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Study on Brewing Quality of Sorghum (*Sorghum bicolor, L. Monech*)

Dev Raj Acharya[1], Rabin Shrestha[2] and Santosh Thapa[2]*

[1]Central Department of Food Technology, Institute of Science and Technology, Tribhuvan University, Nepal
[2]Department of Food Technology, Central Campus of Technology, Tribhuvan University, Dharan, Nepal

***Corresponding Author:** *thapasantosh919@gmail.com*

Abstract
With an aim to bring a variation in quality as well as to reduce the cost of beer, sorghum malt has also gained popularity and thus this study is focused on studying the suitability of sorghum in beer making. Sorghum was malted and different proportions of the malt were mixed with commercial barley malt to produce beer. Germination for 3 days yielded the highest amylase activity in sorghum. Sorghum malt was significantly different ($p<0.05$) from commercial barley malt in moisture, starch content, reducing sugar, protein, % extraction, specific gravity and amylase activity. The proportion of sorghum malt used along barley malt altered the TSS, viscosity, pH and specific gravity of wort significantly ($p<0.05$). Increasing the proportion of sorghum malt resulted in an increase in TSS, pH, ash content, dextrin, acidities, methanol content and tannin content whereas there was a decrease in apparent extract, total reducing sugars and alcohol content of the beers produced. The beer made out of sorghum malt: barley malt (25:75) was found superior to other formulations as well as to barley malt beer both in terms of chemical and sensory properties. Sorghum malt: barley malt (25:75) beer differed significantly ($p<0.05$) from market beer in terms of TSS, real extract %, starch content, ash content, acidity, alcohol content, methanol content and fusel oil content while pH, color, viscosity, original extract %, apparent extract %, real degree of fermentation, reducing sugar, protein, tannin, specific gravity, total aldehyde and ester content were statistically similar. Likewise, sorghum malt: barley malt (25:75) beer had significantly different ($p<0.05$) appearance and clarity, color, flavor and overall acceptance while the mouthfeel was similar to market bought beer.

Keywords: *Sorghum, barley, malt, amylase activity, beer*

INTRODUCTION

Beer is the most widely consumed alcoholic beverage in the world and is the third-most popular drink in overall after water and tea (Nelson, 2005). The consumption of beer is of special interest because of its organoleptic and health-related characteristics and also due to its low cost as compared with other types of Western and European alcoholic beverages, such as wine (Sohrabvandi et al., 2012). Different beer styles are available that derive unique characteristics from the ingredients used and subtle differences in brewing process. But among the variety of options to choose from, malted barley has been the grain of choice in traditional brewing (Goode et al., 2005) which is mainly because of its high hydrolytic enzymes content (Eßlinger, 2009).

In the recent years, the world beer market has become extremely competitive due to which brewers have come under pressure to produce new innovative products as well as to produce high quality beer at lower costs (Bogdan & Kordialik-Bogacka, 2017). This has led to an increased replacement of barley malt with various less expensive malts. According to Annemüller and Manger (2013), up to 85-90% of beer in the world is now produced with adjuncts. In addition to lowering the price of beer produced, use of alternative brewing materials is associated with utilization of cereals available in the region as well as to create innovative beer types (O'Rourke, 1999). Oats, millet, maize, rice, rye, sorghum, wheat, spelt, einkorn, emmer, etc. have been found to be relevant for brewing (Eßlinger, 2009).

Sorghum is the fifth most produced grain globally (Mundia et al., 2019). According to FAOSTAT, 59.34 million tons sorghum was produced in the year 2018. It is an important crop in Africa as it is the staple grain for millions of people (FAO, 1999). It is consumed mainly as grain and is also used in a variety of products such as porridges, breads, alcoholic beverages, etc. (FAO, 1999). In addition to this, sorghum has traditionally been used for brewing opaque and lager beers in Africa as a source of both diastatic malt and adjuncts (Eßlinger, 2009). Although sorghum malts have higher gelatinization temperatures and reduced diastatic power, lower β-amylase activity and lower α-amino nitrogen in comparison to barley malt, the α-amylase activity is quite comparable (Eßlinger, 2009). The fermentable sugar content in sorghum malt is even higher than that of barley malt which makes it a suitable cereal for brewing (Mesta, 2005). In addition to its suitability for brewing, sorghum is a rich source of several macro and micronutrients as well as contains high amounts of phenolic acids, flavonoids and condensed tannins. It is found to possess anti-carcinogenic, anti-bacterial, antioxidant and energizing properties and is widely recognized for the treatment of cholesterol, constipation and colon cancer (Awika & Rooney, 2004).

In context of Nepal, sorghum is a traditional crop of the people living in high altitudes and dry land regions. It is locally called *Junelo* and is commonly used for preparation of *jand* (a cereal-based alcoholic beverage). Use of sorghum either as an alternative to barley malt or as an adjunct along with barley malt could reduce the import of expensive barley malt and thereby promote the utilization of locally available grains in brewing. Thus, besides reducing the cost of beer production, utilization of sorghum for brewing by breweries in Nepal would increase the commercial value of sorghum. This study focuses on comparing the quality of locally available sorghum malt

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with barley malt and determining the suitability of the sorghum malt as an adjunct in beer making.

MATERIALS AND METHODS

Sorghum (*Sorghum bicolor, L. Moench*) locally called "*Junelo*" was collected from Diktel district while commercial barley malt was collected from Chaudhary Udhyog Gram, Nawalparasi, Nepal. Similarly, hops and yeast were supplied by Chaudhary Udhyog Gram, Nawalparasi and Tiger Brewery, Butwal.

