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Correlation of Malt Quality Parameters and Beer Flavor Stability: Multivariate Analysis

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Malt is known to have an impact on beer flavor stability mainly due to the presence of antioxidants. In this study, five barley varieties were malted at industrial and micro scale, and quality parameters of the resulting malts were measured (diastatic power, friability, β-glucan content, antiradical power, reducing power, lipoxygenase activity, and nonenal potential) and correlated with the sensory data obtained for the corresponding fresh and forced aged beers. A statistical strategy using multiple linear regressions was applied to explore relationships between the malt chemical parameters and beer sensory data, showing antiradical power as the major contribution of malt to beer flavor stability. Additionally, the measured antiradical power, which is well correlated with the polyphenolic content, was found to be very similar for malt and barley, emphasizing the key role of barley endogenous polyphenols.

KEYWORDS: Barley; beer; malt; polyphenols; multiple linear regression; antiradical power; nonenal potential

INTRODUCTION

Delaying flavor staling, to prolong shelf life of beer, is one of the greatest challenges facing the brewing industry today. This concern is particularly evident in Mediterranean countries achieving extreme temperatures during the summer period.

Malted barley can have impact on beer stability due to the presence of pro-oxidant and antioxidant activities (*1*). Malt contains various compounds, originated from barley or formed during the malting process, which can play a significant role in malting and brewing through their antioxidant activities (*1*). Malt and barley contain, among others, phenolic compounds, phytic acid, ascorbic acid, melanoidins, and several enzymes which can be responsible for part of this total antioxidant power (*2*). Of these, melanoidins and polyphenols are the most significant sources of natural antioxidants, which can be originated from the malting process or already present in barley (*3*). Accordingly, attention has been paid to raw materials final properties over the past few years (*1*−*2*).

Oxidation can take place during malting, in the brewhouse, or during aging in the brewery or in the package (*4*). Flavor changes are therefore dependent on raw materials, malting and brewing procedures, packaging conditions, and environmental factors.

Despite evidence that both the organoleptic quality of beers and their flavor stability during aging are influenced by the composition of barley and malt, it is difficult to correlate these sensory and analytical data, and relating them to a varietal factor, mainly due to the complexity of controlling all the technological steps involved in the brewing process. This study aims at correlating malt quality parameters potentially relevant for beer flavor stability and sensory data as well as to differentiate a number of barley varieties on the basis of the same parameters. Sensory, compositional, and performance data were collected using experimental protocols specifically devised for the purpose and were examined using multivariate statistical techniques. Multiple linear regression (MLR) was used to determine the malt quality parameters which are most discriminating for differentiating beers of varying flavor stability. A set of industrial scale beers was brewed using six European malting barley varieties. Barley, industrial malt, and micro-scale malt of each variety were monitored for a group of chemical analytes and performance parameters considered significant in terms of their influence on beer flavor stability, such as enzymatic activities, antioxidant capacity, reducing power, and nonenal potential. Fresh beers and forced aged beers (1 week at 37 °C) were then analyzed for several analytical markers of beer aging, as well as by an expert sensory panel, during a long-term storage.

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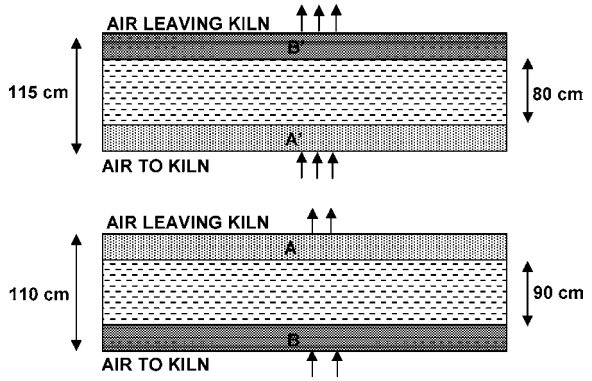

Figure 1. Schematic design of the industrial double-deck kiln. Green malt is dried in the first kiln (bottom) during the withering phase. Malt in then transferred to the second kiln (curing stage), with inversion of the positions of the malt layers A and B.

