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Malt Quality Traits of Different Barley Genotypes from High Growing Potential Areas of Ethiopia

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Ethiopia is the second largest barley producer next to Morocco in Africa. But the quality traits are always influenced by the cultivar itself and growing environment. Thus, the study was targeted on assessment of barley malt quality traits of different genotypes grown at different locations. Sixty barley samples were collected from Holeta, Debre-Birhan and Bekoji. Samples were chemically analyzed in duplicate for five quality traits. The barley FBPVT and NPPT genotypes protein with 10.37% and 10.50% protein mean values respectively shown higher than the other genotypes statistically. Bekoji location reflected higher protein content (11.70%) than Holeta and Debre-Birhan locations with 8.93% and 8.31% protein values respectively. Genotypes NPPT, MBPVT, MBNVT OG and MBNVT N with 79.70%, 80.03%, 80.67% and 80.70% extract content respectively reflected higher value than FBPVT and FBNVTN. Higher extract content was recorded at Holeta and Debre-Birhan locations having 79.91 and 80.3% mean values respectively. Genotypes FBNVT N (68.33%), FBNVT OG (68.67%) and MBNVT OG (73.00%) were higher in friability mean values statistically. Holeta (76.71%) and Debre-Birhan (71.57%) locations scored higher friability content than Bekoji (42.57%). Genotypes like FBPVT with mean 862.7mg/L and FBNVT OG with mean 885.0 mg/L were higher in glucan content as compared to the other genotypes. Holeta and Debre-Birhan locations with 437.3 mg/L and 650.1mg/L value respectively reflected lower value than Bekoji with 958.1mg/L. But no significant variation in moisture content among the genotypes. The same trend was observed also in moisture content over locations similar to genotypes. Therefore, protein, extract and friability traits were in acceptable range according to international malt quality traits standards. But those traits lower in mean value were beyond the acceptable range.

Keywords: Barley, Genotypes, Malt, Trait

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INTRODUCTION

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop worldwide after wheat, corn and rice (Marwat et al, 2012). It is 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). In the country barley has a long history of cultivation in the highlands (Firdissa et al, 2010). Barley has the ability to adapt and survive in a wide range of environmental conditions, but the diversity of barley types found in Ethiopia is probably not expanded in any other region of comparable size (Bekele, 1983). Even though it is most important crop for food in Ethiopia, but it is used mostly for the production of malt (Ullrich, 2002). Barley malt is mainly used as a

source of fermentable sugars for alcoholic fermentation for the production of beer (Kreisz, 2009).

Barley is a complex mixture of many organic components that include protein, starch, water, oil, fiber polysaccharides and sugars (Duffus et al., 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). However, varying the malting process conditions influences the level to which the carbohydrate and protein constituents are modified, which in turn influences beer processing and product characteristics. However, the problem arises in the selection of suitable cultivars for each of these quality compositions that meet required quality specifications.

The ability to predict grain quality for different purposes in early generations would be of great benefit to breeders and maltsters, allowing for selection of suitable lines to deliver product of the highest quality. The fact the production of malt barley is restricted to these specific areas is advantageous with respect to transport, storage and research (Kunze, 2004). However, the problem arises in the selection of suitable cultivars for each of these regions that meet the required quality specifications. Breeding of new cultivars therefore requires the evaluation of many quality characteristics and the testing and selection of thousands of breeding lines, starting with early generation material in the breeding program. Therefore, the barley genotypes grown in different locations in Ethiopia needs to be analyzed for their quality traits to categorize it as malt and food purposes. The barley breeding programs conducted in the country by regional and federal agricultural research centers mainly depends on the selecting materials based on the quality traits and agronomical data to judge it as malt or food. Hence this study supports the breeders in their breeding programs as selection criteria depending on the baseline information generated from this work.

MATERIALS AND METHODS

Sample Source and collection

The samples used in this study were 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 (Holeta, Bekoji and Debre Birhan Agricultural Research Centers). Accordingly, the barley samples were collected representing a range of breeding generations grown at different environments throughout barley growing highland areas specifically from Holeta, Debre Birhan and Bekoji. About 60 samples from 2018-year trials of malt barley were collected from the pre-specified growing areas for the study from the plot of the breeding program-controlled trials depending on genotypes, location and sources from they were obtained.