Preparation of Sorghum Malt

Winnowed and washed sorghum was added to water in the ratio 1:3 and steeped for 12 hours at 27±2°C temperature and 70±5% relative humidity. After steeping, all of the excess water was removed and the grains were spread over plastic trays. The trays were then covered with moistened muslin cloth and germinated at 27±2°C temperature and 75±5% relative humidity for up to 5 days. During germination, the grains were moistened frequently at 4-6 hours interval by sprinkling water and were mixed gently to equalize temperature and to aerate the mass. The germinated grains were then taken at an interval of 24 hours and dried in a cabinet drier to halt the germination process in three stages: first stage at 50-55°C up to 23% moisture content; second stage at 70°C up to 12% moisture content and third stage at 90°C up to 3-5% moisture content. After drying, the rootlets were removed and all of the prepared malt was packed in airtight glass bottles separately.

Preparation of Beer

Sorghum malt and commercial barley malt were ground separately in a grinder to reduce the size to around 1/2 to 1/4th of whole malt kernel. Different samples were then prepared by mixing following proportions on dry matter basis:

Sample A: 75% sorghum malt + 25% barley malt
Sample B: 50% sorghum malt + 50% barley malt
Sample C: 25% sorghum malt + 75% barley malt
Sample D: 100% sorghum malt + 0% barley malt
Sample E: 0% sorghum malt + 100% barley malt

Each sample of malt flour A, B, C, D and E were added to water at the ratio of 1:5 (m/v) inside a stainless steel pot and mashing was performed in the following sequence: mash temperature raised to 45°C and held for 45 minutes (protein rest period), mash temperature raised to 60°C and held for 18 minutes (sugar rest period), mash temperature raised to 72°C and held for 15 minutes (conversion rest period) and finally the temperature was raised to 77°C and held for 8 minutes (mashing off period) (Matz, 1991). After mashing, each wort was filtered separately with double folded muslin cloth. The filtered worts were further boiled for 15 minutes and then filtered through 4 folds of muslin cloth to remove hops and precipitated proteins. It was then left to cool to room temperature. The TSS of each wort was maintained at 14 ° Brix (by adding sugar or water as required) and pH at 4.5 (using 10% citric acid) where were then filled in glass jars for fermentation. Activated Brewery yeast (*Saccharomyces cerevisiae*) was pitched at the rate of 100ml/2L (16×10^6 cfu/ml) wort into the jars. The jars were cotton plugged and left for fermentation for 5 days (active phase of fermentation). The jars were again subjected to passive fermentation where cotton plugs were replaced by air lock dipped in 1% KMS solution and fermentation was continued until bubbling ceased. After fermentation, clear beer was raked and pasteurized at 65°C for 20 minutes followed by immediate cooling to room temperature.

Analytical procedure

Determination of Enzyme Activity of Sorghum malt

5 gram of malt powder was grinded with 50ml of distilled water and filtered through a filter paper. The filtrate was used as enzyme source. Alpha and beta amylase activity was determined as per Malik and Singh (1980). For α-amylase activity, a reaction mixture containing 2 ml of starch (150 mg starch, 600 mg KH_2PO_4, 20 mg anhydrous $CaCl_2$ dissolved in 100 ml distilled water, boiled for 1 min, cooled and filtered) and 1 ml of diluted enzyme was mixed in a test tube and incubated at 40^{o}C for 30 min. At zero and 30 min of incubation, 0.2 ml of the aliquot of reaction mixture was mixed with 3 ml of 1KI solution (254 mg iodine and 4 g KI dissolved in 1 L of water) and absorbance was measured at 620nm. α-amylase activity was expressed in terms of change in optical density at 620nm in 1 ml of enzyme extract from 1% m/v per g of dry matter per unit time.

For β-amylase activity, 1 ml of starch (1% in 0.067 M phosphate buffer, pH 6), 1 mL of undiluted enzyme extract and 1 ml of 0.1 M EDTA were mixed in a test-tube and incubated for 30 min at 37^{o}C. Reducing sugar content in control (0 min incubation) and sample (30 min incubation) was determined as performed by Sadasivam and Manickam (1996) but the Lane and Eynon method was used instead of using the dinitrosalicyclic acid method. β-amylase activity was expressed as mg per 100 gram dry basis.

Proximate evaluation of sorghum grains and malt- Moisture content, ash content, crude fat, crude protein, carbohydrate, crude fiber, reducing sugar and starch (g dextrose/ 100g dry matter) of sorghum grains and sorghum malt was determined as per Ranganna (2001).

Analysis of Malt Extract-

10g malt extract was ground with 40 ml distilled water (46°C). Aroma was taken and the temperature was adjusted to 45°C and held for 30 minutes. Then, the temperature was slowly raised to 70°C, 20ml distilled water was added to it and left for 1 hour followed by cooling and adjusting the weight to 90g. The mixture was then filtered through Whatman No. 4 filter paper and the filtrate was used for analysis AOAC (2005a).

Protein content and reducing sugar content were determined by method described by Ranganna (2001) while the specific gravity, starch-iodine test, filtration rate,

aroma and clarity of extract were determined by pycnometer method as per AOAC (2005a).

Analysis of Wort- TSS was measured by using a hand refractometer (Ranganna, 2001), pH by a pH meter and specific gravity and viscosity by methods as per AOAC (2005a). The color of wort was determined as per AOAC Official Method 976.08 (2005) with slight modification.

Analysis of Beer-
The parameters of beer analyzed and the methods used are shown in Table 1.

