Preliminary Investigation on the performance of malt enzymes for the production of traditional wort from barley and corn

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Abstract

The preliminary investigation contains five trial experiments and was conducted at the following conditions: trial experiment "A" was done by using pure traditional production process, and it gave 22.5 % of malt extract and 2.7 ^{o}Bx of sugar content from barley sourced a place known as "jiga", trial experiment "B" was studied at temperature of 40, 55, 70 ^{o}C, pH value of 4.5, 5.5, 6.5, and time of 120, 180, 240 min using traditional malt. It showed that at the combination factors of 55 ^{o}C, 180 min and pH value of 5.5, there were maximum % extract of 37.35 % and sugar content of 4.5 ^{o}Bx. Trial experiment "C" was conducted at the same temperature, pH, and time as trial experiment "B", but substrate source added of 15, 22.5, 30 gm was considered additionally using traditional malt. It showed that at the combination factors of 55 ^{o}C, 180 min, pH value of 5.5, and substrate source added of 22.5 gm, there maximum % extract of 40.5 % and sugar content of 4.8 ^{o}Bx. Trial experiment "D", was studied by considering factors which were considered in trial experiment "B", but commercial malt was used instead of traditional malt, and the best result % extract of 50.85 % and 6.1^{o}Bx sugar content were observed. Similarly for the final trial experiment "E" all the factors and their value were the same as trial experiment "C", but commercial malt was used instead of traditional malt, and the best % extract of 70.10 % and sugar content of 8.4 ^{o}Bx were obtained.

Keywords: Malt, Substrate, Malt Extract, sugar content, commercial malt, traditional malt

1. Introduction

Malt is the basic raw material, which can be produced from different variety of barley by three step (steeping, germinating and killing) malting procedures. Malt is utilizing by most beer making industries due to it serves as both substrate source and enzyme during mashing process. Furthermore the main reason malt is the most essential raw material in almost all beer making industries, it contain starch as a substrate and different enzymes like starch hydrolyzing (alpha- amylase, beta amylase and limit dextrin's) enzymes as well as protein degrading enzymes. But compare with the beer making industries the utilization of malt is limited in distilled alcoholic beverage industries. This indicated that malt mashing process may need more optimum operating conditions like optimum temperature,

pH, and time in order to have sufficient enzymatic degradation of milled malted barley to attain the best wort composition. The malting industry requires malt with a high extract yield, high levels of enzyme activity, and good modification to manufacture beer of excellent quality. Therefore, barley must be able to germinate vigorously to meet these requirements. The germination energy and germination index both are the most influencing parameters of barley on malt technological parameters. The enzymology of the mashing process of the milled malt for distillery alcoholic production industries is very important for having maximum yield of product from the raw materials. This thesis word is basically intend to increase the consumption of milled malt for the production of distilled alcoholic beverage by considering some influential factors like temperature, pH time and corn meal as substrate source in both mashing and fermentation processes to produce maximum yield of wort and fermented beer. The quality of wort and fermented beer can be achieved in both mashing and fermentation processes by controlling the operating parameters of the processes due to enzymes are much sensitive for temperature, pH, Reaction time and limited substrate source.

2. Materials and Methods

2.1 Description of the study site

The experiment was conducted at Bahir Dar University, institute of technology and Gonder Malt Factory. Bahir Dar University, institute of technology is one of the oldest and famous universities found in the capital city of amhara region bahir dar city, in Ethiopia and located at about 561 km west of Addis Ababa. Gonder malt factory is located 190 km from bahir dar to the south east of bahir dar city.

2.2 Sample and sampling site

For this research work two available varies (Food and beer barely type) were used. Food barley type samples were purchased from five different sites. TMS_1, TMS_2. TMS_3 TMS_4 and TMS_5 were taken from Denbecha, Jigga, Finotoselam, Bahirdarkebela 11 and Bahir Dar Kebela 7, andtwo row barley type (beer barley) was sourced from Gonder Malt Factory (Gonder, Ethiopia) and used for the final Trial Experiment as a source of commercial malt.

2.3 Traditional Malt Preparation

The preliminary investigations were started from malt preparation from food barley type. 250 gram barley was taken from each sample and malted independently by following the same malting procedure (pure traditional malting procedure). The malting process was started from cleaning the raw barley. The cleaned samples were steeped by water at room temperature for three days without air resting and moisture controlling; germination was conducted for five days at room temperature and the green malt was exposed to kilning by sunlight for approximately five days. The finished malt was milled to unspecified particle size and analyzed.

