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Abstract

The study was conducted to develop calibration model for predicting malt barley quality traits of genotypes grown at different locations using near infra-red spectroscopy. For this purpose, 60 barley samples were collected from different growing location: Holeta, Debre-Birhan and Bekoji. Samples were chemically analyzed in duplicate for 5 barley traits. Calibration model was developed using 120 samples spectral data. Partial least squares (PLS) regression method was used for correlating spectral data to wet chemistry data. The selected PLS model had a good predictive power for protein having (R^2_c= 0.97; RPD=5.7 and R^2_c=0.94; RPD=4.16), Extract and Friability (R^2_c= 0.96; RPD=4.54 and R^2_c=0.95; RPD=4.36) respectively whereas β-Glucan calibration model (R^2c= 0.90; RPD=3.18) allowed only for screening purpose. Barley grain dry matter with model parameters result (R^2_c=0.86; RPD=2.69) shown satisfactory result that can be used for rough screening purposes. Thus, the developed prediction model will enable the selection of appropriate food and malt barley genotypes for further breeding program. However, more barely genotypes from different growing conditions will be needed to be included in the calibration data set to capture the whole Ethiopian barley variability.

Keywords: Barley, Genotypes, Malt, Trait, NIRS, Calibration, Validation

Introduction

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop worldwide after wheat, corn and rice (Marwat *et al*, 2012). It is fifth most important cereal crop, as well as a crop of ancient origin in Ethiopia and the country is considered as a center of diversity for barley, because of the presence of great diversity in ecology (Birhane, 1991). It is most widely used for the production of malt worldwide (Ullrich, 2002). Barley malt also mainly used as a source of fermentable sugars for alcoholic fermentation like production of beer (Kreisz, 2009). Barley is a complex mixture of many organic components that include protein, starch, oil, polysaccharides and sugars (Duffus *et a*l., 1992). The amount of each of the constituents will vary due to both the genetic background and the environmental conditions during grain development. The malting process of barley in particular, modifies the grain components during the controlled steeping, germination and drying processes (Bamforth *et al*., 1993). Malt is an essential

ingredient in beer production where soluble components of the malt are extracted into a liquid broth called wort (Briggs *et al*., 1981).

The ability to predict grain quality for different purposes in early generations would be of great benefit to breeders and industries, allowing for selection of suitable lines to deliver product of the highest quality. At later stages in the barley breeding programs, micro-malting can be carried which requires large barley sample sizes, destructive and requires experienced personnel (Marten *et al*, 2009). For this purpose, near infrared spectroscopy is an ideal technique as it is fast, reliable and non-destructive which does not require large sample sizes (Woodcock *et al*., 2008).

Near infrared spectroscopy is a type of vibration spectroscopy that employs photon energy in the range of 7.96×10^{-20} to 2.65×10^{-19} J. The range is higher than necessary to promote molecules to their lowest excited vibrational states and lower than typical values necessary for electron excitation in molecules (Pasquini, 2003). Hence, NIR spectra comprised of broad bands arising from overlapping absorptions corresponding mainly to overtones and combinations of vibration modes involving C–H, O–H, N–H and SH chemical bonds (Huang *et al*., 2008). Thus, sophisticated mathematical techniques, termed chemometrics, are heavily employed to allow calibration for reliable extraction of relevant information encoded in the NIR spectral data (Wang and Paliwal, 2007). Therefore, this study amid to determine constituents of particular interest for the brewing of barley grain quality traits by using NIRS.

Materials and Methods

Study materials

The samples used in this study were obtained from barley breeding program trials of Ethiopian Institute of Agricultural Research Centers having the mandate of barley growing and breeding potential around central highland areas of Ethiopia. Thus, barley samples were collected representing a range of breeding generations fully-fledged at different environments throughout barley growing highland areas, specifically from Holeta, Debre Birhan and Bekoji locations. 60 samples from 2018 year of barley growing season were collected from the pre-specified growing areas for the study, particularly, from the plot of the breeding program-controlled trials depending on genotypes, location, type (food or malt barley) and quality variability.