Sensory evaluation
Sensory evaluation was carried out using 9-point hedonic scale as per Ranganna (2001). A total of 10 semi-trained panelists consisting of administrative staffs, teachers and fellow students of the college were asked to rate the sensory parameters (appearance & clarity, color, flavor, mouth feel and overall acceptability) on a 9-point scale ranging from 1 (dislike extremely) to 9 (like extremely).

Table 1: Analytical methods used for the analysis of beer

Parameter	Reference
Color	AOAC Official Method 976.08 (2005) with slight modification
TSS	Hand refractometer method (Ranganna, 2001)
pH	pH meter
Acidities (total, fixed and volatile)	Ranganna (1986)
Alcohol content	Pycnometer method as per AOAC Official Method 935.21 (2005)
Total nitrogen and protein content	Kjeldahl method (Ranganna, 2001) by taking 5ml of sample for digestion
Total aldehyde content	Kirk and Sawyer (1991)
Total ester content	Kirk and Sawyer (1991)
Fusel oil content	AOAC Official Method 959.05 (2005)
Methanol content	AOAC Official Method 958.04 (2005)
Specific gravity	AOAC (2005a)
Real extract, apparent extract, original extract	EBC (1987)
Real degree of fermentation	EBC (1987)

Statistical analysis

The experiment was conducted in a Completely Randomized Design (CRD) and each analysis was carried out in triplicates. Data were statistically processed by Analysis of Variance (ANOVA) using Genstat (Twelfth Edition developed by VSN International Limited) at 5% level of significance. The mean values were compared by using Fisher LSD method.

RESULTS AND DISCUSSION

Effect of germination time on α-amylase activity of sorghum

Sorghum grains were germinated for 2, 3, 4, 5, 6, and 7 days while steeping and kilning conditions were kept constant. The germination time that gave the highest α-amylase activity was then selected for bulk production of malt. Mean value of α- amylase activity of sorghum malt germinated at ambient temperature for 1, 2, 3, 4, 5, 6 and 7 days were found to be 4.57, 9.6, 21.27, 17.85, 11.4, 8.39 and 5.43 units per g dry matter respectively. As shown in Figure 1, the values increased significantly ($p<0.05$) up to 3rd day and then decreased on further continuing germination process up to 7th day.

During germination, the embryo and endosperm become hydrated thereby starting the synthesis of amylases (Veith, 2009). Similar to our findings, Uvere et al. (2000) found that the α-amylase activity of sorghum malts peaked on the third day of germination after steeping for 18 hours. Veith (2009) reported the α-amylase activity of different sorghum varieties in the range of 71.63-96.44 ceralpha units/g on germination at 26°C for 2.5 days which is much higher than our findings. Similarly, slightly higher values than our findings was observed by Beta et al. (1995) where the α-amylase activity of different sorghum cultivars were 17-91 units/g when steeped for 6 hours and germinated at 28°C for 5 days. The α-amylase activity of cereals is affected by different factors such as the type of cereals, steeping, germination and kilning conditions which may be the reason for lower α-amylase in our findings than reported by other workers. In contrast to our findings, Agu and Palmer (1997) observed that the α-amylase activity kept on increasing even upto 5th day of germination at 30°C. Likewise, Ratnavathi and Chavan (2016) reported the highest α-amylase activity in different sorghum varieties on the 4th day of germination.

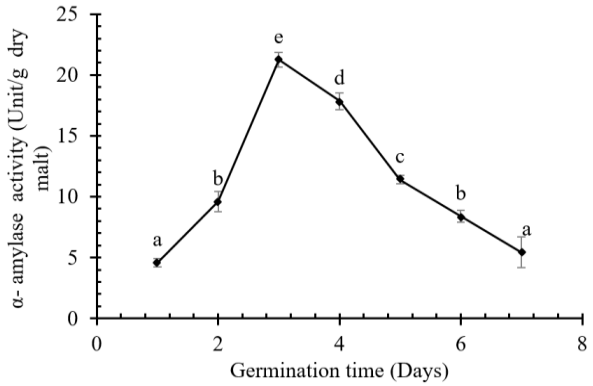

Figure 1: α- amylase activity of sorghum malt

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* Values are the means of three determinations and the vertical error bars represent standard deviations. Values with different superscripts are significantly different at 5% level of significance. (**Note:** 1 unit α-amylase activity = Unit change OD at 620 nm per min under the experiment condition)

Effect of germination time on β- amylase activity of sorghum-

Mean values of β- amylase activity of sorghum malt germinated at ambient temperature for 1, 2, 3, 4, 5, 6 and 7 days were found to be 299.5, 623.9, 846.3, 699, 415.6, 146.4 and 98.6 units per g dry matter respectively. β-amylase activity was affected by germination time and it reached the maximum value on 3 days (846.3 units/g dry malt) and then decreased on further germination (Figure 2). There was no significant difference ($p>0.05$) in β-amylase activity of malt germinated at ambient temperature for 6 and 7 days while there was significant difference ($p<0.05$) in β-amylase activity of malts germinated for other time periods.

Similar to our findings, Uvere et al. (2000) found that the β-amylase activity of sorghum peaked on the third day of germination after steeping for 18 hours. Veith (2009) reported the β-amylase activity of different sorghum varieties in the range of 18.2-38.74 betamyl units/g on germination at 26°C for 2.5 days which is much lower than our findings. Similarly, much lower values than our findings was also observed by Beta et al. (1995) where the β-amylase activity of different sorghum cultivars were 3-34 units/g when steeped for 6 hours and germinated at 28°C for 5 days. In contrast to our findings, Agu and Palmer (1997) observed that the β-amylase activity kept on increasing even upto 5th day of germination at 30°C.