2.4 Preparation of Traditional Wort at different conditions

Trial Experiment 'A'

As described in Table 1.1 the first trial experiment was conducted without considering any factors using the traditional malt. 50 g fine grist was mixed with 300 ml tape water and kept it for three days at room temperature (average atmospheric temperature was recorded 20.5 ^{o}C). After three mashing days, the samples were filtered and the filtrate/supernatant was taken for analysis, the % malt extract was determined from density/specific gravity data, viscosity, sugar content and soluble protein content was determined according to the [1].

Trial Experiment 'B'

The second trial experiments „B" were done using traditional malt as described in Table 1.1.The mash temperature (40, 55 and 70 ^{o}C), mash time (120, 180 and 240 min) and mash pH (4.5, 5.5 and 6.5) were considered. 50 g grist and 300 ml distilled water and mixed; the pH was adjusted by 0.5 M $H_2SO_4$and exposed to the following mashing experimental condition. The first program was 40^{o}C, 120 min and 4.5, the second experimental condition was 55 ^{o}C, 180 min and 5.5, and
the third experimental condition was 70 ^{o}C, 240 min and 6.5. After conducting these experiments
the samples were filtered and analyzed, the % malt extract, sugar content, viscosity was determined according to the [1].

Trial Experiment 'C'

These experiments were conducted using traditional malt by considering a supplementary substrate source in addition to temperature, time and pH. The mash temperature (40, 55 and 70 ^{o}C), mash time (120, 180 and 240 min) mash pH (4.5, 5.5 and 6.5), and supplementary substrate added (15 g, 22.5g and 30g) was considered. 50 g grist and 300 ml distilled water was common; the pH was adjusted by 0.5 M H_2SO_4 and exposed to the following mashing conditions: The first combination was 40^{o}C, 120 min 4.5 and 15 g, the second program was 55 ^{o}C, 180 min 5.5 and,

22.5 g the third experimental condition was 70 ^{o}C, 240 min 6.5 and, 30 g. After conducting these experiments the samples were filtered and the supernatant analyzed, the % extract, sugar content, viscosity was determined according to the [1]

Trial Experiment 'D'

Similarly trial Experiment "D" was also conducted by considering controlling variables which were considered in trial experiment "B", but the barely type was changed from food barely type to beer barely type (tow raw). The mashing experimental condition was also similar with trial experiment "B".

Trial Experiment 'E'

This is the final experiment for the investigation. For this experiment the factors, the mashing program and the grist to water ratio were similar with trial experiment "C", but commercial malt was used instead of traditional malt. The malting condition used to process the beer barley type for the production of commercial malt was similar with the traditional malting process.

2.4 Methods and Procedures of wort Analysis

2.4.1 Determination of % Extract Level

The extract level of the samples was determined by using the density/specific gravity to degree plato correlation. The density/specific gravity of the wort samples were measured using Hydrometer. The density/specific gravity to degree plato data were used to calculate the amounts of dry weight base by using eqn. 1. They the percentage of malt extract present in the filtrates was calculated by equation 2.

$$DWB = \frac{DP*(800+MC)}{100-MC} \quad \text{.....1}$$

Where: DWB is Dry Weight base, DP is Degree Plato, MC is Moisture Content of the samples

$$\%\, Extract\ Level = \frac{DWB*100}{100-MC} \quad \text{.....2}$$

2.4.2 Determination of fermentable Sugar

Wort dissolved sugar content was determined by using AR200 Digital Hand-Held Refractometer. It was also estimated by measurement of specific gravity using a hydrometer. The specific gravity data were used to determine the sugar content in degree plato (^{o}P). The sugar content in degree brix (^{o}Bx) was estimated directly from the digital refractometer.

2.4.3 Determination of Wort Viscosity

Wort viscosity was determined According to [1] using free ball viscometer and the viscosity was determined by using eqn. 3.

$$Viscosity = \eta = K\,(\rho_1 - \rho_2) \cdot t \quad \text{.....3}$$

K = ball constant mPa·s· [$cm^3/g{\cdot}s$], ρ_1 = density of the bail in [g/cm^3], ρ_2 = density of the liquid to be measured at the measuring temperature in [g/cm^3], t = falling time of the ball in seconds.

2.5 Statistical Analysis

Statistical analysis of selected quality parameters were analyzed by using Design expert software.

