and D. After steeping, the barley was only chitting to a small extend and after 48 hours no significant differences could be detected anymore. A delayed growth when applying air rests without a CO<sub>2</sub>-removal could also be observed in several former investigations [1, 8, 11, 42].

In trials A and B, significantly improved germination performances and germination homogeneities, as evaluated by the percentage of germinated kernels (germinative energy) and by the differences between forked and chitted kernels, could be observed for the vibrated samples for both barley varieties.

By implication of the improved germination performance, the results as observed from the germination control were reflected in the final malt quality. The results of the malt analyses are summarised in table 2.

**Table 2** Malt analyses (mean of duplicate analysis) of all trials (dnc: data not collected)

Sample	Trial A (Tipple)		Trial B (Tipple)		Trial C (Tipple)		Trial D (Tipple)			
	control	vibrated (1 <sup>st</sup> wet steep)	control	vibrated (1 <sup>st</sup> + 2 <sup>nd</sup> wet steep)	control	vibrated (1 <sup>st</sup> + 2 <sup>nd</sup> wet steep)	control	vibrated (1 <sup>st</sup> + 2 <sup>nd</sup> wet steep)		
Aeration during wet steep	no		no		no		yes			
Aeration during air rest	yes		yes		no		no			
Analyses	Unit	Repeat-ability r								
Extract (fine)	% (dm)	0.58 <sup>X2</sup>	81.6	81.9* +	83.4	83.5	dnc		83.2	83.4
Filtration time	min	5–25 <sup>X1</sup>	41	38	70	60* +			72	92* –
Turbidity 90°	EBC	0.04 <sup>X2</sup>	8.2	6.3** +	5.9	4.4** +			9.9	14.0** –
Colour of boiled wort	EBC	0.052m <sup>X2</sup>	7.8	5.9** +	6.1	5.6* +			5.7	5.4* +
Kolbach index	%	6.7–0.12m <sup>X2</sup>	42.8	44.0*	43.5	42.5*			45.9	45.7
FAN	mg/100g (dm)	28–0.105*m	142	152*	128	125	128	137*		
Mealiness	%	12–0.11m <sup>X2</sup>	84.1	84.1	87.4	88.0	84.1	87.0* +	79.9	81.6* +
Glassiness	%	0.15+0.35m <sup>X2</sup>	1.4	1.2	1.7	1.6	0.7	0.6	0.7	1.4* –
β-glucan	mg/100g (dm)	11 <sup>X2</sup>	97	204*** –	182	200** –	dnc		409	336*** +
Modification	%	55.3–0.55m <sup>X2</sup>	94	97* +	94	97* +	84	91* +	87	92* +
α-amylase	U/g (dm)	0.05m <sup>X3</sup>	241	249	247	243	273	285* +	295	263* –
β-amylase	U/g (dm)	0.05m <sup>X3</sup>	900	941* +	741	814* +	713	725	773	702* –
β-glucanase	U/kg (dm)	0.05m <sup>X3</sup>	576	518* –	680	546** –	762	794* +	923	856* –
Proteinase	U/kg (dm)	0.05m <sup>X3</sup>	78	62** –	167	92*** –	195	208* +	238	147*** –

repeatability r: X1 = given by each duplicate determination; X2 = given by MEBAK [22]; X3 = given by Megazyme; m=mean of duplicate (confidence coefficient by t-test: \* 95 %, \*\* 99 %, \*\*\* 99.9 %)