Multivariate techniques can be effectively applied to elucidating relationships between instrumental parameters and sensory data. In this work, a statistical strategy using MLR is applied to correlate sensory and chemical data sets. Additionally, the relative significance of each of the chemical data and process parameters on the organoleptic stability of beer is evaluated within the statistical method.

MATERIALS AND METHODS

Chemicals. Acetic acid and methanol of analytical reagent grade were purchased from Prolabo (Fontenay-sous-Bois, France). Sodium acetate trihydrate, 2,6-dichlorindophenolate hydrate, and 1,1-diphenyl-2-picryl-hydrazil (DPPH) were obtained from Sigma-Aldrich Chimie (S^t Quentin Fallavier, France). High-purity water from a Milli-Q185-Plus water purification system (Millipore, Bedford, USA) was used for all chemical analyses and glassware washing.

Malting and Malt Treatment. Single-variety barleys were malted at the same time on industrial (50 tonnes) and micro scale (2 kg), using the conditions defined below. The single-variety industrial malts were then brewed at industrial scale, using the standard procedures of the local brewery (UNICER).

Four spring malting varieties (*Scarlett*, *Prestige*, *Riviera*, and *Nevada*) and one six-row winter barley (*Esterel*) were used in this work. *Scarlett* crops grown in Spain (*Scarlett S*) and France (*Scarlett F*) were studied, in a total of six single-variety barleys studied.

Industrial scale assays were carried out at the Maltiberica malting plant (Poceirão, Portugal). Fifty tonnes of malt of each single-variety were produced in the same conditions. The kilning system is a double-deck kiln with a fully automatic control process. Drying occurs in two distinct stages: the withering phase in the first floor deck is performed by gradually increasing the temperature until 65 °C; malt is then automatically discharged into the second floor deck, where temperature is gradually increased until 70 °C and then until 85 °C staying 3 h at this temperature (curing phase). Malt samples were taken from bottom and top of the bed in the first and second kiln, according to **Figure 1**.

Micro-scale assays (2 kg) were carried out in the automated micro malting equipment of the IFBM (Nancy, France). The procedure used included 46 h steeping at 15 °C with varying wet and dry periods, followed by 5 days germination at 16 °C. Kilning consisted of five successive steps of heating of germinated barley: 50 °C for 8 h, 50−64 °C for 1 h, 64 °C for 10 h, 64−80 °C for 1 h, and 80 °C for 4 h.

Every procedure described below used malt milled in a Bühler (Winnipeg, Canada) universal laboratory disc mill (type DLFU), set with a 0.2 mm disc gap. Moisture content of all malts was determined on a finely ground sample at 104 °C for 3 h in a convection oven (Tripette & Renaud Chopin, France), according to the standard procedure (method 4.2) of the Analytica-EBC (*5*).

Brewing Procedure. A 14 °P lager wort (one degree Plato represents a sugar content equivalent to 1% sucrose by weight) was produced from 10 tonnes of each of the previously mentioned single-variety industrial malt. A total of 21 kg of *Saaz* hops were added to the boiling kettle 15 min before the end of the boiling stage. Hop extract (supercritical CO_2) was added 15 min after starting the boiling stage to achieve the correct bitterness. The concentrated wort (pH between 5.1 and 5.3 and bitterness units between 32 and 36 EBU) was filtered in the Meura 2001 filter system and then cooled. Approximately 1000 HL of the wort was transferred into the cylindroconical ferment and aerate in-line (8 mg/L of dissolved oxygen). Fermentation was undertaken using a bottom fermentating yeast at a pitching rate of 15 $\times$ 10^6 cell/cm^3, and the initial temperature of fermentation was 8 °C. After fermentation, the immature beer was subjected to a lagering stage of 24 h at 0 °C. Beers were bottled in the same filling line, with a final total O_2 content ranging between 0.34 and 0.50 mg/L (**Table 1**).