Table 1: Collected Barley Sample Genotypes from Different Sources

Genotypes	Location	Source	Type	Qnty
NPPT	Holeta, Bekoji & Debre-Birhan	Exotic, ICARDA, Elite	Malt, Food	10
MBNVT N	Holeta, Bekoji & Debre-Birhan	ICARDA	Food	8
MBNVT OG	Holeta, Bekoji & Debre-Birhan	Exotic, Cross	Malt	10
MBPVT	Holeta, Bekoji &Debre-Birhan	Cross, Exotic	Malt	6
FBPVT	Holeta, Bekoji &Debre-Birhan	Cross, ICARDA	Food	9
FBNVT OG	Holeta, Bekoji &Debre-Birhan	Cross, Elite	Food	8
FBNVT N	Holeta, Bekoji &Debre-Birhan	Elite, ICARDA	Food	9

NPPT=National parental performance Trial, MBNVT N=Malt Barley National Variety Trial New, Malt Barley National Variety Trial on Going, MBPVT=Malt Barley Preliminary Variety Trial, FBPVT=Food Barley Preliminary Variety Trial, FBNVT OG=Food Barley National Variety Trial On Going, FBNVT N=Food Barley National Variety Trial New.

Sample preparation

Barley samples which were collected from different locations and different genotypes of breeding trials were selected purposively from different plots depending on agronomical data and source of genotypes from where they originated as well as the history of their quality data. 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. After that, prior to reference samples chemical analysis, malt(after malted) 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.

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). After kilning the rootlets were removed from the malted samples by using mechanical malt cleaner that had been reconfigured to simultaneously process eight 250 g samples (Fraser Fabrications Pty Ltd, Malaga Western Australia).

Wet Chemistry Analysis

Malt quality traits of malt barley were chemically analyzed for reference data at Holeta Food science and Nutrition Laboratory, EIAR (Ethiopian Institute of Agricultural Research) in collaboration with VLB Institute in Berlin (Germany) for the traits mentioned below. But simple quality traits were analyzed at Holetta, EIAR cereal quality laboratory. Blank and known concentration sample were analyzed with the samples to control the analysis biases. The samples also duplicated to reduce the reproduced errors in each sample chemical analysis.

Malt Extract Content

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 Total Protein

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.

Malt 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.

Malt β-glucan

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.

Moisture Content

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

Statistical Analysis

To check the variability of the samples collected from different sources, the traits were compared for each genotype and across location using SAS statistical software version 9. The main effects of the factors data mean were compared using ANOVA statistical tool. The means were separated to their significance level using LSD at $P<0.05$.

RESULTS AND DISCUSSION

Malt Extract Content (%)

Genotypes Variation: Extract content is important trait for malt quality, so that the trait was chemically analyzed for the purpose of NIR model development. According table 2, genotypes NPPT, MBPVT, MBNVT OG and MBNVT N with 79.70%, 80.03%, 80.67% and 80.70% extract content respectively reflected higher value than FBPVT and FBNVT N. But there was statistically significant difference between genotypes at $P<0.05$.

In his study (Swanston *et al*., 2014) also reported that the extract yield varied depending on the extent of enzymatic degradation and the solubility of grain components after malting and mashing. During malting, enzymes that have an impact on the degradation of substrates, were either synthesized or cleaved from their bound forms. The range of enzymes produced included those that degrade cell wall components, proteins and starch. This is also influenced by the nature of the genotypes performance to produce enough enzymes during such processing. As the objective for most maltsters is to maintain high extract levels and yet somehow achieve relatively high extract content according to EBC standard from 70-80% based on genotypes.

Location Variation: On the other hand, as mentioned in table 3 there was statistically significant difference between the locations Holeta, Debre-Birhan and Bekoji at ($p<0.05$). Higher extract content was recorded at Holeta and Debre-Birhan having 79.91 and 80.3% mean values respectively at constant genotypes. Therefore, location effect was observed in the result as the sample selection depended on creating variability between samples to capture minimum and maximum values later in calibration. As reported by (Fox *et al.,* 2003) extract variability occurred as influenced by several factors such as environmental, growing conditions, temperature, available nitrogen and moisture. The author reflected that these factors were different with location, as the result it created variation in the mean of the traits.

