Physico-Chemical and Malting Properties of Barley Cultivars

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Abstract

Barley (HordeumvulgareL.) is the traditional cereal used in the production of malt; the principal material for both alcoholic and non-alcoholic beverages. In this study, sixteen malt barely cultivars (four-varieties under production namely Holker, Traveller, EH1847 and IBON174/03) and twelve promising genotypes) harvested in 2014 main seasons were investigated for their grain, malt and wort quality parameters over three locations. The grain quality parameters were sieve size, hectoliter weight, thousand kernel weight, germination energy, moisture content and protein content and malting and wort quality parameters were hot water extract, soluble protein content, friability, diastatic power, free amino nitrogen and zinc content using standard methods. The result showed that cultivar MB1, MB3, MB5, MB7, MB9 and MB4 have better grain and malt quality as compared to the standard check over the three locations (Bekoji, Holetta and Ankober). Among the three locations, Kulumsa (Bekoji) was suitable for malt barley production as the grain and malt quality fulfil the standard requirements of European Brewery Convention (EBC) and Asela Malt factory standard (Ethiopia).

Introduction

Barley (*Hordeumvulgare*L.) is the traditional cereal used in the production of malt; the principal material for both alcoholic and non-alcoholic beverages. Ethiopia is ancient origin of barley and considered as a center of diversity for the crop, because of the presence of great diversity in ecology (Berhane,1991). The diversity of barley types found in the country is probably not exceeded by any other region of comparable size (Bekele, 1983). It is mainly produced in the southeastern part of Ethiopia in Arsi and Bale zones (Getachew *et al.*, 2007) and mainly used for making local recipes and drinks such as Bread, kolo, genfo, beso, tela and borde.

Malt is the second largest use of barely and now a daysit is considered as one of the cash crops and its demand by malt factory is increased due to expansion of breweries and beer consumption levels in the country (Tura, 2015). Getachew *et al.* (2007) have reported that malt barely is among crops demanded in good quantity and quality. However, inadequate supply to the brewery industries, which hindered the growth of the sector.Due to this, in 2011, Breweries in Ethiopia, imported 60% of the malt primarily from international producers (Ethiopian Barely Business Association, 2012). To this end, the agriculture research system is expected to develop high yielding malt barley varieties that satisfy standard requirements of the brewery industry. Therefore,

this study was conducted to investigate grain physico-chemical and malting properties of promising malt barley cultivars that help to select best cultivars that fulfil both European and Ethiopian brewery standards and for further use in breeding programs

Material and Methods

Plant materials and locations

The experiment was conducted on sixteen malt barley cultivars collected from Arsi zone of Oromia region (Kulumsa agricultural research center), north shewazone of Amahara region (Deberebrhan regional research center) and central high land of Ethiopia (Holetta agricultural research center)during 2014/2015 cropping season. Four of the cultivars, whichwere used as standard checks (Holker, Traveler, EH 1847, and IBON174/03) have been previously released and are under production and the remaining twelve were promising cultivars under National Variety Trial. Planting and field management was carried out as per the recommendation for the crop.

Physico - chemical analysis

Grain size of samples was determined using 2.8mm, 2.5mm and 2.2mm vibrating sieves. Germination energy was determined by taking 100 barley kernels and spreading on wetted (4ml distilled water) filter paper lined on petri dishes (90mm).The kernels were allowed to germinate at nearly 100% relative humidity set at a temperature of 16°C in germination cabinet for 3 days (Analytica - EBC, 2013). Moisture content was determined using oven drying method, where 3g barley flour was weighed on analytical balance and oven dried at $105^{O}C$ for 3 hours. The moisture lose on drying was calculated and expressed in percentage of the pre-drying sample mass (Analytica - EBC, 2013).Thousand kernel weight was determined by taking 100 barley kernel samplesweighed using analytical balance and multiplied by 10. Hectoliter weight was determined for dockage free samples using a standard hectoliter weight apparatus (grain analysis computer (GAC) 2100) (AACC, 2000). Kjeldahl method (AACC (2000) determined protein content of each barley variety.