Sample preparation

For barley reference and spectral data analysis 150g per sample was taken after manually cleaned and graded. Then the samples were packed into plastic bag. Before malt quality traits analysis, the malt barley samples were malted according

to Phoenix Automated Micro malting system (Phoenix Bios stems, Adelaide, Australia) designed to process 300g of 24 barley samples per batch (Nilsen and Panozzo, 1995). Then malted barley were ground using a Laboratory Sample Mill3100 (Perten Instruments, Hagersten, Sweden) to pass through 0.5mm sieve for calibration reference data. But for malt friability determination the malt sample was not grounded, because the friabilimeter machine itself grounds the sample for the ratio of friability measurement.

Wet chemistry analysis

Malt quality traits of malt barley were chemically analyzed for reference data set at Holeta Food Science and Nutrition Laboratory, Ethiopian Institute of Agricultural Research (EIAR) in collaboration with VLB Institute in Berlin (Germany) for the malt barely quality traits. The analysis was carried out in duplicated to reduce the reproduced errors in each sample chemical analysis.

Malt hot water extract

Malt extract content was determined according to a small-scale version of the European Brewery Convention (EBC, 1998) Methods Manual, Section 4.9.1. Fine grind malt was extracted using a hot water mashing bath (SIEMENS Mashing Machine, Germany). For extraction, 50 g of finely ground malt was mixed with 200 mL of distilled water and mash at 45°C with continuous stirring. After 30 minutes of mashing, the temperature was increased by 1°C/min until 70°C. As temperature reaches 70°C, there was added of 100 mL distilled water. After 1 hour, the mash was cooled to 30°C and adjusted to a volume of 515 mL or a weight of 450g. The extract was filtered using whatman 12cm filter paper into 500ml cylinder and specific gravity was measured at 20^{o}C using a DMA5000 density meter (Anton Paar GmbH, Graz, Austria). Therefore, the following formula was used to put the end result. E = P (800+M)/ (100-P); where, E= Extract content, P= Wort Density (^{o}Plato), M= Malt Moisture content.

Malt protein content

The malt protein content was determined using kjeldhal method (Digester SBS 2000, Distillation Unit 5000DL, FoodALYT GimbH, Germany) according to (AOAC, 2005). For analysis one gram ground sample of malt barley was measured and transferred into completely dry kjeldhal flask. Ten gram of kjeldhal tablet was added to the sample inside the flask. Twenty milliliter of 98% concentrated sulphuric acid was mixed with the sample. The sample digestion was started by connecting the kjeldhal flasks with the digestion rock. The digestion was completed when the brown color of the sample completely disappeared. After the digested sample was cooled, 100 ml of distilled water and 80 ml of sodium hydroxide (32%) were added and distilled into 25 ml of excess boric acid containing 0.5 ml of screened indicator. The distillate was titrated with 0.1N hydrochloric acid to the methyl red end point. The protein was calculated by using

this formula: CP%= [(T-B)*14*6.25)]/[W(100-MC)]; where CP=Crude Protein, T= Volume of HCl used in Titration, B= Blank used as control and W= Weight of sample taken for analysis.

Friability

Malt grain samples were analyzed using a friability measuring machine (Pfeuffer Friabilimeter GmbH, Germany) which used a pressure roller to grind the sample against a rotating screen. Low, medium and high friability malts were tested according to EBC method 4.15 (EBC, 1998). 50g malt sample was run in the friabilimeter for 8 min and the non-friable fraction was weighed to get the final result.

β-glucan Content

The malt β-glucan content was determined using the Megazyme kit method (Megazyme, Bray, Ireland) according to EBC, 1998 Method 4.16.1. For the analysis 100 mg sample was suspended and hydrated in a buffer solution of pH 6.5 and incubated with purified lichenase enzyme and filtered. An aliquot of the filtrate was then hydrolyzed to complete with purified β-glucosidase. The D-glucose produced was assayed using a glucose oxidase/peroxidase reagent. The final prepared aliquot was measured by spectrophotometer at absorbance 510 nm against reagent blank within one hour. Finally the beta-glucan was calculated using the formula; B-glucan (%W/W) = ΔA * (F/W) * 27; Where, ΔA= Absorbance after β-glucosidase treatment (reaction) minus reaction blank absorbance, F = Factor for the conversion of absorbance values to μg of glucose, W = The calculated dry weigth of the sample analyzed in mg.