Table 1. Results of the Total Oxygen Content and the ANOVA Statistical Test Performed for the Sensory Scores of Fresh and Forced Aged Beers

barley variety	total oxygen content (mg/L)	fresh beers	forced aged beers[a]
Scarlett S	0.42	–0.64	–0.82 a, b
Prestige	0.50	–0.42	–0.80 a, b
Esterel	0.40	–0.50	–1.55
Riviera	0.38	–0.47	–0.53 a
Scarlett F	0.34	–0.36	–0.70 a, b
Nevada	0.40	–0.67	–1.05 b
mean		–0.51	–0.91
significance	–	0.207	0.007

[a] Mean values with no common letters differ significantly at the 95% confidence level.

***Tepral* Filtration.** The *Tepral* mashing and filtration was based on the original method described by Moll et al. (*6*) with the modifications reported by Guido et al. (*7*). The filtrate (*Tepral* wort) was weighed in a digital balance, and the filtration performance was measured by calculating the filtration velocity. The *Tepral* wort was immediately cooled in ice and kept at −20 °C until further analysis.

Determination of Nonenal Potential. Nonenal potential, an indicator of how a beer will release *E*-2-nonenal during storage, was first reported by Drost et al. (*8*). In this work, the nonenal potential was determined using the protocol described by Guido et al. (2005) (*7*). Briefly, the pH of *Tepral* wort was adjusted to 4 and purged for 3 min with nitrogen (99.5%, Air Liquide). The sample was heated at 90 °C for 2 h and then cooled to 4 °C. *E*-2-Nonenal released was then extracted by a liquid−liquid extraction with carbon disulfide and quantified by GC-MS using 1-octanol as an internal standard.

Determination of Friability. Malt friability, the degree to which the endosperm has been broken down (modified) during the germination, was determined according to the method 4.15 of Analytica-EBC (*5*).

Determination of β-Glucan Content. The β-glucan content was determined according to the method 4.16 of Analytica-EBC (*5*).

Determination of the Lipoxygenase (LOX) Activity. LOX activity was determined according to the protocol reported by Guido et al. (2005) (*7*).

Determination of Total Polyphenol Content. The total phenolic content was determined according to the Folin−Ciocalteau method, described by Singleton and Rossi (*9*). Phenolics from malt were extracted in methanol by ultrasonication (30 min at 30 °C) and determined spectrophotometrically at 740 nm using a calibration curve was prepared with gallic acid.

Wort Reducing Power Assay. The reducing power of worts was assessed by a modified version of the ITT (Indicator-Time-Test) (*10*) adopted for wort properties (namely pH). This assay is an established method specifically developed to measure the oxidation state of beers and commonly used nowadays by the brewing industry. This essay is based on the reaction of the electron acceptor 2,6-dichlorophenolindophenol (DCPIP) with the reducing compounds present in wort. The measured value is the percentage of the indicator decolorized during a

precise time period, which is specifically defined for fast, intermediate, and slow reducing agents. A 0.15% (w/v) DCPIP stock solution in water was 20-fold diluted in sodium acetate buffer (0.1 M, pH 4.3). After selecting the wavelength to 520 nm, a blank test tube was prepared with 2.00 mL of the wort and 2.00 mL of sodium acetate buffer (0.1 M, pH 4.3) for each wort sample to be analyzed. The spectrophotometer (Varian, Cary 50 UV−visible) was set to read zero of absorbance using the blank. A reference test tube containing 2.00 mL of the DCPIP solution and 2.00 mL of sodium acetate buffer (0.1 M, pH 4.3) and a sample test tube with 2.00 mL of the DCPIP solution and 2.00 mL of the wort sample were then placed into the test tube holder. A_{520} readings (I) were continuously recorded every 30 s over the course of the reaction for up to 60 min. Using a daily calibration curve, absorbances were converted into micrograms of DCPIP, which were related with the concentrations of fast, intermediate, and slow reducing compounds as follows: fast: $I_{\text{Reference}} - I_{30''}$; intermediate: $I_{30''} - I_{5'}$; and slow: $I_{5'} - I_{60'}$. Results are reported for the average of three readings.