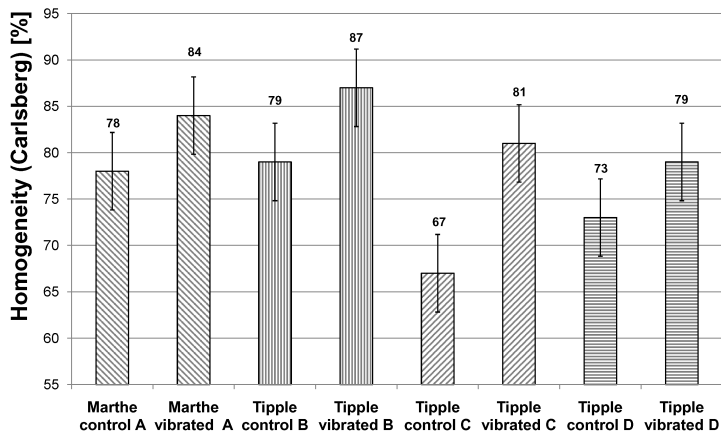


Fig. 3 Evaluation of the homogeneity according to the Carlsberg Calcofluor method. (mean of duplicate analysis; standard deviation of the MEBAK analysis [22] included)

In general, treating with specific sonic waves during wet steeping led to adequate malt quality. Slightly higher extracts could be observed when treating with vibrations, but the  $\alpha$ - and  $\beta$ -amylase activities were not markedly influenced by the different steeping procedures. The enzyme  $\alpha$ -amylase is not formed before the second day after steeping in [15, 16, 25], and thus the steeping affected the  $\alpha$ -amylase formation only marginally. Also, the enzyme  $\beta$ -amylase, which is already present in barley and released from the kernel's aleurone layer during germination [15, 16, 28], was not clearly affected by the sonical treatment during wet steeping. In contrast to this, the activities of the  $\beta$ -glucanases and proteinases were clearly reduced when applying vibrations during wet steeping. However, the reduced proteinase activities did not negatively influence the Kolbach indices and the FAN contents which are crucial parameters for the protein degradation. The parameters of the cytolytic modification showed contrary results. For the first series of trials (A, B), the results of the modification according to Carlsberg was slightly higher and the mealinesses and glassinesses were quite similar although the  $\beta$ -glucan contents were higher within the malts which were treated with sonication during steeping. The reason for this may lie in remarkably reduced  $\beta$ -glucanase activities of the malts which were sonicated during steeping.

It was hypothesized that advantages of the faster water uptake and the faster germination were compensated by the lower  $\beta$ -glucanase activity. Therefore, the second series of trials (C, D) were done applying reduced treatment time during wet steeping which yielded a clearly improved cytolytic modification as indicated by all three parameters (friability, modification,  $\beta$ -glucan content).

There is evidence that the colour of wort is in strong correlation to the oxidative wort stability. Lower colours thereby suggest a lower oxidative deterioration [18, 19]. In all trials, the colour of the boiled worts of the treated malts was lower which gave a first hint to an improved oxidative stability when using malt vibrated during wet steeping.

The standard methods [22] Calcofluor according to Carlsberg and development of the acrospires in the final malts were used to assess the homogeneity of the produced malts. For clarification of the trends,

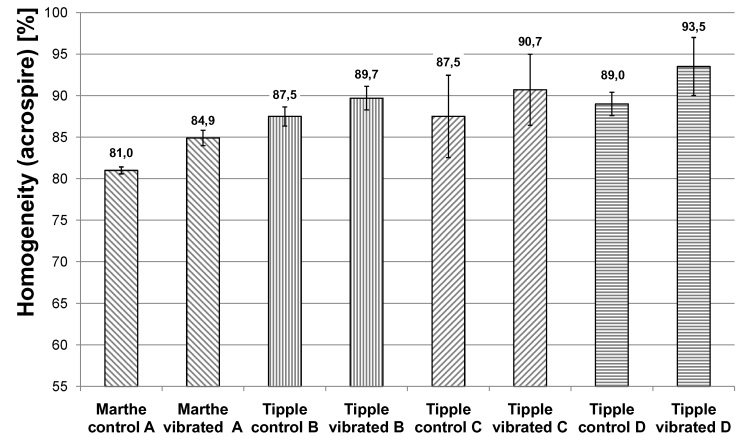


Fig. 4 Evaluation of the homogeneity of the acrospire length. (mean of duplicate analysis; standard deviation of duplicate analysis [22] included)

the homogeneities according to the Calcofluor method and the homogeneities of the acrospire length are shown in figure 3 and 4, respectively.