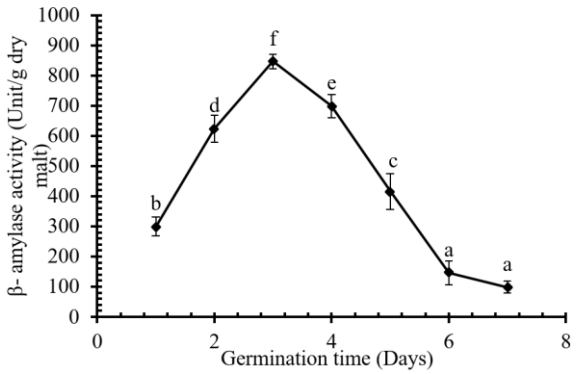

Figure 1: β- amylase activity of sorghum malt

* Values are the means of three determinations and the vertical error bars represent standard deviations. Values with different superscripts are significantly different at 5% level of significance. (**Note:** 1 unit β- amylase activity= 1 mg reducing sugar produced in 30 mins at 30 °C)

Chemical composition of sorghum grain and sorghum malt

The chemical constituents of sorghum grain and sorghum malt are tabulated in Table 2. Malting was found to have a significant impact on chemical constituents of sorghum. Moisture, crude fiber and starch content of sorghum were found to be reduced significantly ($p<0.05$) while the ash content and reducing sugar content increased significantly ($p<0.05$) due to malting. Although not significant, crude fat content was found to be reduced whereas crude protein and carbohydrate content slightly increased on malting.

Although the moisture content increased during germination, kilning process reduced the moisture drastically. The hydration process during germination activates a wide array of enzyme systems that hydrolyze and solubilize food reserves. Increase in ash content may be due to enzyme solubilization and leaching out of anti-nutrients binding with the minerals (Alemu, 2009; Idris et al., 2007). A decrease in starch content and corresponding increase in reducing sugars may be attributed to starch hydrolysis by hydrolytic enzymes activated during germination (Mella, 2011). Similarly, decreased quantity of crude fiber may be due to leaching out of water soluble fibers, mainly β-glucan, as well as due to the activity of β-glucanase activated during germination. Similar changes have been observed by Idris et al. (2007), Alemu (2009) and Ogbonna et al. (2016) during malting of sorghum.

Table 2.: Proximate composition of sorghum grain and malt (% dry basis except moisture)

Chemical constituent	Sample	
	Sorghum grain (unmalted)	Malted sorghum
Moisture content (%)	10.34^{a}(0.265)	8.01^{b}(0.327)
Ash content (%)	0.86^{a}(0.015)	2.55^{b} (0.05)
Crude fat (%)	2.31^{a}(0.032)	1.8^{a} (0.10)
Crude protein (%)	9.66^{a}(0.158)	9.9^{a}(0.05)
Carbohydrate (%)	74.27^{a}(0.383)	74.96^{a}(0.236)
Crude fiber (%)	2.55^{a}(0.05)	2.00^{b}(0.1)
Reducing sugar (%)	0.32^{a}(0.010)	1.6^{b}(0.1)
Starch (g dextrose/ 100g)	70.74^{a}(0.41)	65.76^{b}(0.21)

*Values are the means of triplicate analysis. Means with different superscripts on the same row are significantly different at 5% level of significance. Values in the parentheses represent Standard Deviation.

Analysis of malt extract of sorghum malt and commercial barley malt

The comparative study of sorghum malt and commercial barley malt was done and the findings are tabulated in Table 3. Statistical analysis showed that sorghum malt had a significantly ($p<0.05$) higher moisture and starch content whereas the reducing sugar content, protein content, percentage extraction, specific gravity and amylase activity of commercial barley malt were significantly

($p<0.05$) higher. Similarly, the pH and ash content were not significantly different ($p>0.05$). The filtration rate of barley malt extract was relatively higher than sorghum malt which may be due to presence of hulls in barley malt that aids in filtration (Lewis & Young, 1996).

Comparable observations were reported by Ratnavathi and Chavan (2016) who found the amylase activity of different sorghum varieties malted for 72 hours ranged from 22.5-75 units/g/20 min. Similarly, the crude protein content, ash content, starch content and reducing sugar content in the sorghum malt was reported to be 11%, 0.93% 50.03% and 4.73% respectively (Ratnavathi & Chavan, 2016). The protein content, starch content and reducing sugar content were higher whereas the ash content was lower than our findings.

Table 3: Chemical Composition of Malt Extract

Parameter	Sorghum malt	Commercial barley malt
Aroma	Slightly Aromatic	Aromatic
Saccharification time	40-45 min	10-15 min
Filtration rate	Slow	Normal
Clarity of extract	Haze	Clear
Starch iodine test	-	+
pH of extract	6.5^{a}(0.3)	6.2^{a}(0.26)
Moisture Content (%)	8.01^{a}(0.125)	6.25^{b}(0.28)
Starch content(g dextrose/100 g)	65.76^{a}(0.21)	55.06^{b}(1.25)
Reducing sugar(g dextrose/100 g)	1.6^{b}(0.1)	2.80^{a}(0.222)
Ash content(%m/m)	2.55^{a}(0.05)	2.60^{a}(0.05)
Protein (%)	9.9^{b}(0.05)	11.36^{a}(0.49)
% of extract(dry basis)	29.45^{b}(0.5)	57.98^{a}(2.23)
Sp. Gr. of extract (%)	1.0016^{b}(0.003)	1.02678^{a}(0.004)
Amylase activity(g dextrose/100g)	29.45^{b}(0.5)	50.05^{a}(2.14)

* Values are the means of triplicate analysis. Means with different superscripts on the same row are significantly different at 5% level of significance. Values in the parentheses represent Standard Deviation.