Wort and Malt Antiradical Power (ARP). The following procedure was based on the work of Goupy et al. (1999) (*8*) with minor modifications. Briefly, malt extracts were prepared by stirring 2.0 g of ground malt (barley or spent grains) with methanol (4 °C, 20 min). The extraction procedure was repeated twice, and the methanolic extracts combined, filtered, and evaporated to dryness under vacuum. This residue was redisolved in 5.00 mL of methanol and sonicated if necessary for complete dissolution. For the test, these extracts were diluted 4, 8, 10, and 20 times with methanol, 0.15 mL of these solutions was added to 2.85 mL of a DPPH solution, and the absorbance was determined against a blank solution at 515 nm immediately (A_0) and after 120 min (A_{120}). For reference, the absorbance after 120 min of a solution consisting of 2.85 mL of DPPH solution and 0.15 mL of methanol (A_{ref}) was measured. For each test, the percentage of DPPH in the radical form was calculated ($A_{120}/A_0 \times 100$), and a calibration curve was obtained plotting concentration of malt on a dry basis (barley or malt) against the percentage of unreacted DPPH. Using this calibration curve it was possible to determine the concentration of malt capable of reacting with 50% of DPPH (EC_{50}). For convenience, the value of $1/EC_{50}$ is used, which is called the antiradical power (ARP). In practice, higher ARP values correspond to more efficient scavenging capabilities. In the case of wort there was no need for extraction procedures. First, wort was diluted 3, 6, 8, and 10 times in acetate buffer 0.1 M, pH 4.3; then 0.2 mL of this solution was added to 2.8 mL of DPPH (1.87×10^{-4} M in acetate buffer 0.1 M, pH 4.3) and the absorbance was measured at 515 nm immediately (A_0) and after 120 min (A_{120}) against a blank solution.

Sensory Analysis. Eight tasters of the UNICER's internal sensory panel with at least 1 year of experience were recruited. The panel was contracted for three 1 h sessions per week, which took place on the morning at an adequately isolated taste room. Panelists were asked to comment the general quality of bottled beer (discrimination test) as well as to describe the flavor profile according to a special form (description test). The beers were tasted in random order, fresh (1 week at 4 °C) and after forced aging (1 week at 37 °C) by the Duo−Trio test. All beers were tasted at 4 °C and evaluated for the degree of staling on a five-point scale defined as follows: (+1) no sign of oxidation; (0) very slight oxidation symptoms; (−1) slight oxidation symptoms; (−2) enough level of oxidation to reject the beer; and (−3) very strong level of deterioration by oxidation. The sensory classification of each beer consisted of the mean value of the scores provided by all tasters, with an adopted sensory rejection limit of −1.5.

Statistical Analysis. The data obtained from the experiments were analyzed using MLR. The general purpose of MLR is to study the relation between a dependent variable (Y) and other independent variables (X_n). The general mathematical function is a first-degree equation specified as

$$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + ... + \beta_m X_m + \epsilon \quad (1)$$

where X_m represents the m experimental variables tested, α is the constant term, and ϵ the predictive error. β_m represents the regression coefficients of the independent variables, and each represents the "weight" (correlation) of the respective independent variable (*12*).

To measure the strength of relationship between the measured variables, the two most commonly used correlation coefficients were calculated. Pearson's correlation coefficient (r) requires both variables to be measured on an interval or ratio scale, and the calculation is based on the actual values. Spearman's correlation coefficient (ρ) is a nonparametric statistic and so can be used when the data have violated parametric assumptions and/or the distributional assumptions. Spearman's rank coefficient requires data that are at least ordinal, and the calculation, which is the same as for Pearson's correlation, is carried out on the ranks of the data. Each variable is ranked separately by putting the values of the variable in order and numbering them (*13*).