Malt quality analysis

Malt was prepared using a Phoenix Automated Micro malting system (Phoenix Bios stems, Adelaide, Australia). The mashing process was done according to the Analytica - EBC mashing method. Friability was analyzed using a PfeufferFriabilimeter, which uses 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 (Analytica - EBC, 2013). Soluble protein content was determined by taking 20 ml wort, which was transferred in to kjeldahl flask containing 3ml of sulfuric acid and antifoam was added to prevent excess foaming. After drying, 20ml sulfuric acid and 10g of catalyst was added. The digestion, distillation and titration were completed as described in Analytica - EBC method. The diastatic power of the malt was determined according to ASBC (2008). The free amino nitrogen value was determined from the wort sample based on a small-scale version of the IoBNinhydrin method. Concentrationsof zinc was determined by shaking, first the wort was homogenizing the wort, which was then filtered through dry zinc free filter paper, and the first 4 ml filtrate was discarded.Then,

2.0 ml of wort sample and 8.0ml 0.01 mol/l HCl successively pipetted in a test tube and mixed well. The solution was subjected to atomic absorption spectrophotometer analysis and zinc content was determined using calibration curve made by standard concentration of reference zinc solution.

Results and Discussion

Grain quality

Grain size (sieve size*):* There was significant difference ($P < 0.05$) among varieties for grain size. MB1 had the highest mean grain size (92.66%), while varieties MB12 had the lowest value (Table 1). It has been reported that the grain size percentage should be >90% for 2-rowed barley and >80% for 6-rowed barley (Anonymous, 2012).Hence, grain size of most of the materials in the present study fulfills the standard requirement of the industries and Ethiopian malt factory except, variety MB12 (78.83%). Growing location showed significant difference ($P<0.05$) in grain size.Varieties grown at Bekoji had higher mean grain size than cultivars grown at Ankober and those grown at Ankober had in turn higher mean value than did those from Holetta (Table 2). Industry standards for grain size are set as total percentage of grain > 2.5mm, while Ethiopian standard requirement for malt shows that>80 % must be above 2.8 and 2.5mm sieve size. Therefore, Bekoji and Ankober were found to be suitable for the production of malt barley and Holetta was not. Similarly, Fox *et al*. (2003) have demonstrated that both genetic and environment affect grain size of malt barley.

Table1: Effect of barley variety on grain size, germination and moisture content of grain.

Genotype/Variety	Grain size (%)	Germination energy (%)	Moisture content (%)
1.MB1	92.66±3.46^{c}	89.33±5.13^{e}	11.46±2.84
2.MB2	86.00±15.82^{b}	64.33±23.54^{c}	11.10±2.65
3.MB3	90.8±7.70^{b}	96.33±1.15^{f}	11.30±2.72
4.MB4	80.50±25.89^{a}	87.66±9.29^{d}	10.83±2.85
5.MB5	91.93±5.74^{c}	93.66±7.09^{f}	11.00±3.00
6.MB6	83.40±13.13^{a}	68.66±27.30^{c}	11.26±2.96
7.MB7	89.90±7.18^{b}	65.00±34.04^{c}	10.96±2.70
8.MB8	86.06±9.30^{b}	76.33±10.69^{d}	11.33±2.85
9.MB9	87.96±10.48^{b}	96.00±6.08^{f}	11.83±2.70
10.MB10	85.80±20.36^{b}	99.33±1.15^{f}	10.73±2.55
11.MB11	80.10±17.95^{a}	94.33±8.14^{f}	11.10±3.05
12.MB12	78.83±17.87^{a}	87.00±13.52^{e}	11.33±2.80
13.MB13	83.63±10.53^{a}	98.33±2.08^{f}	11.33±2.99
14.MB14	86.60±18.62^{b}	13.00±4.58^{a}	11.73±2.22
15.MB15	92.56±3.63^{c}	55.00±6.55^{b}	11.10±2.88
16.MB16	87.63±9.036^{b}	91.33±9.01^{e}	11.00±2.68