Chemical composition of wort-

Wort prepared from five different combinations of sorghum and barley malt were analyzed and the findings are tabulated in Table 4. Statistical analysis showed that worts having different proportions of sorghum and barley malt are significantly different ($p<0.05$) in terms of TSS, viscosity, pH and specific gravity. Wort samples with higher proportion of sorghum malt were found to have lower TSS, higher viscosity, higher pH and lower specific gravity in comparison to wort samples with lower proportion of sorghum malt.

Specific gravity is a measure of density of wort which is measured to indicate the amount of sugar in solution. Similarly, TSS is also the measure of dissolved solids, which eventually indicates the amount of sugar in wort (Veith, 2009). Higher specific gravity and TSS of wort samples having lower proportion of sorghum malt in comparison to wort samples having higher proportion of sorghum malt may be correlated with lower reducing sugar content of sorghum malt than that of commercial barley malt. Similarly, higher viscosity of worts containing higher proportion of sorghum might be accounted to lower amylase activity of sorghum malt than commercial barley malt.

For 100% sorghum malt wort, values higher than our findings have been reported for TSS (Veith, 2009) and specific gravity (Ortega Villicaña & Serna Saldivar, 2004; Veith, 2009) whereas values lower than our findings have been reported for pH (Ortega Villicaña & Serna Saldivar, 2004; Osorio-Morales et al., 2000) and viscosity (Osorio-Morales et al., 2000). Similarly, Dhamija and Singh (1978) observed that the wort containing barley and sorghum malt (50:50) had a pH value of 5.5 which is lower than the value observed in this study for same proportion of malt.

Chemical composition of beer

Beer prepared by the fermentation of worts containing different fractions of sorghum malt were subjected to chemical analysis and the results are tabulated in Table 5. Similarly, the optimum formulation was then compared

Table 4: Physiochemical Properties of Wort

Attributes	Wort samples				
	A	B	C	D	E
T.S.S(°Bx)	5.81^{c}(0.076)	8.06^{b}(0.46)	13.46^{a}(1.092)	5.76^{c}(0.12)	13.07^{a}(0.124)
Viscosity (cP)	1.78^{ac} (0.095)	1.56^{bc}(0.05)	1.28^{bd}(0.098)	2.04^{a} (0.336)	1.18^{d}(0.01)
pH	5.94^{ab} (0.055)	5.8^{bc}(0.05)	5.77^{c} (0.026)	6.066^{a}(0.163)	5.73^{c}(0.05)
Specific gravity	1.0106^{d} (0.002)	1.0156^{c} (0.00)	1.027^{b} (0.0029)	1.0015^{e} (0.0003)	1.0343^{a} (0.003)
Starch iodine test	+	-	-	+	-

*A, B, C, D, and E indicate sorghum: barley (75:25), sorghum: barley (50:50), sorghum: barley (25:75), sorghum, and commercial barley wort respectively. Values are the means of triplicate determinations. Figures in parentheses are the standard deviations. Means having similar superscripts within a row are not significantly different ($p>0.05$) by LSD.

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Table 5: Physicochemical properties of sorghum malt incorporated beer

Parameter	Beer samples			
	A	B	C	D
TSS (ºBx)	5.85^{cb}(0.05)	5.63^{b}(0.15)	5.1^{c}(0.057)	7.066^{a} (0.15)
pH	5.1^{a}(0.35)	4.917^{a}(0.16)	4.13^{b}(0.057)	5.13^{a}(0.05)
Color (ASBC unit)	3.03^{bd} (0.15)	3.2^{bc}(0.05)	3.41^{ac}(0.08)	3.08^{ab} (0.19)
Viscosity (cP)	1.35^{a} (0.01)	1.39^{a} (0.015)	1.21^{b} (0.025)	1.22^{b} (0.025)
Original extract %	9.27^{ab}(0.06)	9.36^{a} (0.076)	9.25^{ab} (0.045)	9.253^{ab} (0.101)
Real Extract %	2.91^{b}(0.060)	3.26^{a} (0.051)	2.38^{c} (0.14)	2.33^{c} (0.152)
Apparent extract (%)	2.14^{b}(0.169)	2.333^{b} (0.115)	4.02^{a} (0.096)	1.58^{c} (0.075)
Real degree of fermentation %	75.46^{a}(0.85)	74.34^{a}(1.033)	74.06^{a} (1.33)	76.073^{a} (2.197)
Reducing sugar (% as maltose)	1.546^{c}(0.09)	1.866^{b} (0.15)	2.45^{a} (0.132)	0.936^{d}(0.058)
Starch (g dxtrose/100ml)	4.68^{b}(0.066)	4.69^{b}(0.173)	4.486^{a} (0.035)	4.866^{a} (0.041)
Dextrin (g/100ml	2.63^{b}(0.152)	2.45^{c} (0.05)	1.89^{d} (0.134)	3.3^{a} (0.085)
Ash content (%)	0.701^{b}(0.004)	0.596^{b}(0.181)	0.103^{c}(0.006)	0.941^{a}(0.023)
Protein %	0.24^{b}(0.1)	0.27^{b}(0.017)	0.59^{a}(0.119)	0.25^{b} (0.04)
Acidities (%m/v)				
a. Total as acetic	0.258^{bc}(0.00)	0.249^{c}(0.007)	0.202^{d}(0.001)	0.271^{ab}(0.015)
b. Fixed as acetic	0.246^{ab}(0.00)	0.203^{c}(0.005)	0.193^{d}(0.0006)	0.260^{a}(0.005)
c. Volatile as acetic	0.008^{b}(0.001)	0.011^{ab}(0.003)	0.0094^{b}(0.006)	0.013^{a}(0.0005)
Tannin (mg/L)	187.87^{a}(0.05)	180.56^{b}(0.03)	178.77^{b} (4.87)	190.3^{a} (1.307)
Specific gravity	1.046^{a}(0.050)	1.076^{a} (0.030)	1.015^{a}(0.0005)	1.012^{a} (0.019)
Alcohol (% v/v)	3.92^{bc}(0.08)	3.95^{bc} (0.3)	4.85^{a} (0.1)	3.7^{c} (0.076)
Methanol (g/100L alcohol)	100.8^{b}(1.56)	97.11^{b} (5.63)	87.12^{c}(1.02)	116.11^{a} (2.96)
Fusel oil (g/100L alcohol)	131.7^{a}(2.48)	109.61^{c}(4.43)	126.81^{b} (3.49)	134.28^{a} (1.51)
Total aldehyde (g/100L alcohol)	1.58^{b}(0.062)	1.42^{c} (0.055)	1.70^{a} (0.06)	1.38^{c} (0.003)
Ester (g/100L alcohol)	16.75^{c}(0.43)	17.52^{c} (0.96)	18.43^{b} (1.61)	21.37^{a} (0.33)

