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Optimization of Local Wort and Fermented Beer from Barley as Substitute Raw Material for Ethanol Production Using Response Surface Methodology

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ABSTRACT

The research work was aimed to find the exact optimized operating temperature, time, pH and substrate which is important for the production of wort and fermented beer in both mashing and fermentation processes. Here, the barley was used as a basic source of substrate and enzymes. To determine the optimum operating temperature, pH, time and substrate under mashing and fermentation processes, Central Composite Experimental Design (CCD) was used. The results showed that, the maximum % malt extracts (92.36 %), fermentable sugar (10.53 oBx) were observed at 70oC, 120 min., pH value of 4.5 and 30 gm substrate source addition. After optimizing the wort, the fermentation experiment was conducted accordingly the combination which was given by design expert software. The maximum degree of attenuation value (86 %) was observed at 20oC, 96 hr, and pH value of 4.4 and 75 gm substrate source added. Therefore, good barley type and optimum condition for mashing and fermentation process were found to be significant effect for high wort, and distillery beer.

Keywords: Mashing, Attenuation, Fermentable sugar, Optimization, Wort

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Introduction

The distillery mashing process involves the conversion of milled malted barley into a fermentable extract or wort [1]. The mashing temperature, PH and Mash time are extremely important in the enzymatic degradation of the milled malted barley and is essential to attain the best wort composition[2]. Heat labile enzymes can be quickly denatured if the temperature is too high, **[2]** and at lower temperatures starch gelatinization may not be fully achieved. Although the mashing process is a simple and straight- forward procedure, it includes a complicated system of chemical and biochemical reactions [8] Starch granules become hydrated and soluble in the mash liquor and are made available to the malted barley enzymes. Any insoluble polymers such as cell-wall glucans still associated with the insoluble milled malted barley particles may be attacked by enzymes during the mashing process, thus heterogeneous mixture of enzymes and substrate molecules are found within the wort. These may contribute to the yield of soluble sugars available to the fermentation.[2]

Barley is used for a wide range of traditional [9] and novel end uses [10]. Barley is fed to animals as a significant part of the diet of cattle, pigs, and poultry. Malting barley varieties are usually soft, whereas non-malting varieties are hard. According [11] report, 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.

A large number of parameters have been proposed to define malting quality, it is also a fact that the texture of the endosperm influences the malt modification process by affecting water uptake and consequently enzyme synthesis and movement within the endosperm [12, 13] studied the variation and correlation between chemical and physical characteristics of barley samples including kernel hardness, but found only a low correlation between kernel hardness and physical and chemical grain properties.

Malting is a controlled germination process consisting of steeping or hydration of grains (to ensure good absorption of water by the grain from 12% to at least 45% of moisture)[14]. Fundamentally, the aim of malting is to unmask starch granules from the surrounding cell walls and protein matrix so that fermentable sugars can be optimally released from starch during the mashing process [14]. Due to this Enzymes are synthesized or activated in the aleurone and scutellar cells by the action of embryonic gibberellin activated signal transduction pathways and are secreted into the starchy endosperm [15, 16]. Thus, the main objective of the research work was to determine the optimum local wort and fermented beer from barley as substitute raw material for ethanol production.

Materials and Methods

Raw Materials

The food barely type was purchased from Gayent wereda south Gonder and packed through plastic bag and transported to Bahirdar institute of Technology, Bahirdar University (BiT-BDU).The purchased barely used as source of malt with 10.4 % moisture content, 11.4 % protein content, 96 % germination capacity and energy, thousand kernels weight, and 2.5mm sieve size.

Supplementary Substrate Preparation/Adjuncts

For this research, corn grain was selected as source of supplementary substrate. The raw corn was collected from the local market and cleaned in order to remove metal, dirt, cobs, etc. This corn grain sample was prepared by pre-cooking the corn sample until changed its color to slightly dark. Then, the cooked corn ground to a diameter of 0.5 mm particle size. The ground meal was used as a source of additional substrate source on both mashing and fermentation processes.