Figures followed by same letter (s) within a column are not significantly different ($P\leq0.05$)

Table 2: Effect of location on grain size, germination and moisture content of grain of malt barley

Location	Grain quality parameter		
	Grain size	Germination energy	Moisture content
Holetta	72.62±11.38[a]	76.87±24.58[a]	11.44±.51[b]
Debrebrhan (Ankober)	92.87±4.19[b]	85.93±26.34[a]	8.36±.62[a]
Kulumsa (Bekoji)	94.07±2.96[b]	76.37±24.45[a]	13.83±.34[c]

Means followed by same letter (s) within a column are not significantly different (P≤0.05)

Germination energy (GE): The Germination energy is the total number of grains that germinate over 72hr of incubation under specified conditions (Woonton*et al*., 2005). Average germination energy of barley cultivars grown at the three locations was not significantly different (P≤0.05), though it ranged between 76.37% for Bekoji and 85.93% for Ankober (Table 2). On the other hand, germination energy was significantly different (P<0.05) among varieties and ranged between 13% for MB14 and 99.33% for MB10 (Table 1). A minimum of 95% germination on a 3-day germination test is an absolute requirement. Any factor which interferes with the uniformity of germination or reduces the vigor of kernel growth during processing will reduce the quality of malts produced (Michael, 2014). In agreement with the findings of the present study, Swanston *et al.*, (1995) noted differences in the genetic factors determining germination after three days of incubation and environmental effects on their expression.

Moisture content: There were significant differences between locations for grain moisture content (P≤0.05). The average moisture content of grain was higher at Bekoji (13.83 %) and lowest at Ankober (8.36%) (Table 2). In contrary, moisture content was not significantly different (P≤0.05) for the varieties and varied between 10.00and 11.9% (Table 1). These results suggest that moisture content of grain is mainly more affected by the environment than by variety. 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-causingmicroorganisms. According to Fox *et al.* (2003), the maximum reasonable industrial specification of malt barley content for safe storage is 12.5%, whereas moisture content of 12 -13% is accepted for EBC standard.

Thousand grain weight (TKW*):* Thousand kernel weight was significantly (P≤0.05) affected by locations (Table 4). Cultivars grown at Bekoji exhibited greater TKW than did those from Ankober and Holetta (52.4 g 48.0gand 44.2 g, respectively). On the other hand, there was no significant difference (P≤0.05) among the cultivars for thousand-kernel weight (Table 3). It has been reported that thousand grain weight should be >45 g for 2-rowed barley and > 42 g for 6-rowed barley (Anonymous, 2012).

Table 3: Effect of variety on Hectoliter weight, thousand kernel weights and protein content of malt barley grain

Genotype/Variety	Grain quality parameter		
	Hectoliter weight(kg/hl)	Thousand kernel weight(g)	Protein (%)
1.MB1	64.36±4.350[a]	48.96±3.53[c]	10.03±1.501[a]
2.MB2	67.00±4.026[b]	46.80±5.23[b]	10.43±2.064[b]
3.MB3	66.66±3.82[b]	47.80±3.56[b]	10.80±1.41[b]
4.MB4	65.73±2.36[a]	50.13±6.21[c]	10.23±2.48[a]
5.MB5	65.86±3.34[b]	47.53±3.66[b]	10.50±1.91[b]
6.MB6	66.60±2.94[b]	50.63±4.25[c]	11.10±1.45[b]
7.MB7	66.20±3.85[b]	46.50±3.051[a]	11.50±1.45[c]
8.MB8	65.93±3.11[b]	45.40±4.51[a]	10.23±.92[a]
9.MB9	67.90±3.06[c]	47.43±4.90[b]	9.86±1.30[a]
10.MB10	65.33±3.80[a]	51.60±5.55[d]	10.46±1.27[b]
11.MB11	65.30±4.38[a]	47.83±2.61[b]	10.33±1.87[a]
12.MB12	66.56±1.98[b]	44.56±7.83[a]	10.96±2.00[b]
13.MB13	64.566±2.87[a]	50.00±5.56[c]	9.96±1.46[a]
14.MB14	64.53±3.35[a]	48.13±8.46[b]	9.66±.66[a]
15.MB15	65.43±3.16[a]	46.66±3.00[b]	11.30±1.55[c]
16.MB16	64.66±2.84[a]	51.10±1.94[d]	10.46±1.78[b]