In correlation to the more homogenous and sooner germination (Fig. 2), a higher homogeneity of all vibrated malts was observable. Due to high standard deviations of both methods, the differences were not significantly for all sample pairs but a trend of improvement by applying vibrations during wet steeping was clearly detectable. Comparing the Calcofluor results of the Tipple malts (Trial B with C/D), it could be shown that the lack of aeration during the air rest led to a reduced homogeneity, whereas the growth was not affected (see the results of the acrospire length's homogeneity in figure 4).

'Good' malt qualities as suggested by analytical data do not necessarily imply a tolerable lautering time in the breweries. The filtration time of the congress mash according to MEBAK [22] often gives no adequate information about the real lautering performance [31]. Also in these investigations, the filtration time of the congress worts gave no evidence for an effect of the vibrations on the lautering properties of the produced malts (Table 2). For evaluating the influence of the sonication on the lautering performance of the produced malts in the present study, an in-house developed

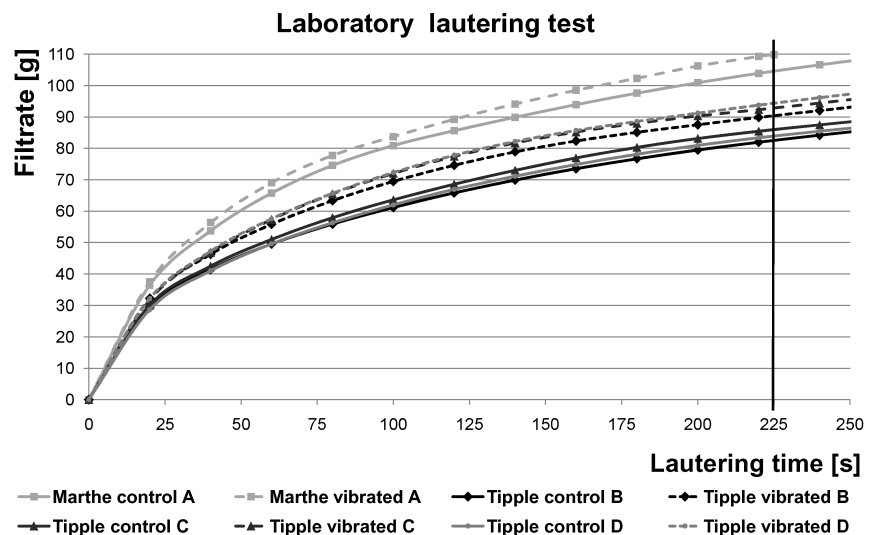


Fig. 5 Comparison of the lautering properties of the final malts, evaluated by an in-house laboratory lautering test

Laboratory lautering test

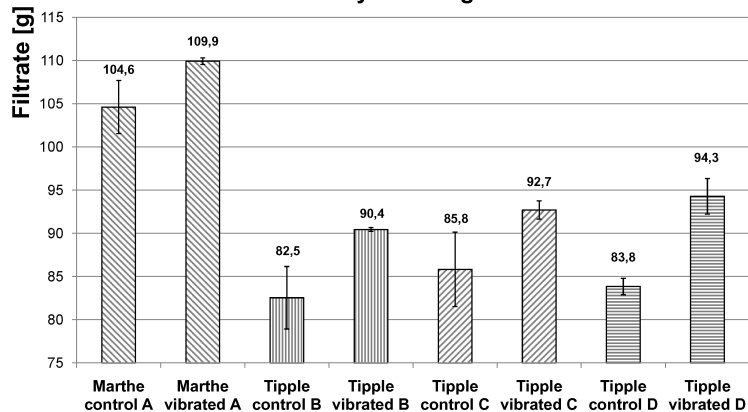


Fig. 6 Results of the laboratory lautering test (mean of duplicate or triplicate analysis; standard deviation included; sample "Tipple control (C)" only single analysis with 5 % standard deviation)

method was used to check the produced malts (see materials and methods section). The malt's lautering properties were evaluated by comparing the curve shapes as received when plotting collected wort volume against filtration time and by analysing the total volume after 225 seconds of filtration. The obtained curves of the tests are displayed in figure 5 and the results are summarised in figure 6.