*Values are the means of three determinations. Figures in the parentheses are standard deviation. Figures in the row bearing different alphabet in superscript are significantly different at $p<0.05$. A, B, C and D indicate sorghum: barley (75:25), sorghum: barley (50:50), sorghum: barley (25:75) and (100:0) Sorghum respectively

with commercial barley malt beer and market beer and the results are tabulated in Table 6.

Beers with different proportions of sorghum malt varied significantly in almost all of the parameters studied except color, viscosity, specific gravity and real degree of fermentation (Table 5). Increasing the proportion of sorghum malt resulted in an increase in TSS, pH, ash content, dextrin, acidities, methanol content and tannin content whereas there was a decrease in apparent extract, total reducing sugars and alcohol content of the beers produced. The results for original extract %, real extract %, starch content, protein content, fusel oil content, total aldehyde content and ester were not found to be correlated with the proportions of sorghum malt used for fermentation. Similar pattern was demonstrated for TSS and alcohol content by Kullar (2018) for beer made of sorghum and pilsner malt where increasing sorghum malt fraction resulted in increased alcohol and decreased TSS of beer. Comparable values for ethanol content (3.28-4.17%) were reported by Veith (2009) in sorghum beers after 8 weeks of fermentation. Similarly, analogous values for TSS (5.8 ± 0.28), pH (4.16 ± 1.25), original extract (11.10 ± 0.247) and real extract (4.38 ± 0.26) have been reported by Acharya (2007) for naked barley beer. In addition to this, Dhamija and Singh (1978) observed a pH range of 4.1-5.15 in beer prepared from barley using sorghum as adjunct which is similar to our findings. (Roger et al., 2013) found that the total titratable acidity, volatile acidity, alcohol content, methanol content, acetaldehyde content and pH of commercial sorghum beer in Cameroon was 0.82%, 0.03%, 5%, 161g/100 liter, 134.5g/100 liter and 6.2 respectively which are higher than the values observed in our study for sorghum beer. Alcohol content in 100% sorghum malt beer was found to be 4.39% after 7 days of fermentation by Mesta (2005) which is higher than the alcohol content in this study and this variation may be due to variation in the amount of fermentable sugars in wort, sorghum variety, type of inoculum used and fermentation conditions. Small amounts of tannins is favorable in beer for good color and flavor whereas high amounts make the beer bitter and inhibits amylase enzymes (Mesta, 2005). Since sorghum is found to contain the highest amount of tannin among cereals, use of higher proportion of sorghum malt might have resulted in higher

tannin content in beers produced from such malt. Similarly, aldehyde content is of special interest in beer as acetaldehyde, which is one among the aldehydes, is the precursor of ethanol and has an unpleasant grassy flavor and aroma which has a flavor threshold of 10-20ppm (Briggs et al., 2004).

Table 6: Physicochemical properties of the best sorghum malt incorporated beer in comparison to commercial barley malt beer and market beer