The statistical package, *StatBox* 2.1 (Grimmer Logiciel, Paris, France) was used for all statistical calculations.

RESULTS AND DISCUSSION

Effect of Barley Variety on the Beer Flavor Stability. To evaluate the influence of barley variety on the sensory attributes of beer during storage, a two-way ANOVA (barley variety and beer taste score were considered as the variables 1 and 2, respectively) was run for each barley variety. The ANOVAs of sensory analysis (mean scores of three or four sensory evaluations) of fresh and forced aged beers showed significant differences between barley varieties only for aged beers. The results of the ANOVA statistical test are given in **Table 1**.

With a high p-value (0.207), the null hypothesis may not be rejected and the variety was shown to not have a statistically significant influence on the sensory quality of fresh beers (total taste score ranged between −0.67 for *Nevada* and −0.36 for *Scarlett F*). On the contrary, important differences for forced aged beers were observed between barley varieties, with *Esterel* clearly showing the lowest taste score (−1.55) and *Riviera* considered the best by the sensory panel (−0.53). The null hypothesis was here clearly rejected ($p = 0.007$), and the sensory scores of forced aged beers were examined to evaluate the barley varieties significantly different among them at the 0.05 significance level by a t-test. *Esterel* was the only variety showing significant differences with respect to all other varieties for the forced aged beers, as may be seen in **Table 1**. *Esterel* differs significantly from *Scarlett S* ($p = 0.016$), *Prestige* ($p = 0.042$), *Riviera* ($p = 0.000$), *Scarlett F* ($p = 0.001$), and *Nevada* ($p = 0.025$). In addition, the effect of the barley variety on the sensory scores of forced aged beers was found statistically significant between *Riviera* and *Nevada* ($p = 0.031$). These differences cannot be attributed to the total oxygen content, which varies between 0.34 mg/L for *Scarlett F* and 0.50 mg/L for *Prestige*.

Relationship between the Malt Quality Parameters and the Beer Flavor Stability. Malt quality parameters were assessed for the upper and lower kilning layers of the industrial malts (**Figure 1**) corresponding to each of the barley varieties. Additionally, each barley variety was then micro-malted (2 kg) under as near as possible identical conditions in order to try to eliminate potential variations related to the depth of bed in the kiln (*7*). Industrial and micro malts were analyzed for LOX activity, ARP, β-glucan, and friability. *Tepral* worts were assessed for the nonenal potential, ARP, and reducing power, and the velocity of the *Tepral* filtration was calculated. The relationship between the total taste score for forced aged beers and the malt parameters has been estimated using the Pearson correlation coefficient (r) and the Spearman's rank correlation coefficient (ρ). Correlation coefficients for industrial and micro malts are given in **Table 2**.

The Pearson's linear correlation coefficient (r) was used to determine the degree of relationship between the variables. Pearson's correlation coefficient may provide the best estimate of the population correlation coefficient if the data are normally

Table 2. Pearson's Correlation (r) and Spearman's Rank Correlation (ρ) Coefficients between the Total Taste Score Obtained for Forced Aged Beers and the Analytical Parameters for Industrial and Micro Malts[a]

	industrial malts		micro malts	
	r	ρ	r	ρ
Tepral wort nonenal potential	0.49	–0.12	–0.68	–0.46
LOX activity	–0.25	–0.20	–0.30	0.03
malt ARP	–0.02	–0.09	0.86*	0.84*
Tepral wort ARP	–0.34	–0.41	–0.19	0.14
Tepral wort reducing power	–0.15	–0.03	–0.60	–0.23
β-glucans	0.48	0.46	0.77	0.20
friability	–0.16	–0.12	–0.23	–0.03
filtration velocity	–0.66	–0.49	–0.58	–0.49