Preparation of kilned malt

Two raw barley samples of 5 kg (2.5 mm Size)

were malted at (Faculty of Chemical and Food Engineering (FCFE) Research Grade Laboratory, Bit-BDU. The malt was produced by applying the pure traditional malting procedure:-

First, the malting procedure consists of steeping of the raw barley at room temperature (an average atmospheric temperature was 20.5 ^{o}C) for 2.5 days to achieve an out-of- steep moisture of at least 42-45% and the moisture content was calculated at each steeping day (at day1, day 2 and day 2.5).

Second, the germination phase was lasted for 5 days at room temperature (an average atmospheric temperature was 20.5 ^{o}C) in a flow of fully humidified air.

Third step of malting process was kilning, it was conducted by exposing the sample to sunlight for five days to produce malt around 4% moisture.

Milling condition and Sieving analysis

All the malt samples were milled using disk mill. The desired particle size for this study was 0.2 mm sieve size. Sieving analysis of the milled malt was performed in order to achieve the desired particle size distribution. The sieve sizes used were 0.2mm, 0.5mm, 0.75mm, 1mm, 1.5mm and 2 mm. All the required samples of milled malt were sieved to have 0.2 mm size.

Experimental Design

This research was carried out to optimize dependent process variables in two steps of experiments i.e at mashing and fermentation unit operations. Response Surface Methodology/central composite design (CCD) was used to identify the relationship between the response functions and the process variables, as well as to determine those conditions that optimize the malt enzymes activity by measuring the response variables from the two step experiments. The research has four factors for each step experiment, then the experiment was designed according to; 2^4 factorial points, 2*4 axial points and 6 center points. So the RSM/CCD contain 16 factorial points, 8 axial points and 6 center points, totally 30 number of runs for each step experiment. The independent variables or factors studied for the first step experiment were temperature (X_1: 40-70 ^{o}C), pH (X_2: 4.5-6.5), residence time (X_3:120 -240 min), and supplementary substrate added (X_4:15-30 gm) and for the second step experiment were temperature (X''_1:20-40 oC),pH (X''_2: 4.0-4.8), substrate concentration (X''_3:50-100 gm) and residence time (X''_4:48-96 hour). The response variables from the first step experiments were: % extract content (Y_1),and dissolved sugar content (Y_2). The second step experiment has two responses which are percent alcohol by volume (Y''_1) and Apparent Attenuation Limit (AAL).

Malt Analysis

Malt samples were analyzed according to [7] in Gonder Malt Factory (Gonder, Ethiopia).Sieve test, moisture content, fine-coarse extract, friability, color, PH, thousand kernels, Glassiness (wholly unmodified grain), partly unmodified grain, odor, appearance, and wort viscosity was analyzed.

Mashing condition

Wort was produced in a small-scale by temperature type mashing. The mash temperature, pH, mash time and amount of supplementary substrate conditions were taken according to the design expert version 7 output data in the experimental design section 2.4. After conducting each combination of experiment the mash products were filtered with filter paper and the spent grain was separated from the supernatant. The following Filtrate wort properties such as: % extract, soluble protein, sugar content or fermentability, wort viscosity were analyzed according to [7].

Determination of % Extract Level

The extract level of the samples was determined 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. They the percentage of malt extract present in the filtrates was calculated [7].

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 as shown in fig.2.1 (b), 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 as shown in Figure 2.1(a).

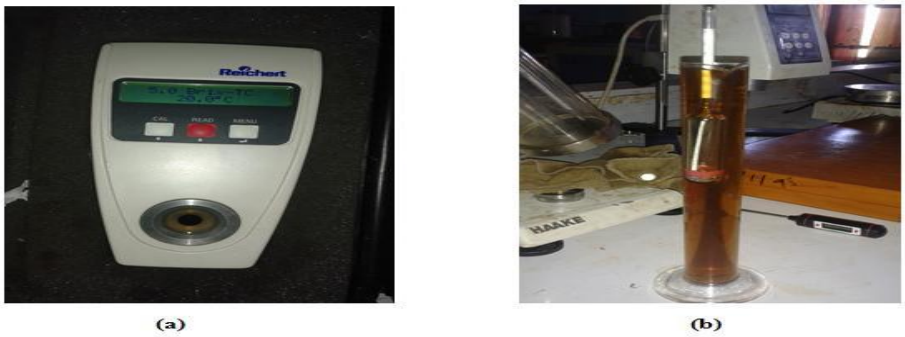

Figure 2.1: (a) Sugar content determination in ^{o}Bx by Refractometer, (b) Sugar content determination from SG in ^{o}P

Determination of Wort Viscosity

Wort viscosity was determined According to [7] using free ball viscometer as described in section 3.8.3 and the viscosity was determined.