Means followed by same letter (s) within a column are not significantly different (P≤0.05)

Hectoliter weight*:* Hectoliter weight of malt barley grown in different locations was significantly different (P≤0.05) andcultivars from Holetta (67.88 kg/hl) had the highest hectoliter weight and those from Bekoji (62.33 kg/hl) had the lowest (Table 4). Hectoliter weight (HLW) has been shown to be influenced by growing environment (Molina-Cano et al., 2001), which supports the result obtained in this study. Test weight (TW) (bulk density or HLW) is an industry standard for classifying malt and feed barley. Barley with plumper grains and a higher test weight should have a greater percentage of starch orenergy in the grain and should be lower in fiber (Shewry and Morell, 2001). Hectoliter weight is one of the best-correlated parameters for malt quality and significantly affected by location.

Protein content: The analysis of variance revealed significant differences between locations for grain protein content (P≤0.05), which was higher at Kulumsa (Bekoji)(11.87 %) than at Holetta and Ankober (10.6% and 9.0%, respectively) (Table 4). It has been reported that grain protein content is influenced largely by both genotype and environment (Bathgate, 1987). The protein content of malt barley cultivars in the present study showed no significant difference (P≤0.05), though it ranged from9.66% for MB14 to 11.5 % for MB7 (Table 3). In line with this, it has been reported that desirable protein content range for 2-rowed barley is 9.0-11.0% and for 6-rowed barley is 9.0-11.5% (Anonymous, 2012) and barley used for malt should have a grain protein concentration (GPC) below11.5percentage, as higher protein content will deteriorate malting and lead to poor beer quality.

Table 4: Effect of location on hectoliter weight, thousand kernel weight and protein content of malt barley grain.

Location	Grain quality parameter		
	Hectoliter weight(kg/hl)	Thousand kernel weight (g)	Protein (%)
Holetta	67.88±1.61^{c}	44.17±3.62^{a}	10.60±.95^{b}
Debrebrhan(Ankober)	67.16±1.63^{b}	48.01±2.62^{b}	9.00±.60^{a}
Kulumsa(Bekoji)	62.33±1.71^{a}	52.39±2.93^{c}	11.87±.88^{c}

Figures followed by same letter (s) within a column are not significantly different (P≤0.05)

Malt quality

Hot water extract (HWE): Fine ground hot water malt extract of cultivars grown at different locations exhibitedsignificant difference (P≤0.05). The extract amount was higher forHolettasamples (72.74%) than for Bekoji (70.66%) and Ankober (68.04%) (Table 6), and indicats that variation in growing condition resulted in a wide range of malt extract values. In line with this, Fox *et al.* (2003) have reported that quality of the extract is influenced by several factors such as environmental and growing conditions, including temperature, fertilizer application and availability of nitrogen or moisture. On the other hand, the variation among cultivars was not significant for HLW (P≤0.05), but ranged from 67.18 % for MB1 to 72.91% for MB1 (Table 5). Hence, the extract content of promising cultivars was found to be similar with that of the released varieties. The extract yield reflects the extent of enzymatic degradation and the solubility of grain components after malting and mashing (Swanston *et al.*, 2014).EBC requirement for hot water extract value ranges from 75.0-80.7%, but all cultivars in this study did not fulfill this standard.