Dry Matter

Barley grain dry matter content was determined according to AOAC (2005) international standard method from grain flour prepared using the above sample preparation method. 5g of barley flour was taken using a sensitive analytical balance and oven dried at 105^{O}C temperature for 3 hours. After the dried sample was cooled in a desiccator, the final measurement was taken using the same analytical balance to get the result using the following known formula for moisture content. MC% = $(W_i-W_f)/W_i*100$; where W_i is initial weight, W_f is Final Weight; DM = (100-MC)%.

Spectral data acquisition

Before scanning the samples, the spectroscopic performance of the device was checked using gold standard (1.038) and light trap standard (0.00065) provided by Bruker company of Germany. Then 60 barley samples were scanned twice for spectral data using near infrared spectrometer (Tango2017, Bruker Optics GmbH, Germany) which uses scan and rotating mode within 16 seconds, sample presentation with rotating accessory having 600 mL sample cuvette. It used 32

amounts of scans between 867 –2535 nm wave length ranges with 16 cm^{-1} wave number band resolution. The NIR sensor used in this experiment is capable of recording spectra by diffusive reflectance measurements at the mentioned spectral wave length range. Spectra were assessed from untreated grains for barley before malting using a Bruker Tango. The device recorded spectra in diffusive reflectance by using an integrating sphere. Measurements were done in duplicate for each sample, leading to a total number of 120 spectral data.

Spectral data pre-processing

There were spectral biases and overlaps of wave length bands due to matrix effect, different particle size and spectroscopic condition. For this matter spectral data obtained from the tango direct measurement were pre-processed using OPUS software version 7.5.1. Therefore, first Derivative plus Standard Normal Variate (1^{st} D/ve+SNV) transformations, the 1st derivation with 17 smoothing points were found to be the best method for spectral treatment of samples.

Calibration and validation

The laboratory reference data was correlated with spectral data using the software OPUS version7.5.1 of Tango (Bruker, Optics GmbH, Germany). In total, 120 spectral data were used for calibration and validation. From 120 spectral data, two thirds of the samples were used in the calibration set and one third in the validation set. Validation method used to check the performance of the calibration model was test set validation method. The calibration set was checked to cover the whole variation in terms of spectral data, traits and locations. The following numbers of factors were used for the traits of interest: protein content 8, extract content 8, friability 9, ß-Glucan 10 and dry mater 10. The calibration model result was evaluated by using OPUS software statistical systems (chemometrics) (Krapf *et al*., 2011).

Calibration model statistical evaluation

Statistical tools were used to evaluate the efficiency of NIR calibrations and various terms are important in understanding the performance of a calibration model as described by (Williams, 2001). This includes statistics of calibration as well as statistics of validation. For developing calibration model a Partial Least Square (PLS) regression was performed using OPUS software.

The Coefficient of Determination ($R^{2)}$, Root Mean Square Error Estimation (RMSEE), Root Mean Square Error of Prediction (RMSEP), Ratio of performance to Deviation (RPD), Standard Deviation (SD) and Standard Error of Prediction) were used to evaluate the model performance depending on the reference data and spectra. The calibration was automatically tested by test set validation method (Martens, 2001). The final calibration was determined from an optimization routine of OPUS after the removal of the outliers. During the optimization step,

various frequency regions and also spectral pre-treatments was systemically tested to determine the optimal calibration that was directly stored in the device memory for future quantitative analysis as used by (Krapf et al., 2011).

Results and Discussion

Calibration model development highly depends on selecting a set of good calibration samples data obtained by wet chemistry analysis method. The set of calibration samples used in this study also contained the range of chemical and physical variations for which calibration model was applied. Accordingly, the calibration experiment was established using a mathematical relationship between the NIR spectrum and physical/chemical properties determined by wet chemistry methods. Similarly, comparing the chemical reference data variability and model accuracy is vital point as reported by (Cen, 2007).