[a] Asterisk (*) indicates significant values with 95% of confidence.

distributed. This correlation has two limitations: it is neither robust nor resistant. It is not robust because a strong yet nonlinear relationship between the two variables may not be recognized. It is not resistant since it can be extremely sensitive to one or a few outlying point pars. Since the value of the correlation is markedly influenced by extreme values, it does not provide a good description of the relationship between the two variables when the distribution of the variables is skewed or it contains outlying values (*12*). Thus, the data were converted into ranks, and Spearman's nonparametric correlation coefficients (ρ) were also calculated. As may be seen in **Table 2**, a clear difference for industrial and micro malt correlation coefficients was obtained. It is important to mention that the mean values between the upper and lower kilning layer were used for all industrial malt parameters assessed, due to the unfeasibility of collecting an homogeneous sample of the malt representative of the malt bed in the kiln. It might be assumed that this simplification may induce a lack of consistency between the computed correlations, but in our view this was the most suitable approach to overcome the problem of heterogeneity. There was no significant correlation ($p > 0.05$) between the beer flavor stability and the malt parameters measured for each variety of industrial malts. Moreover, the nonenal potential determined after bench-scale mashing and filtration (*Tepral* wort) of industrial malts does not correlate at all with the total taste score. On the contrary, better relationships were obtained when micro malts are considered (**Table 2**). Malt ARP is the only variable significantly correlated to the beer flavor stability at a 95% confidence level ($r = 0.86$ and $\rho = 0.84$). Nonenal potential of *Tepral* wort is the second variable most strongly correlated, although this correlation is not significant ($r = -0.68$ and $\rho = -0.46$; $p > 0.05$). These results show that barley variety has a marked influence on the beer flavor stability, but treatments in the industrial malting plant may have much higher influence than do varietal factors. In fact, higher dissimilarities for the malt analytical parameters were obtained between the upper and lower bed in the kiln than between the barley varieties, as may be seen in **Table 3**. Nonenal potential is the parameter contributing higher for the dissimilarities between the kilning layers (χ^2 distance = 0.04). The potential factors causing this observed heterogeneity related to the depth of bed in the kiln were already discussed elsewhere (*7*).

To show the strong connection of micro-malt variables with the sensory scores of aged beers, a MLR was run taking into account the micro-malt parameters (eq 2). The malt ARP and nonenal potential of *Tepral* wort were able to predict 73% of

Table 3. Dissimilarity between Variables Calculated by the χ^2 Distance

	χ^2 distance	
	lower/upper malt bed	barley variety
Tepral wort nonenal potential	0.04	0.01
LOX activity	0.02	

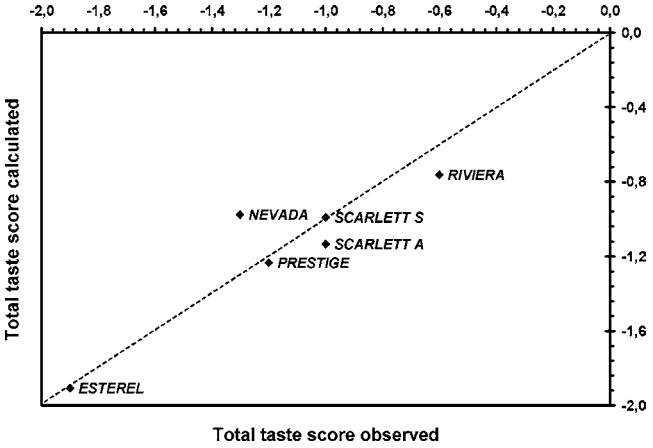

Figure 2. Comparison between sensory scores calculated by the proposed model and observed for the forced aged beers.

the variation found for the total taste scores of aged beers. The adjusted R^2, which takes into account the number of independent variables, obtained for the following linear regression model was 0.73 ($p = 0.06$):

$$\text{Total Taste Score} = -0.159 + 0.117\ \text{NP } \textit{Tepral}\ \text{Wort} - 0.062\ \text{Malt ARP} \quad (2)$$

The correlation between the observed and calculated total taste score is depicted in **Figure 2**. The model seems to effectively explain the variability for all the studied varieties, except for *Nevada*. The environmental effects (temperature, relative moisture content) seem to be more evident on barley malting quality than on beer flavor stability, as inferred by comparing the Spanish and French grown *Scarlett*.