In direct comparison, the Marthe malts generally showed a better lautering performance than the Tipple malts. When comparing the different reference malts with the corresponding vibrated malts, it was observable that the only varied malting parameter of the vibration treatment during wet steeping was responsible for an improvement of the lautering performance for all vibrated samples. The three reference samples as well as the three vibrated samples of the Tipple malts (trial B–D) showed very similar lautering curves and lautering times.

When comparing the turbidities of the steeping waters visually, it was obvious that the sonication led to an extreme washing effect on barley. Therefore, the chemical oxygen demand (COD) and the iron content of the steeping waters were additionally analysed in the second series of trials (C, D). The results of the measurements confirmed the washing effect and are summarised in table 3.

The CODs of the vibrated steeping water increased by about the threefold as compared to the reference values. When aerating during wet steeping, the abrasion caused by the air bubbles supported

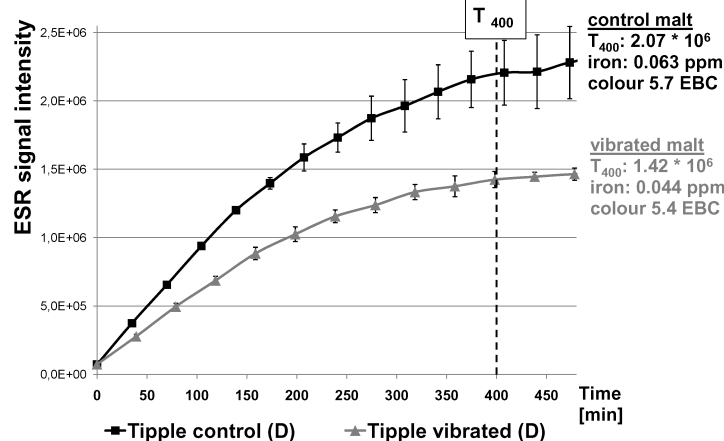


Fig. 7 EAP determination [MEBAK (Methodensammlung der Mitteleuropäischen Brautechnischen Analysenkommission), Band Rohstoffe, (2006).] of congress worts (origin extract 12.1 %) produced from the Tipple malts of trial D; comparison between radical generation, as indicated by the T400-values, iron content and colour

the COD increase by about 20–25 %. This cleaning effect of the vibrations may explain the slightly higher extracts (Table 2) found for all vibrated samples due to a removal of useless impurities resulting in a higher extract per mass malt.

Additionally, the influence of the sonic waves on the microbial contamination of the finished malts was assessed (see materials and methods section). The results are summarised in table 4.

With the exception of trial A, the significant decrease of the microbial count demonstrates that a contamination reduction of about 50–60% could be achieved when applying sonication during wet steeping. Analogically to COD measurements, this can be explained by an extreme washing effect of the vibrations. The washing effect seemed to be more effective when applying vibrations during the second wet steep because the reduction of the Marthe malts' microbial contamination (trial A) was negligible. However, the Marthe barley was much lower contaminated as compared to the Tipple barley. Comparing the results of the Tipple malts of trial B and C, whose wet steeps were unaerated, it is observable that an aeration during air rest (trial B) led to higher microbial counts which can probably be explained by the increased availability of oxygen. The abrasion of air bubbles during an aerated wet steep (trials C, D) most likely also supports a reduction of the microbial counts.