Protein (%)

Genotypes Variation: The Protein content of barley throughout genotypes varied from 8-16%. But according to EBC standard range malt barley protein content ranges from 9-11.5%. Similarly, in this study a mean value from 8.50-10.50% was obtained according to table 2 results. Depending on the result FBPVT and NPPT genotypes with 10.37% and 10.50% protein mean values respectively shown higher than the other genotypes as statistical analysis showed. Other genotypes relatively have lower in protein content as compared to the two. This means that there was significant difference between the genotype's protein content mean values statistically.

In the brewery standard protein content is not needed to be higher as well as lower, but need to be in the range of 9-11.5% as mentioned above, study reported a similar trend in normal malt barley commercial requirement protein content is a maximum of 11.5% protein in the dry matter as (Kunze, 2004). However, in this study since some food barley genotypes were included in the samples the values reflected less than 9% protein content. (Emebiri *et al*.,2007) reported that protein variability occurred due to genotypes variability. Also, he reported negative correlation between protein and extract, a positive correlation between protein and diastatic power, using a low protein breeding population, mean that the quality traits correlation of genotypes could affect the protein variation among genotypes.

Location Variation: Also, the location wise study reflected in table 3, that there was significant difference between Holeta, Bekoji and D/Birhan at $p<0.05$ statistically. Accordingly, Bekoji location reflected higher protein content (11.70%) than Holeta and Debre-Birhan locations with 8.93% and 8.31% protein values respectively. Because of purposive sample collection variability was occurred between the genotypes and locations. These variability reasons were also reported by Emebiri *et al*. (2007) that barley type (one and two rowed, malt and food type) and a parental irrelative affects protein content. Again, he reported that protein variability occurred due to environment and nitrogen fertilizer application. My suggestion was also similar to the literatures soil type, growing season, agricultural practices, amount of rain fall and maturity mainly affected the protein content to be varied among locations and between genotypes.

Table 2: Malt Quality Traits Wet Chemistry Result of Genotypes

Genotypes	Extract (%) Mean±SE	Protein (%) Mean±SE	Friability (%) Mean±SE	β-glucan (mg/L) Mean±SE	Moisture (%) Mean±SE
FBPVT	75.10±1.05^{b}	10.37±0.09^{a}	45.33±5.46^{b}	862.7±7.06^{a}	8.75±0.01^{a}
FBNVT N	77.50±1.9^{ab}	9.60±0.95^{ab}	68.33±13.19^{a}	638.0±183.8^{ab}	9.00±0.05^{a}
FBNVT OG	79.38±0.65^{a}	8.50±0.76^{b}	68.67±4.41^{a}	885±109.55^{a}	9.21±0.43^{a}
NPPT	79.70±1.55^{a}	10.50±1.22^{a}	56.33±11.46^{ab}	756±123.78^{ab}	8.85±0.32^{a}
MBPVT	80.03±1.47^{a}	9.80±1.33^{ab}	66.33±16.91^{ab}	528.7±247.45^{b}	10.93±0.30^{a}
MBNVT OG	80.67±2.04^{a}	9.10±1.60^{b}	73.00±18.33^{a}	529.3±239.28^{b}	8.65±0.28^{a}
MBNVT N	80.70±2.10^{a}	9.60±1.82^{ab}	67.33±20.67^{a}	573.3±256.88^{b}	9.77±0.02^{a}

Results were expressed mean of mean with standard error; means were compared using ANOVA LSD multiple comparison at significance level P<0.05. Means indicated with similar superscript lower case letters in the same column were statistically not significant.

Location was one of the important variations, because the genotypes trials were grown at the following different locations using the same package of seed rate, fertilizer rate and genotypes. The only variations occurred might be from soil types, variation of season and other practices. Therefore table 6 described that the locations selected were Holeta, Debre-Birhan and Bekoji for which the comparisons were made.