Calibration model

NIRS calibration models were developed for five barley traits determining quality for malt and food. The model performance was assessed by the following parameters: coefficient of determination of the calibration (R^2c) and validation set (R^2v), standard errors of prediction (SEP) as well as root mean square error of prediction (RMSEP). The RPDv value indicates the suitability of the calibration for the prediction. With a higher RPDv value the calibration will more likely be able to predict the right sample values (Duffus *et al*, 1992).

The calibration and validation statistical description was very important to see the feasibility of each traits data to build calibration model. Table 1 indicated that the general features of descriptive statistics of traits to detect the right data variability for developing strong calibration model. Good data variability was observed in all traits except for DM. Therefore, expected calibration model is good for all traits

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Table 1: Descriptive Statistics of Calibration and Validation Reference Data

		Calibration Data				Validation Data			
Traits	Units	Mean	Range	SD	N_C	Mean	Range	SD	N_V
Extract	%	78.1	73-83.5	2.93	120	78.1	69.9-84	3.12	60
Protein	%	10.4	7-14.1	1.88	120	10.5	6.8-13.3	2.08	60
Friability	%	61.0	29-97	18.96	120	59	20-98	22.13	60
β-glucan	Mg/L	685.0	158-1000	279.5	120	699	50-1000	358.6	60
DM	%	91.34	90.6-92.3	0.4	120	91.28	90.8-91.9	0.26	60

DM=Dry Matter; SD=Standard Deviation; N_C=No of Spectral Data Used for Calibration; N_V=No of Spectral Data Used for Validation.

Extract content

Extract content prediction gave good calibrations for whole grain samples (R^2_c = 0.956; RPD_C = 4.54 with variable samples) (Table 2). The model major

parameters R^2 and RPD reflect acceptable for most applications and screening purposes. Results from this study was comparable and much better than that of previous researchers who developed promising calibrations for predicting the extract of whole grain (R^2_c = 0.78 - 0.85) (Black & Panozzo, 2001) and ground barley (R^2_c = 0.77 - 0.96) (Tragoonrung *et al*., 1990). Because this property is highly influenced by the malting process since enzyme activity during malting influences the malt extract which limits the accuracy of any NIR prediction based on unmalted barley (Henry, 1985). This is why different calibration model performance is reported by different scientists, even if the accuracy of reference sample analysis data is very important.

Protein content

Similarly calibration model results obtained for whole barley grain samples for protein content with (R^2_c = 0.97; RPD = 5.7 of variable samples) (Table 2). The model is more acceptable than the models for other traits which could be usable in most applications, quality assurances and quality control. This prediction of nitrogen content from whole grain barley is well established in the literature and the results from this study compared well with those of previous reports for whole grain barley with R^2_c = 0.94 (Edney *et al.*, 1994) and R^2_c = 0.95 (Sohn *et al.*, 2008). Because NIR prediction is more effective in predicting biochemical properties than physical properties.

Friability

Friability prediction performance model was found to be promising with R^2_c=0.95; RPD=4.3), which was excellent like the protein model which could be used in most applications including in quality assurances, but in our case it was trustful model to identify excellent barley friability for malt factories at breeding final stage. Almost similar performed model was also reported in the literature with parameters value (R^2_c=0.91; RPD=3.33) by (Selioni, 2011). Therefore, the friability calibration model was not as such challenging like in dry matter and beta-glucan as observed from similar model reported by different authors.

β-

Beta-glucan prediction model was also successful in this study for whole grain barley samples having (R^2_c=0.90; RPD=3.18) as indicated in table 2. According to Williams (2001) this type of model performance is usable with caution especially for screening purposes such like in early stage breeding lines. But a similar study have been reported in the literature with much lower (R^2_c=0.25) as compared to this study by (Black & Panozzo, 2001). On the other hand Roux(2011) reported (R^2_c=0.61) and he referred that the poor distribution of reference values in the sample range may be the reason for the poor results in Beta-glucan model of barley grain that were obtained in his study. But in this study the good modifying

malting process, excellent sample variability selection and moderate accurate chemistry analysis made the model better as compared to the reported literature.