The iron contents of the steeping waters (Table 3) correlated well with the results of the COD. The applied vibrations yielded a higher

Table 3 Chemical oxygen demand (COD) and iron content of steeping water (mean of duplicate analysis) – Trial C–D

Steeping water	Wet steep	COD [mg/L]	Total COD [mg/L]	Iron entry [mg/kg barley]	Total iron entry [mg/kg barley]
Tipple control (trial C), unaerated wet steeps	1 <sup>st</sup>	2725	5853	1.66	2.9
	2 <sup>nd</sup>	3129		1.25	
Tipple vibrated (trial C), unaerated wet steeps	1 <sup>st</sup>	7319	18491	6.66	10
	2 <sup>nd</sup>	11172		3.29	
Tipple control (trial D), aerated wet steeps	1 <sup>st</sup>	3629	7149	3.96	5.9
	2 <sup>nd</sup>	3520		1.98	
Tipple vibrated (trial D), aerated wet steeps	1 <sup>st</sup>	10888	23039	10.74	15.1
	2 <sup>nd</sup>	12151		4.39	

iron content in the steeping water and thereby consequently suggesting lower iron concentrations remaining in the malts. It was noticeable that the iron entry into the water was much higher during the first wet steep. Several research groups [12, 18, 19, 38, 39] have shown that the reduced iron entry by raw materials like malt results in a higher oxidative beer stability. This positive effect of an iron reduction can be explained by the catalyzing effect of metal ions on the oxygen activation per electron transfer and the prooxidative acting radical generation caused by the Fenton-/Haber-Weiss reaction system. Lower iron values in the malts used for brewing may therefore yield an improved flavour stability of the beers produced thereof. To investigate the influence of vibrations during steeping and potentially lowered iron values of the malts on the oxidative stability, worts were produced and the wort's flavour stability was evaluated using ESR spectroscopy. In this context, the wort's iron content and colour was also determined. The results from the ESR measurements and the corresponding colour and iron contents are shown in figure 7.

The enhanced washing out or elution of iron led to an iron reduction of about 30 % in the wort produced of the vibrated malt. In direct correlation, the radical generation in the worts during forced aging at 60 °C, as indicated by the ESR signal intensity after 400 minutes of measurement ( $T_{400}$ -value), was lowered by 31.5 % by the treatment with sonic waves during wet steeping.

## 5 Conclusion

Taking together, these data give evidence that the application of vibrations or sonication at 180–200 Hz during steeping results in a remarkably improved cleaning effect of the barley. Consequently, this yields a diminished microbial contaminations of the malt, lower iron contents and therefore also an increased flavour stability of the worts produced thereof.

In combination with the lower microbial contamination, the treatment with vibrations also promote a faster water uptake, both leading to

a significantly sooner and more homogeneous start of germination of the barley thereby yielding more homogenous malt. In general, it could be shown that the application of vibrations do not negatively influence any parameter of the standard malt analyses which all ranged in specifications recommended by MEBAK. However, significantly reduced lautering times, assessed by a laboratory lautering test, could be observed for all four sample pairs, consisting of a reference and a vibrated malt. Applying vibrations during wet steeping, thus, seem to be advantageous for an increasing the capacity of a brewhouse due to a shorter lautering process which is the most time consuming step in the wort production.

However, the improved cleaning effect on barley also resulted in much dirtier steeping waters, indicated by threefold higher CODs. This has to be taken into consideration due to possible higher costs for the sewage water of a malting plant.

Another advantage of the cleaning effect resulting in the enhanced iron reduction in vibrated malts could be of value for the production of special malts like crystal or roasted malts. Recent investigations of *Kunz et al.* [20] showed that a significant increased release of iron and copper ions is caused by roasting processes leading to a higher entry into the wort during mashing. This metallic ions are probably bound in the covering of the barley kernels and are not released during the kilning process of "normal" pale malt. Hence, a reduced iron content in the green malt before a roasting process could lead to a further reduced iron entry during mashing and thus to an improved oxidative wort and beer flavour stability when using "vibrated" special malts.