Table 5: Varietal effects on malt quality of fine ground hot water malt extract (%), friability and solubleprotein content.

Genotype/Variety	Malt quality parameter		
	Fine ground malt extract (%)	Friability (%)	Soluble protein content of wort (%)
1.MB1	72.91±3.475^{c}	54.73±7.5^{b}	4.66±1.18^{c}
2.MB2	71.70±4.978^{c}	62.56±7.88^{c}	3.94±0.60^{b}
3.MB3	70.91±1.940^{b}	69.60±10.71^{d}	4.21±0.62^{b}
4.MB4	67.18±4.69^{a}	61.00±3.29^{c}	4.80±0.39^{d}
5.MB5	71.21±2.23^{b}	52.96±15.02^{a}	4.25±0.69^{b}
6.MB6	71.90±1.20^{c}	51.80±0.91^{a}	4.79±0.68^{d}
7.MB7	71.86±2.71^{c}	53.23±11.47^{a}	5.43±0.86^{e}
8.MB8	68.67±4.614^{a}	54.00±9.6^{a}	4.61±0.38^{c}
9.MB9	71.98±1.88^{c}	60.50±0.7^{c}	5.06±1.28^{d}
10.MB10	72.19±4.96^{c}	60.60±0.72^{c}	4.46±1.22^{c}
11.MB11	69.75±3.28^{b}	55.92±4.53^{b}	3.51±0.83^{a}
12.MB12	68.59±4.37^{a}	61.40±4.97^{c}	5.29±1.45^{e}
13.MB13	70.77±3.16^{c}	61.65±1.81^{c}	4.82±0.42^{d}
14.MB14	71.70±1.52^{c}	67.16±10.42^{d}	4.09±0.22^{b}
15.MB15	68.74±6.11^{a}	50.90±14.16^{a}	4.38±0.82^{c}
16.MB16	67.65±3.40^{a}	71.70±5.99^{e}	4.56±1.29^{c}

Figures followed by same letter (s) within a column are not significantly different (P≤0.05)

Friability: Friability is a measure of the breakdown of malt endosperm cell wall components. Malt friability should be >60% (Anonymous, 2012). In the present study, variation for friability was not significant (P≤0.05) between locations. The mean value

of friability forHoletta, Ankober and Bekojisamples was 56.95%, 57.65% and 63.46% respectively (Table 6). Cultivars grown at Bekoji were best in friability. An increase in friability reflects a more extensive modification of the endosperm during malting. mostly with respect to degradation of the protein matrix and cell walls (Chapon*et al*., 1979).The results also showed that, there was no significant difference($P \leq 0.05$) among cultivars for friability content, though the mean values ranged from 50.9% for MB15 to 71.7% for MB16 (Table 5) with about 44% of the varieties had friability percentage of <60%. When barley endosperm is properly modified during malting, the resulting malt is soft and friable. Factors that interfere with endosperm modification, such as poor germination, large kernel size and high protein, are expected to reduce malt friability (Edney*et al.,* 2014).

Table 6: Location effect on malt quality of fine grind hot water malt extract (%), friability (%) and soluble protein content of wort.

Location	Malt quality parameter		
	Fine grind malt extract (%)	Soluble protein contentof wort (%)	Friability (%)
Holetta	72.74±1.74^{b}	4.40±0.69^{a}	56.95±9.95^{a}
Debrebrhan(Ankober)	68.04±2.77^{a}	4.41±1.19^{a}	57.65±7.31^{a}
Kulumsa(Bekoji)	70.66±3.99^{a}	4.85±0.61^{b}	63.46±9.37^{b}

Figures followed by same letter (s) within a column are not significantly different ($P \leq 0.05$)