Parameter	Beer samples		
	C	E	F
TSS (ºBx)	5.1^{b}(0.057)	6^{a} (0.17)	4.8^{c}(0.1)
pH	4.13^{a}(0.057)	4.02^{a} (0.12)	4.12^{a}(0.1)
Color (ASBC unit)	3.41^{a}(0.08)	2.97^{b} (0.032)	3.581^{a}(0.19)
Viscosity (cP)	1.21^{b} (0.025)	1.35^{a} (0.015)	1.19^{b} (0.05)
Original extract %	9.25^{bc} (0.045)	11.43^{a} (0.101)	9.0821^{c} (0.115)
Real Extract %	2.38^{c} (0.14)	3.206^{a}(0.100)	2.474^{b} (0.474)
Apparent extract (%)	4.02^{a} (0.096)	3.16^{b}(0.0655)	4.0745^{a}(0.135)
Real degree of fermentation %	74.06^{a} (1.33)	74.01^{a} (0.7008)	74.19^{a}(4.24)
Reducing sugar (% as maltose)	2.45^{a} (0.132)	2.28^{a}(0.16)	2.356^{a} (0.095)
Starch (g dxtrose/100ml)	4.486^{c} (0.035)	4.776^{a} (0.11)	4.670^{b} (0.010)
Dextrin (g/100ml	1.89^{c} (0.134)	2.076^{a} (0.0838)	1.936^{bc} (0.0512)
Ash content (%)	0.103^{a}(0.006)	0.1026^{b} (0.0015)	0.1026^{b} (0.0025)
Protein %	0.59^{a}(0.119)	0.316^{b} (0.0064)	0.6^{a} (0.04)
Acidities (%m/v)			
a. Total as acetic	0.202^{b}(0.001)	0.199^{c} (0.0002)	0.282^{a} (0.007)
b. Fixed as acetic	0.193^{b}(0.0006)	0.1866^{c}(0.003)	0.247^{a} (0.0170)
c. Volatile as acetic	0.0094^{c}(0.006)	0.01098^{b} (0.0109)	0.035^{a} (0.005)
Tannin (mg/L)	178.77^{a} (4.87)	148.85^{c} (2.724)	174.71^{a} (5.983)
Specific gravity	1.015^{a}(0.0005)	1.014^{a} (0.019)	1.015^{a} (0.00152)
Alcohol (% v/v)	4.85^{a} (0.1)	4.18^{c}(0.076)	4.386^{b} (0.12)
Methanol (g/100L alcohol)	87.12^{a}(1.02)	85.33^{b} (2.92)	48.72^{c} (0.12)
Fusel oil (g/100L alcohol)	126.81^{a} (3.49)	114.74^{b} (2.03)	110.36^{bc} (1.10)
Total aldehyde (g/100L alcohol)	1.70^{a} (0.06)	1.53^{b} (0.096)	1.78^{a} (0.057)
Ester (g/100L alcohol)	18.43^{a} (1.61)	14.706^{b} (0.21)	19.81^{a} (0.808)

*Values are the means of three determinations. Figures in the parentheses are standard deviation. Figures in the row bearing different alphabet in superscript are significantly different at $p<0.05$. C, E and F indicate sorghum: barley (75:25), commercial Barley and market beer respectively.

In terms of superiority sorghum and pilsner malt where increasing sorghum malt fraction resulted in increased alcohol and decreased TSS of beer. Comparable values for ethanol content (3.28-4.17%) were reported by Veith (2009) in sorghum beers after 8 weeks of fermentation. Similarly, analogous values for TSS (5.8 ± 0.28), pH (4.16 ± 1.25), original extract (11.10 ± 0.247) and real extract (4.38 ± 0.26) have been reported by Acharya (2007) for naked barley beer. In addition to this, Dhamija and Singh (1978) observed a pH range of 4.1-5.15 in beer prepared from barley using sorghum as adjunct which is similar to our findings. (Roger et al., 2013) found that the total titratable acidity, volatile acidity, alcohol content, methanol content, acetaldehyde content and pH of commercial sorghum beer in Cameroon was 0.82%, 0.03%, 5%, 161g/100 liter, 134.5g/100 liter and 6.2 respectively which are higher than the values observed in our study for sorghum beer. Alcohol content in 100% sorghum malt beer was found to be 4.39% after 7 days of fermentation by Mesta (2005) which is higher than the alcohol content in this study and this variation may be due to variation in the amount of fermentable sugars in wort, sorghum variety, type of inoculum used and fermentation conditions. Small amounts of tannins is favorable in beer for good color and flavor whereas high amounts make the beer bitter and inhibits amylase enzymes (Mesta, 2005). Since sorghum is found to contain the highest amount of tannin among cereals, use of higher proportion of sorghum malt might have resulted in higher tannin content in beers produced from such malt. Similarly, aldehyde content is of special interest in beer as acetaldehyde, which is one among the aldehydes, is the precursor of ethanol and has an unpleasant grassy flavor and aroma which has a flavor threshold of 10-20ppm (Briggs et al., 2004).

In terms of superiority of beer made out of formulations A, B, C and D with respect to the chemical parameters

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studied, formulation C (sorghum malt:barley malt::25:75) was found to be superior to other formulations and it was further subjected to comparison with commercial barley malt beer and market beer. The results are shown in Table 6. Statistical analysis revealed that the sorghum beer (sorghum malt:barley malt::25:50) was similar ($p>0.05$) with commercial barley malt beer in terms of pH, real degree of fermentation, total reducing sugar content and specific gravity. Likewise, the beer was similar ($p>0.05$) to market beer in terms of pH, color, viscosity, original extract %, apparent extract %, real degree of fermentation, total reducing sugar content, dextrin content, protein content, tannin content, specific gravity, total aldehyde content and ester content. All of the parameters of beer analyzed were found to be within the lager beer specification and also within the range of Nepal standards.

Sensory evaluation of beer

Beers made from the samples containing sorghum malt and commercial barley malt in different fractions, sample A, B, C and D were subjected to sensory evaluation and the mean sensory scores are shown in Figure 3. There was a significant difference ($p<0.05$) among these beer samples in terms of all the sensory parameters. The following conclusion was drawn among the samples in terms of superiority at 5% level of significance:

Appearance and clarity : [C]> [A/D]> [B/D]
Color : [C]> [A]> [B/D]
Flavor (taste and aroma) : [C]> [A/D]> [A/B]
Mouth feel : [C]> [A/D]> [A/B]
Overall acceptance : [C]> [D]> [A/B]

For all of the sensory parameters studied, beer made out of sample C stood out among the other samples with significantly ($p<0.05$) higher scores than other samples. The sensory scores for beer was not found to be correlated with the proportion of sorghum and commercial barley malt used for beer making. The panelists involved in this analysis were consumers of light colored beer. Among the beer samples, sample A, B and D beer were quite white in color and opaque whereas sample C was quite clear and light colored which was more or less similar to market beer. This might be the reason for the preference of sample C beer.