For applying vibrations during wet steeping, it can be suggested to aerate the steeping water continuously due to desorption of the dissolved oxygen. In steeping systems without the possibility of pressure aeration, vibrations should be applied during the first wet steep and/or at the end of subsequent wet steeps to diminish a lack of oxygen. During the first wet steep the barley only needs little oxygen supply due to low activity and during an unaerated second or third wet steep the oxygen is consumed very fast.

**Table 4** Microbial analyses of the final malts (mean of duplicate analysis).

Sample	Microbial count (wort agar) [ $\times 10^6$ ]	Reduction [%]	Coliform count (Chromocult agar) [ $\times 10^6$ ]	Reduction [%]
Marthe control (trial A) unaerated wet steeps, aerated air rest	7.9	6	2.7	0
Marthe vibrated (trial A) unaerated wet steeps, aerated air rest	7.4		2.7	
Tipple control (trial B) unaerated wet steeps, aerated air rest	23.0	57	7.1	46
Tipple vibrated (trial B) unaerated wet steeps, aerated air rest	9.8		3.8	
Tipple control (trial C) unaerated wet steeps, unaerated air rest	16.5	56	6.2	29
Tipple vibrated (trial C) unaerated wet steeps, unaerated air rest	7.2		4.4	
Tipple control (trial D) aerated wet steeps, unaerated air rest	12.9	51	7.1	83
Tipple vibrated (trial D) aerated wet steeps, unaerated air rest	6.3		1.2	