Table 3: Samples Wet Chemistry Result across Location

Location	Extract (%) Mean±SE	Protein (%) Mean±SE	Friability (%) Mean±SE	β-glucan (mg/L) Mean±SE	Moisture (%) Mean±SE
Holeta	79.91±1.33^{a}	8.93±0.31^{b}	76.71±7.37^{a}	437.3±100.54^{b}	8.61±0.11^{a}
Debre-Birhan	80.34±0.93^{a}	8.31±0.52^{b}	71.57±5.97^{a}	650.1±107.39^{b}	9.00±0.16^{a}
Bekoji	76.78±0.71^{b}	11.7±0.52^{a}	42.57±6.04^{b}	958.1±24.09^{a}	9.03±0.15^{a}

Results were expressed mean of mean with standard error; means were compared using ANOVA LSD multiple comparison at significance level P<0.05. Means indicated with similar superscript lower case letters in the same column were statistically not significant.

Friability (%)

Genotypes Variation: Measuring the friability of commercial malt has increasingly been used as an indicator to malting and brewing quality as well as trouble shooting on samples of poor malt quality. According to the result shown in table 2, there was significant difference between friability mean value of genotypes statistically at P<0.05. Genotypes FBNVT N(68.33%), FBNVT OG(68.67%) and MBNVT OG(73.00%) were higher in mean values as LSD comparison. But FBPVT (45.33%) was lower in mean value as compared to others. Friability potential of genotypes needs to be higher in breeding lines for the purpose of barley malt commercial as set European Brewery Convection (EBC, 1998). The lower and varied value of friability occurred among genotypes could not be only the genotype variation, also occured because of relationship with other malt quality parameters and malting process as reported by (Chapon *et al.,* 1978). These relationships between friability and key malt traits like wort β -glucan, Kolbach Index, wort viscosity along with other malt quality parameters have been studied through detailed experiment examining malt quality (Chapon *et al.* 1980).

Location Variation: Location wise variation studied in this study for Goleta, Bekoji and Debre-Birhan locations were showing significant difference among the friability mean for the specified location at P<0.05 statistically. Holeta (76.71%) and Debre-Birhan (71.57%) locations scored higher friability content than Bekoji(42.57%) which scored significant lower friability content mean as illustrated in table 3. However, variations between locations were better than the variation that occurred between genotypes for friability content as the results mean reflected in the table statistically. Even if the friability content scored in both locations were varied as need for model calibration, still it was less than the EBC standard range from 78-81%. Other factors also could affect the result variation due to endosperm modification, such as poor germination, large kernels and high protein which was expected to reduce malt friability as (Edney, 2014) reported in his study rather than locations.

β-Glucans (mg/L)

The major constituent of barley endosperm cell wall is -D-(1-3), (1-4)-glucans (75%), with a minor component identified as arabinoxylans (20%) (Fincher and Stone 1986). The range in barley for glucan is 2 to 10% of total grain weight (Henry 1987). β-Glucan content was determined as the method described previously in the materials and method.

Genotypes Variation: Depending on the result table 2, β-Glucan content mean values were significantly different among genotypes at P<0.05 statistically. Genotypes like FBPVT with mean 862.7mg/L and FBNVT OG with mean 885.0 mg/L were higher in glucan content as compared to the other genotypes. But even if there was significant difference between the means, other genotypes scored lower glucan content. But the significant variation between genotypes was very important for further breeding works.

Location Variation: The same trend as in genotypes was observed in table 3 between location variation that the glucan mean values were significantly different at P<0.05 statistically. Holeta and Debre-Birhan locations with mean value 437.3 mg/L and 650.1mg/L respectively reflected lower value than Bekoji with mean value 958.1mg/L. Higher value of β-Glucan content is not needed for malt commercial for it contributes undesirable effect in other malt quality. The same as in genotypes significant variation between locations were very important to consider factors in breeding programs.