Since beer made out of sample C (sorghum malt: barley malt:: 25:75) was found to be superior to other samples, this beer was further subjected to comparison with commercial barley malt beer and market beer. The mean sensory scores and the statistical analysis for the scores are presented in Fig 4. The beer purchased from the market stood out among the others with a significantly higher scores in all the parameters except mouth feel after taste. This may be obviously due to highly sophisticated brewing methods in commercial breweries. Also, sample C was found to have significantly ($p<0.05$) higher scores for flavor, mouth feel and overall acceptance than for commercial barley malt beer. Although statistically insignificant, the scores for other parameters were also higher for sample C in comparison with commercial barley malt beer.

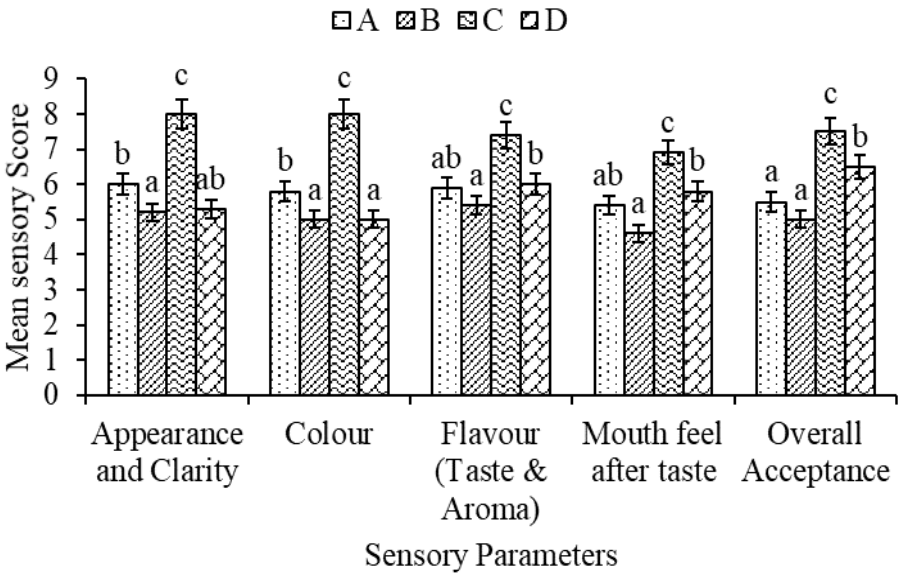

Figure 3: Sensory characteristics of beer made of different proportions of sorghum and commercial barley malt

*Here, the samples A, B, C and D represent beer made out of sorghum malt: barley malt (75:25), sorghum malt: barley malt (50:50), sorghum malt: barley malt (25:75) and 100% sorghum malt respectively. Bars with the same letter for any sensory parameter are not significantly different at 5% level of significance.

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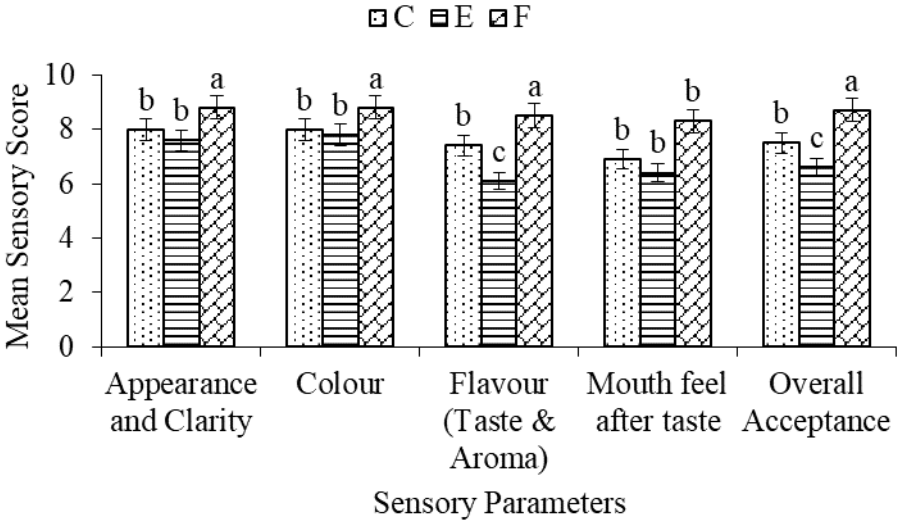

Figure 4. Sensory comparison of sorghum malt incorporated beer with commercial barley malt beer and market beer

*Here, the samples C, E and F represent beer made out of sorghum malt: barley malt (25:75), commercial barley malt beer and beer bought from market respectively. Bars with the same letter for any sensory parameter are not significantly different at 5% level of significance.

CONCLUSION

The malt prepared from locally available sorghum was compared with commercial barley malt and the former was found to be inferior to the latter in terms of the chemical parameters studied. Similarly, among worts prepared from different fractions of sorghum malt and commercial barley malt, the wort from sorghum malt: commercial barley malt (25:75) was comparable to commercial barley malt wort while the other worts containing higher fractions of sorghum malt were significantly different. Among the beer samples produced, the one with 25 parts sorghum malt and 75 parts commercial barley malt was found to be superior to other beer samples containing higher proportion of sorghum malt in terms of both physicochemical and sensory properties. Beer made from the combination of sorghum malt and barley malt (25:75) was found to be more appealing to sensory panelists than the beer made from commercial barley malt alone. Similarly, even though the market bought beer stood out among the others, beer made from sorghum malt: barley malt (25:75) was also acceptable to panelist. Thus, local sorghum malt can be used as adjunct (up to 25 parts) along with commercial barley malt to produce cheaper beer along with no major compromise in the quality of beer.

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