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## 7 References

1. Aalbers, V.-J.; Drost, B.-W. and Pesman, L.: Aerated Steeping Systems, MBAA Technical Quarterly **20** (1983), pp. 74-79.
2. Annemüller, G.; Bauch, T.; Nagel, R. and Böhm, W.: Der Endo- $\beta$ -Glucanasegehalt – ein Maß für die „cytolytische Kraft“ des Malzes?, BRAUWELT **135** (1995), pp. 206-210.
3. Axcell, B.; Jankovski, D. and Morrall, P.: The Crucial Factor in Determining Malt Quality, The Brewers Digest, **58** (1983), pp. 20-23.
4. Baxter, E.-D.; Reeves, S.-G. and Bamforth, C.-W.: The Effects of Increased Steeping Temperature on Enzyme Development in Malt, J. Inst. Brew. **86** (1980), pp.182-185.
5. Baxter, E.-D. and O'Farrell, D.-D.: Effects of Raised Temperatures During Steeping and Germination on Proteolysis, J. Inst. Brew. **86** (1980), pp. 291-295.
6. Bloch, F. and Morgan, A.-I.: Germination Inhibition in Wheat and Barley During Steeping, and Alpha-amylase Development in Presence of Gibberellic acid, Cereal Chem. **44** (1967), pp. 61-69.
7. Briggs, D.-E.: Accelerating Malting a Review of Some Lessons of the Past from the United Kingdom, ASBC Journal **45** (1986), pp. 1-6.
8. Brookes, P.-A.; Lovett, D.-A. and Mac William, I.-C.: The Steeping of Barley – A Review of the Metabolics Consequences of Water uptake and their Practical Implications, J. Inst. Brew. **82** (1976), pp. 14-26.
9. Clasen, C. and Kulicke, W.-M.: Zum Gelbildungsprozeß von (1 $\rightarrow$ 3) (1 $\rightarrow$ 4)- $\beta$ -D-Glucanen, Monatsschrift für Brauwissenschaft **56**, (2003), no. 9/10 pp. 161-171.
10. COFCO MALT DALIAN COLTD.: Method for Ultrasonically Processing Barley to Improve Quality of Malt, Patent CN 102174345 A (2011).
11. Dietrich S.: Einfluß und Bedeutung der Belüftung während der Weicharbeit und Vermälzung von Braugerste, BRAUWELT **106** (1966), pp. 1-4.
12. Frederiksen, A. M.; Festersen, R.-M. and Andersen, M.-L.: Oxidative Reactions during Early Stages of Beer Brewing Studied by Electron Spin Resonance and Spin Trapping, J. Agric. Food Chem. **56** (2008), pp. 8514-8520.
13. Gordon, A.-G.: The use of Ultrasound in Agriculture, Ultrason., **1/2** (1963), pp. 70-77.
14. Home, S.; Stenholm, K. and Oikku, J.: Measuring and Evaluating Malt Homogeneity, Proc. 27<sup>th</sup> EBC Congr., Fachverlag Hans Carl, Nürnberg, Germany, Cannes (1999), pp. 365-375.
15. Kleinwächter, M.; Meyer, A.-K. and Selmar, D.: Malting Revisited: Germination of Barley (*Hordeum vulgare* L.) is Inhibited by Both Oxygen Deficiency and High Carbon Dioxide Concentrations, Food Chemistry **132** (2012), pp. 476-481.
16. Kleinwächter, M.; Müller, C.; Methner, F.-J. and Selmar, D.: Biochemical Heterogeneity of Malt is Caused by both Biological Variation and Differences in Processing: I. Individual Grain Analyses of Biochemical Parameters in Differently Steeped Barley (*Hordeum vulgare* L.) Malts, J. Agric. Food Chem. **147**, 25-33.
17. Kunz, T.; Methner, F.-J.; Kappl, R. and Hüttermann, J.: Verfahren zur Bestimmung des endogenen antioxidativen Potenzials von Getränken mittels ESR-Spektroskopie, Deutsche Patentanmeldung U30121, TU-Berlin/Universität des Saarlandes, IPAL Offenlegungsschrift Deutsches Patent- und Markenamt DE 10 2005 043 113 A1, 2005
18. Kunz, T.; Müller, C. and Methner, F.-J.: EAP Determination and Beverage Antioxidative Index (BAX) – Advantageous Tools for Evaluation of the Oxidative Flavour Stability of Beer and Beverages (Part 1), BrewingScience – Monatsschrift für Brauwissenschaft **66** (2013), no.1/2, pp. 12-22.
19. Kunz, T.; Strähmel, A.; Cortés, N.; Hense, W.; Kroh, L.-W. and Methner, F.-J.: Influence of Intermediate Maillard Reaction Products with Endiol Structure on the Oxidative Stability of Beverages, J. Am. Soc. Brew. Chem. **71** (2013), pp. 114-123.
20. Kunz, T.; Stoffregen, T.; Cruz Palma, C.; Xu, C. and Methner, F.-J.: Influence of Special Malt and Coloring Agents on the Oxidative Beer Stability and Aging Components, presentation at MBAATQ, **50/3** (2013), ISSN 0743-940, p.126.
21. MacLeod, A.-M. and Millar, A.-S.: Effects of Gibberellic Acid on Barley Endosperm, J. Inst. Brew. **68** (1962), pp. 322-332.
22. MEBAK (Methodensammlung der Mitteleuropäischen Brautechnischen Analysenkommission), Band Rohstoffe, (2006).
23. Müller, C.; Kleinwächter, M.; Selmar, D. and Methner, F.-J.: The Influence of Elevated Steeping Temperatures on the Resulting Malt Homogeneity and Malt Quality, BrewingScience – Monatsschrift für Brauwissenschaft **66** (2013), no. 7/8, pp. 114-122.
24. Narziß, L.: Moderne Mälzungsmethoden I, BRAUWELT **105** (1965), pp. 1506-1515.
25. Narziß, L.; Friedrich, G.: Der Einfluß des Mälzungsverfahrens auf die Steigerung der Enzymaktivität I–IV, Monatsschrift für Brauwissenschaft **23** (1970), pp. 123-142, 167-175, 229-234, 265-271.
26. Narziß, L.; Reicheneder, E. and Brauchle, R.: Untersuchungen zur Cytolyse des Malzes, BRAUWELT **127** (1987), pp. 1453-1461.
27. Palmer, G.-H.: Increased Endosperm Modification of Abraded Barley Grain after Gibberellic acid Treatment, J. Inst. Brew. **75** (1969), pp. 536-541.
28. Pollock, J.-R.-A. and Pool, A.-A.: Enzymes of Barley and Malt III – The Latent  $\beta$ -amylase of Barley, J. Inst. Brew. **64** (1958), pp. 151-156.
29. Pool, A.-A.: Re-appraisal of the Usefulness of Warm-Water Steeping in Malting in the Light of the Effect of Gibberellic Acid, J. Inst. Brew. **70** (1964), pp. 221-225.
30. Reeves, S.-G.; O'Farrell, D.-D. and Wainwright, T.: The Effect of Increased Steeping Temperature on Malt Properties, J. Inst. Brew. **86** (1980), pp. 226-229.
31. Sarx, H.-G. and Rath, F.: Filtration Risk Analysis - New Method for Predicting Problems in Wort and Beer Filtration, Proc. 25 EBC Congr. IRL Press: Oxford, Brussels, (1995), pp. 615-620.
32. Schneid, R.: Verfahrenstechnische Beeinflussung des Maischens sowie dessen Auswirkung auf den Brauprozess und die Bierqualität, dissertation, TU Berlin, 2009, pp. 28-32.
33. Segal, B.; Dan, V. and Grager, W.: Über den Einfluß von Ultraschall auf die Keimfähigkeit und Enzymaktivität der Gerste, Monatsschrift für Brauwissenschaft **24** (1971), pp. 241-244.
34. Shors, J.-D.; Soll, D.-R.; Daniels, K.-J. and Gibson, D.-P.: Method for Enhanced Germination, Patent US 5,950,362, 1999.
35. Sims, R.-C.: Germination of Barley: Effects of Varying Water Contents upon the Initiation and Maintenance of Growth, J. Inst. Brew. **65** (1959), pp. 46-51.
36. Specht, W. and Schultheiss, H.: Ultrasonic Irradiation in Preparation