Relationship between the Malt Antiradical Power and the Beer Flavor Stability. Malt antiradical power and the nonenal potential of *Tepral* worts were seen before to correlate with the organoleptic qualities of aged beer. Previous studies have shown that the ARP measured by the DPPH-method is mainly due to the polyphenols which promptly react with the stable radical 1,1-diphenyl-2-picrylhydrazyl (*14*−*16*). Furthermore, it was reported by Kaukovirta-Norja et al. (*17*) that the ARP does not correlate with malt color, which is mainly due to melanoidins. The wort reducing power assessed by the ITT method, on the other hand, gave very good correlations with malt color, mainly due to the heat-induced Maillard reaction products (*18*). Moreover, recent data on the reducing power of different tea extracts suggested that the antioxidant activity of tea extracts likely involves other mechanisms in addition to those of reductones (*19*). In order to confirm the previously reported observations, 15 randomly chosen malts were checked for the total phenolic content. A positive correlation was found between total phenolics and the ARP of different malt samples ($r = 0.88$, $N = 15$). It might be inferred from the positive correlation found between the micro-malt ARP and the total taste score of aged beers (**Table 2**) and the positive correlation observed between

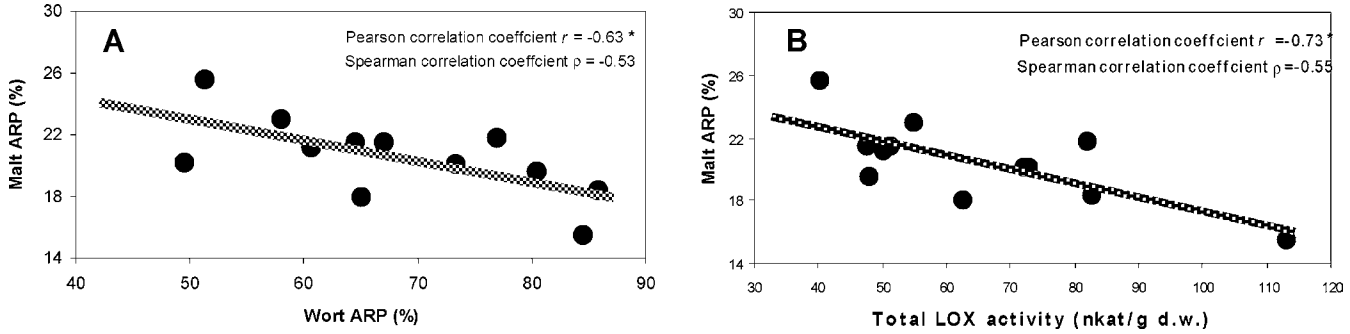

Figure 3. Scatter plot of malt ARP versus the *Tepral* wort ARP (**A**) and the total LOX activity (**B**) for industrial malts ($N = 12$). Pearson's and Spearman's correlation coefficients are indicated (*significant correlation at 95% confidence level).