Soluble protein content: Soluble protein content of malt was not significantly ($P \leq 0.05$) affected by location. However, the highest soluble protein content was obtained from Bekoji samples (4.85%), followed by those from Holetta and Ankober (4.40 and 4.41%, respectively) (Table 6).Similarly, there was no significant difference ($P \leq 0.05$) among the cultivars for soluble protein content, though the mean values ranged from 3.51% for MB11 to 5.43% for MB7 (Table 5). In protein-protein linkages, the stabilize foams are responsible for mouth feel and flavor stability, and in combination with polyphenols, they are thought to form haze. As amino acids and peptides, they are important nitrogen sources for yeast (Steiner *et al.,* 2011).

Diastatic power: The variation for diastatic power (DP) was not significant ($P \leq 0.05$) between locations, though the mean value was higher for Ankober (372.01WK) than for Holetta and Bekoji samples (370.69 and352.97WK, respectively) (Table 7). Diastatic power, the total activity of starch degrading enzymes in barley malt, is considered an important quality characteristic for malting and brewing (Allison, 1986). The conversion of barley into beer represents humankind's oldest and most complex example of applied enzymology. Indeed, historically some of the most significant advances in enzymology have been linked to the world of brewing, such as Eduard Buchner's extraction of enzymes from brewing yeast and Adrian Brown's kinetic analysis of invertase (Brown, 1992). The results of the present study also showed that there was no significant difference ($P \leq 0.05$) among varieties for diastatic power.However, mean values for the varieties ranged from 288.80WK for MB11 to 428.60WK for MB16. The desirable range for diastatic power is 90-110^{o}L or 200-300WK for 2-rowed cultivars and 90-120^{o}L for 6-rowed ones, hence, most of the cultivars in the present study had the desirable range for DP.

Table 7: Effect of location on diastatic power andfree amino nitrogen content of malt (wort)

Location	Malt quality parameter	
	Diastatic power	Free amino nitrogen
Holetta	370.69±57.68a	275.29±54.06b
Debrebrhan (Ankober)	372.01±69.77a	260.90±40.57b
Kulumsa (Bekoji)	352.97±49.87a	235.71±49.74a

Figures followed by same letter (s) within a column are not significantly different (P≤0.05)

Free amino nitrogen (FAN): There was no significant difference (P≤0.05) among varieties for FAN, but the values ranged from 223.48ppm for MB6 to 357.06 ppm for MB7. Similarly, there was no significant difference between locations (P≤0.05) though; the value of free amino nitrogen was the highest (275.29ppm) for Holetta samples and the lowest for Kulumsa (bekoji) (235.71ppm) (Table 7). Generally, the specifications for a normal fermentation require FAN levels between 140-160 mg/l(250-400ppm).Hence, cultivars grown at Holetta and Ankober fulfil this requirement. High FAN value is considered a good index for potential yeast growth and fermentation. Protein modification also involves the production of wort amino acids and small peptides (dipeptides and tripeptides), collectively known as free amino nitrogen (FAN). Adequate levels of FAN in wort ensure efficient yeast cell growth and, hence, a desirable fermentation performance (Enari, 1974).

Conclusion

The result of the present study showed that cultivar MB1,MB3,MB5,MB7,MB9,MB10 and MB4 exhibited acceptable grain quality (grain size, germination energy, moisture content, hectoliter weight, thousand kernel weight, and protein content). Malt quality (hot water extract amount, soluble protein, free amino nitrogen, diastatic power, and zinc content) as compared to the standard checks (MB13, MB1, MB14 and MB16). However, some did not fulfil the standard requirements of brewery industries. Among the locations,Kulumsa (Bekoji) was found to be suitable for quality malt barley production, as the grain and malt quality traits were in the acceptable range, followed by Debrebrhan (Ankober).Therefore, it was concluded that there are appreciable genetic variations among the malt barleycultivars and, growing environment greatly affects both grain and malt quality attributes.

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