- of Malt, Patent US 2,745,789, (1956).
37. Sommer, G.: Weichtemperatur und Malzqualität, Mschr. Brauerei. **24**, 205-210, (1971)
38. Uchida, M. and Ono, M.: Improvement for Oxidative Flavour Stability of Beer - Role of OH-Radical in Beer Oxidation, J. Am. Soc. Brew. Chem. **54(4)** (1996), pp. 198-204.
39. Uchida, M. and Ono, M.: Determination of Hydrogen Peroxide in Beer and Its Role in Beer Oxidation, J. Am. Soc. Brew. Chem. **57(4)** (1999), pp. 145-150.
40. Voborsky, J.: Der Einfluss der Sprühweiche und der Wassertemperatur auf die Qualität des Malzes, Lebensmittelindustr. **18/10** (1971), pp. 377-380, pp. 428-431.
41. Weidinger, A.: Neue Wege der Weichgutbelüftung, BRAUWELT **106** (1966), pp. 1171-1178.
42. Wilhelmson, A.; Laitila, A.; Vilpola, A.; Olkku, J.; Kotaviita, E.; Fagerstedt, K. and Home, S.: Oxygen Deficiency in Barley (*Hordeum vulgare*) Grain during Malting, J. Agric. Food Chem. **54** (2006), pp. 409-416.
42. Yaldagard, M.; Mortazavi, S.-A. and Tabatabaie, F.: Application of Ultrasonic Waves as a Priming Technique for Accelerating and Enhancing the Germination, J. Inst. Brew. **114** (2008), pp. 14-21.

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