2.6 Experimental Design, Treatment and Layout

Table 1.1: Summary of trial experiments for preliminary investigation

Trial Experiments No.	*Barley Type*	*Malt Type*	*Factors which were considered for preliminary investigation at each trial experiments*
TEsA	Food Barley Type	Traditional Malt	Mash time = 3 days No additional factors were considered
TEsB	Food Barley Type (TMS1)	Traditional Malt from TMS1	Temperature = 40, 55 and 70^{o} C Time = 120, 180 and 240 min pH = 4.5, 5.5 and 6.5
TEsC	Food Barley Type (TMS1)	Traditional Malt from TMS1	Temperature = 40, 55 and 70o C Time = 120, 180 and 240 min pH = 4.5, 5.5 and 6.5
TEsD	Two raw Barley (Beer Barley)	Commercial Malt	Temperature = 40, 55 and 70o C Time = 120, 180 and 240 min pH = 4.5, 5.5 and 6.5
TEsE	Two raw Barley (Beer Barley)	Commercial Malt	Temperature = 40, 55 and 70o C Time = 120, 180 and 240 min pH = 4.5, 5.5 and 6.5

3. Results and Discussions

3.1 Performance evaluation of barley from different locations

The preliminary investigation was intended to evaluate the performance of malt enzymes (starch Hydrolyzing enzymes) indirectly by measuring the malt extract and sugar content in the wort. As described in table 1.1, the preliminary investigation contained five trial experiments and the results are discussed below.

Table 3.1 Result for sugar content and extract level for traditional wort from different barley type.

Sub-TE No.	Sample No.	Specific Gravity	Degree Plato (^{o}p)	Sugar content (^{o}Bx)	Viscosity(cp)	Extract Level (%)
STE11	TMS1	1.010±	2.6±	2 ± 0.7	3 ± 0.9	22 ± 0.50
STE12	TMS2	1.008±0.07	2.1±0.21	2 ± 0.2	2.9± 0.12	18 ± 0.16
STE13	TMS3	1.005±0.02	1.3±0.31	1 ± 0.3	2.78±0.87	11 ± 0.25
STE14	TMS4	1.006±0.09	1.5±0.55	1 ± 0.6	2.69±0.97	12 ± 0.98
STE15	TMS5	1.007±0.01	1.8±0.82	1 ± 0.9	3.11±0.22	15 ± 0.57

Table 3.1 presented the % malt extract value and the fermentable sugar level obtained after operating the mashing process at atmospheric conditions. The maximum value of % malt extract and fermentable sugar level were observed to be 22.5 % and 2.7 ^{o}Bx, respectively. Since the wort quality depends on the malting barley type, the quality of selected malting barley type, which is TMS1, was better than the other malting barley type. In addition to that the starch content of the malting barley has a significant effect on the % malt extract during mashing process.

These different values were raised primarily from the poor barley type and unfavorable mashing condition. However, the rate of hydrolysis was slow at all TEsA which results in a lower degree of hydrolysis. This lower degree of hydrolysis may be due to the insufficient energy provided by the temperature, the mash pH and reaction time.

Table 3.2: TEsB Preliminary Result of evaluation of diastatic enzymes activity using TMS1 after considering temperature (^{0}C), pH and time (minute)

Sub-TE No.	Condition	Specific Gravity	Degree Plato (^{o}P)	Degree Brix (^{o}Bx)	Viscosity (cp)	Extract Level (%)
STE21	T = 40 ^{o}C t = 120 min PH = 4.5	1.012±0.05	3.1±0.35	3 ± 0.2	3.30±0.01	26 ± 0.93
STE22	T = 70 ^{o}C t =180 min PH = 5.5	1.017±0.2	4.3±0.29	4 ± 0.5	3.53±0.08	37 ± 0.35
STE23	T = 55 ^{o}C t = 240 min PH = 6.5	1.014±0.09	3.6±0.21	3 ± 0.7	3.42±0.99	31 ± 0.27

Table 3.2 presents results of preliminary investigations of % malt extract and sugar content at temperature, time and pH combination. In TEsB, there were three different parameters considered and studied which were temperature, pH and time. The maximum % malt extract content and the fermentable sugar level were recoreded at 70 ^{o}C, 180 min and pH of 5.5 which is 37.35 % and 4.5 ^{o}Bx, respectively. It was better result comparing with the value which was obtained from the TEsA shown in Table 3.1. The result in Table 3.2 also indicated that the high value of malt extract and fermentable sugar level were recorded at temperature 70 ^{o}C, pH 5.5 and time 180 min.

Table 3.3: TEsC Preliminary Result of evaluation of diastatic enzymes activity using TMS1 after considering temperature (^{0}C), pH time (minute) and S.S.A (gram).