As the study of (Henry, 1985) reflected both genotypes and location influenced the content of glucan as it has been shown to have a relationship with other malt quality traits. Importantly, high glucan levels may not result in higher or lower extract but relate to other malt quality traits such as Kolbach Index (ratio of soluble to total protein), viscosity or the speed of filtration as (Evans *et al.* 1999) studied. There was a contradicting idea between that the higher glucan content as lower the amount of extract and indirectly contributing for reducing extract content rather having direct relation with wort speed of filtration and viscosity. But in my opinion, I support the idea that the glucan content reduces directly the extract content, because glucans are not easily broken-down during mashing to release starch so that the extraction amount will be increased in the mashing time limit given according to the method.

Moisture Content (%)

The moisture content of barley is 8-15% on average. The moisture content can vary between 12% in very dry harvesting conditions and over 20% in wet conditions. More precisely, it is less than 13% in the South region of the European Brewery Convention (EBC) barley and malt committee, and it is more than the 16% in the North region, where consequently the barley should be dried before long term storage.

Genotypes Variation: In fact, barley must have moisture content below 15% for long term storage. But according to the study results in table 2 no variation of moisture content among the genotypes was observed. As compared by using LSD value there was no significant difference between the mean of genotypes at P<0.05. But as different literatures mentioned there was moisture

content in the accepted range genotypes having 8.65-10.93% range. Normally moisture levels need to be low enough to inactivate the enzymes involved in seed germination as well as to prevent heat damage and the growth of disease microorganisms. Quality and germination capacity may also significantly deteriorate as (Plankinton *et al*., 2014). Similarly, the moisture content result obtained was maximum not higher than 11%.

Location Variation: As shown in table 3 there was also no significant difference between the mean of locations similar to genotypes moisture content at $P<0.05$. But as the literatures mentioned there was moisture content in the accepted range for location having since the mean ranged from 8.61 to 9.03% similar to genotypes variation. Moreover, the determination of the moisture content is important when the amounts of the other components are related to the dry weight according to (Kunze, 2004, Vijaya, 2003) report.

CONCLUSION

Barley is the fourth most important cereal crop worldwide. Most of the Ethiopian barley production is consumed as food at home also indicating the status of barley as “poor man’s bread”. At the same time using barley for malt production establishes new value-added chains from which Ethiopian small holders can benefit substantially. The main objective of this study was enabling the breeding programs to select the appropriate genotypes by generating quality trait data information for breeding materials. Depending on the objective, samples quality traits were analyzed using the international official methods described in materials and method part. According to the results FBPVT and NPPT genotypes were higher in protein content as well as in acceptable range. Extract content was highest for NPPT, MBPVT, MBNVTOG and MBNVTN genotypes. Genotypes like FBNVTN, FBNVTOG and MBNVTOG were higher in friability value than other genotypes while FBPVT and FBNVTOG recorded higher value in beta-glucan content, but it needs to be lower as malt quality criteria. Incase of location variation Holeta and Debre-Birhan locations resulted higher extract and friability content, while Bekoji location resulted higher for protein and beta-glucan content statistically. But no significant different for moisture content both for locations as well as for genotypes. Therefore, protein, extract and friability traits were in acceptable range according to international malt quality traits standards. But those traits lower in mean value were beyond the acceptable range. Currently barley breeding programs conducted in the country regionally and by federal agricultural research centers mainly depends on the selecting materials based on the quality traits and agronomical data to judge materials as malt or food. Hence this study supports the breeders in their breeding programs as selection criteria depending on the baseline information generated from this work.

RECOMMENDATION

All parameters that could have direct or indirect correlation with these identified traits should be included for full malt quality traits to reduce traits matrix effect in the future study. For some of the traits like dry matter and beta-glucan further study is very important to distinguish between sample variability range and wet chemistry analysis inaccuracy which could contribute to be less trusted result observed in most studies. The study focused on evaluating different genotypes across location to see the variation of quality traits due to the factors. But no significant effect between the interaction of genotypes and location so that the completely randomized design was used to compare the results statistically in this study. Also, not all barley growing areas were included in this research work, only high growing potential areas were considered. So, some one can think of other locations of barley growing areas for further information.

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Conflict of Interest

This manuscript is the original work of the author which is not planned, studied and written by any other body. Therefore, the author declares that there is no conflict of interests regarding the publication of this paper.

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