Dry Matter

Moisture predictions model from the same whole barley grain samples were good, but was only acceptable for some screening purposes, because R^2_c = 0.86 and RPDc=2.69 was less as compared to the recommended range by (Williams, 2001). Results of moisture content from this study are not comparable to literature reports, this is due to the smaller sample moisture content ranges (90.6-92.3 % DM) utilized compared to those used by previous researchers. Similar problem was also observed in literatures with the small sample range (78.4 - 83.4% DM) with in reference values obtained as studied by (Roux, 2011). The range of samples needs to be expanded in order to obtain acceptable calibrations model.

Generally, calibration model for the three traits extract content, protein content and friability were found to be a promising model for value predicting in some applications while beta-glucan and DM gave satisfactory result. But calibration model alone does not enough to judge traits model to be applicable for targeted purpose. It is very important to see the validation parameters result side by side with the calibration parameters result as clearly shown in table 5 for all selected traits in this study.

Table 2: Barley Traits Calibration and Validation Model Statistical Parameters

	Calibration Statistical Parameters					Validation Statistical Parameters				
Traits	R^2	SEC	RMSEE	RPD	slope	R^2_V	SEP	RMSEP	RPD	Bias
Extract	95.62	0.65	0.67	4.54	0.95	0.81	1.37	1.34	2.28	0.062
Protein	96.93	0.33	0.34	5.7	0.97	0.93	0.55	0.54	3.78	-0.03
Friability	94.75	4.35	4.5	4.36	0.95	0.59	14.10	13.8	1.57	0.32
β-glucan	90.11	87.90	91	3.18	0.90	0.49	254.33	249	1.41	-22.6
DM	86.23	0.15	0.153	2.69	0.86	0.51	0.18	0.29	1.43	-0.001

SD=standard Deviation of reference data; R^2 =Coefficient of determination; SEC=Standard Error of Calibration; Standard Error of Prediction; RMSEE=Root Mean Square Error of Estimation; RPD=Ratio of Performance to Deviation.

Validation model

Model evaluation was performed by test set validation; because test set validation is a more independent validation method than cross-sectional validation as (Williams, 2001). In this method, the set of calibration samples is divided into a calibration set and validation set. According to this study from 60 samples, 20 samples spectral data and chemistry data was set for validation. Using this process, the models were validated and checked for their prediction capacities. The principle was predicting quantitative value using the model from the validation set spectral data and comparing the predicted value against the chemistry data set for validation.

Validation parameters for each barley traits were acceptable as in table 2 for Extract content, protein content and friability having (R^2_V = 0.81 & RPD=2.28), (R^2_V=0.93 & RPD_V=3.78) and (R^2_V=0.59 & RPD_V =1.57) values respectively. These models could be applied for quality control and screening purposes in breeding programs or any other applications. But as previously observed in calibration model above validation result for beta-glucan (R^2_V = 0.49 with RPD_V = 1.41) and DM (R^2_V = 0.51 with RPD_V = 1.43) was not reflected good model even though could be used in some rough screening purposes. This kind of model was not widely usable for prediction as (Black & Panozzo, 2001) reported unless for simple rough screening purpose. Also, similar study reported correspondent result with this study (Martens *et al.,* 2015). Other parameters like SEP\RMSEP and Bias with lower value reflected that the model was well performed. These validation parameters showed very convincing value that the user could believe the model to use for prediction of protein, extract and friability in general. Similarly, a study by (Edney *et al.,* 1994) delivered excellent prediction models for these traits that could be used in most applications. This should be due to the nature of the sample and less modifying malting process occurred (Selioni, 2011).

Conclusions and Recommendation

Calibration models were developed for extract, protein, friability, beta-glucan and moisture content using NIR OPUS software statistical parameters. The developed calibration model showed promising result for predicting quality traits of Ethiopian malt barley. Therefore, this prediction model will enable the selection of appropriate food and malt barley genotypes for further breeding. However, more data set needed to be included in the calibration data set to capture the whole Ethiopian barley variability.

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