Sub-Trial Experiment No.	Condition	Specific Gravity	Degree Plato	Degree Brix	Viscosity	% Extract
STE31	T= 40 ^{o}C t=120 min. PH = 4.5 S.S.A = 15 gm	1.013±0.11	3.3±0.08	3±0.58	2.97±0.25	28±0.66
STE32	T= 55 ^{o}C					

STE33	t=180 min. PH = 5.5 S.S.A = 22.5 gm	1.018±0.09	4.6±0.02	4±0.85	2.85±0.45	40±0.53
	T= 70 °C t=240 min PH = 6.5 S.S.A = 30 gm	1.015±0.1	3.8±0.06	4±0.71	3.19±0.32	33±0.82

Table 3.3 demonstrates preliminary investigation results of % malt extract and sugar at temperature, pH, time and substrate concentration. The wort quality still needs improvement and an additional factor which is substrate concentration was considered in TEsC. From Table 3.3 at 55 ^{o}C, 180 min, pH of 5.5 and 22.5 gm, the value of % mat extract and fermentable sugar content was recorded 40.5 % and 4.8 ^{o}Bx, respectively, which is greater than the value recorded in TEsB presented in Table 3.2 at 70 ^{o}C,180 min and pH of 5.5. This shows that considering substrate concentration as influencing factor for wort optimization is essential in order to obtain the maximum wort quality for fermentation process The major reason for maximum % malt extract and fermentable sugar level observed at 55 ^{o}C, 180 min, pH of 5.5 and 22.5 gm. was the amount of substrate which was considered as a factor at TEsC.

Table 3.4: TEsD Preliminary Result of evaluation of diastatic enzymes activity for malt from the two raw barley type with considering temperature (^{o}C), PH and time (minute).

Sub-TE No.	Condition	Specific Gravity	Degree plato	Degree Brix(^{o}Bx)	Viscosity (cp)	% Extract
STE41	T = 40 ^{o}C t = 120 min PH = 4.5	1.018 ± 0.2	4.6 ± 0.9	4 ± 0.8	3.1± 0.01	40 ± 0.31
STE42	T = 55 ^{o}C t =180 min PH = 5.5	1.023 ± 0.13	5.8 ± 0.7	6 ± 0.10	2.9 ± 0.05	50 ± 0.82
STE43	T = 70 ^{o}C t = 240 min PH = 6.5	1.020 ± 0.21	5.1± 0.5	5 ± 0.3	3 ± 0.08	44 ± 0.69

The quality of wort is still unsatisfactory, which means that in order to produce a high quality fermentable product, the content of % malt extract and fermentable sugar level should have maximum value. As a result the final two trial experiments (TEsD &TEsE) are proposed to conduct with the new barley type (two raw barley) by keeping temperature, pH, time and substrate concentration constant. As observed from table 3.4 at 55 ^{o}C, 180 min, pH of 5.5, the maximum % malt extract and sugar content was recorded 50.82 % and 6.1 ^{o}Bx for TEsD, respectively. This maximum value was obtained due to the fact that malting barley type used for TEsD was commercial barley type which has a good quality. Wort quality depends on the properties of the barley type used. Barley type differs markedly in their Beta-glucan content in wort which is given by the content of polysaccharides in a barley caryopsis and capacity of the enzymatic apparatus of the caryopsis to degrade Beta- glucan in the course of malting.

Malting barley varieties are usually soft, whereas nonmalting varieties are usually hard [2] also reported significant relationships between hardness of barley grain as assessed using the particle size index and hot water extract of malt as well as the malt quality index of barley malt. Other structural and compositional characteristics of barley endosperm could contribute to grain hardness, including proteins, starch, β-glucan, and their interactions, and packing during grain filling [3]. With the same malt type and considering one additional factor for TEsE, the values of % malt extract and fermentable sugar content were found 70.1 % and 8.4 ^{o}Bx , respectively, as shown in Table 3.5 at 55 ^{o}C, 180 min, pH of 5.5 and 22.5gm.

Table 3.5: TEsE Preliminary Result of evaluation of diastatic enzymes activity for malt from the two row barley type with considering temperature (oC), PH time (minute) and S.S.A. (gram).

Sub-TE No.	Condition	Degree plato(^{o}P)	Degree Brix(^{o}Bx)	Viscosity (cp)	N (mg/l)	% Extract
STE51	T = 40 ^{o}C t = 120 min PH = 4.5 S.S.A. = 15 gm	6 ± 0.3	6 ± 0.6	2 ± 0.97	nd	57 ± 0.57
STE52	T = 55 ^{o}C t =180 min PH = 5.5 S.S.A. = 22.5 gm	8 ± 0.1	8 ± 0.4	2 ± 0.86	nd	70 ± 0.10
STE53	T = 70 ^{o}C t = 240 min PH = 6.5 S.S.A. = 30gm	7 ± 0.8	8. ± 0.1	3 ± 0.19	nd	68 ± 0.39

Conclusions

From the investigation we found that the best wort quality was observed from the final trial experiment (TEsE) at a combination treatment of 55 ^{o}C temperature, 240 min mashing time, pH of 6.5 and substrate source added 30 gm by using the commercial barley variety. The qualirt of wort is basically the most significant factor for the production of fermented beer and the distilled alcoholic beverage. There for we can take the optimized value of the factors and conduct fermentation experiment to produce the beer having maximum % alcohol by volume.

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