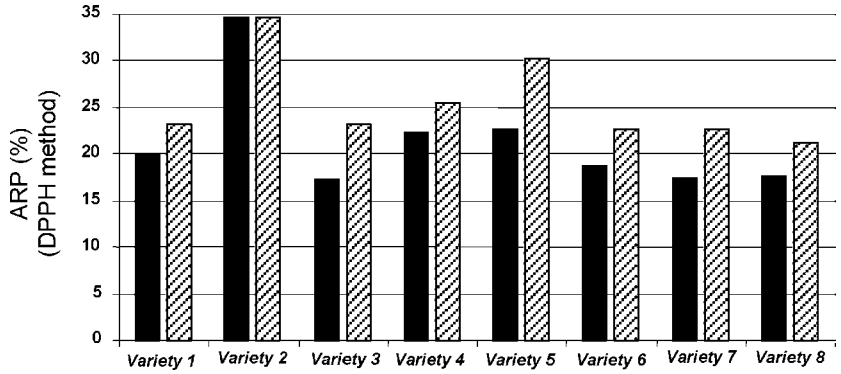

Figure 4. Comparison between the antiradical power (assessed by the DPPH-method) of barley (full black bars) and malt (black lined bars) for eight different varieties of malting barley.

the malts ARP and the total phenolic content that malt polyphenols may have a beneficial function retarding the development of typical oxidized flavors in beer. This emphasizes the important role played by the malt polyphenols in inhibiting the generation of off-flavors compounds or their precursors throughout the malting, mashing, or filtration steps. **Figure 3B** plots malt ARP vs LOX activity in the finished malt ($r = -0.73$, $p < 0.05$), showing the inhibitory action of malt polyphenols toward the LOX activity. The elucidation of the mechanism by which phenolic compounds exert their inhibitory action toward LOX may provide an important contribution to understand oxidative events that occur during malting and mashing. Surprisingly, the ARP of the *Tepral* wort not only showed a negative correlation with the total taste score obtained for forced aged beers but still shows an inverse correlation with the ARP of malts (**Figure 3A**). Additionally, a weak positive correlation was found between the LOX activity and the *Tepral* wort nonenal potential for the same malt samples ($r = 0.58$, $p > 0.05$)

As shown in **Figure 4**, the ARP of the barley is very similar to that of the malt, suggesting a major contribution from the natural polyphenols found in barley. It was shown by Goupy et al. (*11*) that the antiradical power is positively correlated with the total amount of flavan-3-ols and increased with the degree of polymerization. The high negative correlation found between the ARP of malt and wort following mashing and filtration (**Figure 3A**) may be explained if higher molecular weight polyphenols are present in malt. These highly polymerized phenolic compounds exhibiting higher antiradical capacity have an increased tendency to form insoluble complexes with proteins during mashing. Moreover, the solubility of the polyphenols decreases with increasing molecular weight (*3*), and the highly polymerized polyphenols have a higher antioxidant activity than the monomeric counterparts (*20*−*21*). Oxidative polymerization of polyphenols, and the increased propensity of these larger polymerized species to form complexes with proteins, results in the formation of insoluble species (*22*). These are lost due to precipitation and filtration, which can lead to the observed decrease for the antiradical capacity in the *Tepral* wort. Conditions during mashing can also result in the breakdown of dimeric polyphenols, releasing smaller and more soluble polyphenols into the wort. Only this free fraction and water soluble polyphenols will persist in the *Tepral* wort.

It was recently reported that the major portion of phenolics in grains existed in the bound form and that this fraction is the major contributor to the total antioxidant activity (*23*). The presence of phenolic acids bound to the cellular walls of germinated barley has been previously reported in barley (*24*). The role of these insoluble bound phenolic acids of malt and their behavior during mashing and filtration remains to be elucidated.

The results presented and discussed in this section clearly impute the antioxidant activity and ARP of malt polyphenols as a key role in improving the beer flavor stability. High polymerized phenolic compounds and/or insoluble bound polyphenols from malt are effective against oxidation reactions during the malting and the mashing stages. The fraction extracted during mashing and found in wort is, on the other hand, not effective and even seems to show pro-oxidant activity. Whether the remaining phenolic compounds may act as antioxidants or pro-oxidants during the course of the brewing process and in the final beer needs to be further investigated.

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