Distillery Fermentation Condition

The fermentation process was conducted by taking the wort samples with high % malt extract and sugar content from the mashing condition. The controlling factors were according to the design expert software output data which was described in section 2.4.

Determination of Apparent Attenuation Limit (AAL)

Fermentability /Apparent Attenuation Limit (AAL) is the malt, wort and beer evaluating parameter that describe the ability of yeast to turn sweet (sugar) in wort into alcohol. It is also used to measure the performance of starch converting enzymes (degree of hydrolyzing) and the amount of sugar level present in the wort.

The density/specific gravity of wort and beer samples was determined by Anton paar DMA 4500M density meter. Samples was taken from the clear wort and injected to the density meter. This experiment was used for the samples after fermentation (determination of density for sample after fermentation) and the data/values were used to determine the degree of attenuation.

Calculating attenuation: Attenuation refers to the percentage of original extract (the level of fermentable sugar) found in the wort sample that has been converted by the fermentation process. It can be calculated as:

$$Attenuation = \frac{(OG-FG)}{OG-1.0} * 100\ \%. \quad (1)$$

Where: OG is the Original Gravity of the wort, FG is Final Gravity of the beer/ sample after fermentation process

Data Analysis

RSM is a tool, which was applied to designed the mathematical relationship to link the controllable parameters to the experimental responses so as to explore the effect of parameters on responses. In the thesis work, Design Expert software, Response Surface Methodology (RSM) and central composite design (CCD) was used to analyze the data.

Result and Discussion

Effect of Mashing Parameters on Wort Quality

The discussion part was based on interaction effect of factors on the responses of mashing experiment using 3-D plot of response surface graphical representation. The second one, which was fermentation results, discussed by 3-D plot response surface graphical representation, and also based on the combined effect of factors (temperature, pH, time and amount of substrate source added) on the responses of fermentation experiment.

Table 3.1: Main Experimental Result for mashing process (on wort Optimization)

Run No.	*Factors*				*Responses*			
	Temperature (^oC)	Time (min.)	pH	S.S.A. (gm)	% Extract (%)	Sugar Content (^oBx	Protein content (g/l)	Viscosity (cp)
1	70.00	240.00	5.50	22.50	68.35	8.1	0.794	4.1
2	55.00	240.00	6.50	15.00	59.76	7.1	0.879	2.7
3	70.00	180.00	5.50	22.50	66.25	7.9	1.734	3.9
4	55.00	120.00	4.50	15.00	56.08	6.4	1.65	2.9
5	70.00	180.00	6.50	30.00	76.93	8.84	2.188	3
6	55.00	180.00	6.50	15.00	68.35	7.86	1.144	2.4
7	40.00	240.00	4.50	30.00	83.33	9.57	1.988	1.3
8	40.00	240.00	6.50	15.00	76.93	8.84	1.063	1.9
9	55.00	240.00	6.50	22.50	72.64	8.35	1.64	2.58
10	70.00	120.00	5.50	22.50	81.23	9.32	1.406	3.2
11	55.00	180.00	4.50	30.00	83.33	9.57	1.216	2.2
12	70.00	240.00	6.50	15.00	61.95	7.12	0.881	3.8
13	40.00	120.00	4.50	30.00	66.24	7.61	2.138	1.5
14	55.00	120.00	6.50	30.00	76.79	8.84	0.653	2.1
15	40.00	180.00	5.50	22.50	74.83	8.6	1.766	1.9
16	70.00	240.00	4.50	30.00	85.44	9.8	2.719	4
17	40.00	240.00	4.50	15.00	76.79	8.84	1.263	1.3
18	70.00	120.00	4.50	30.00	92.36	10.53	1.734	4.1
19	40.00	240.00	6.50	30.00	89.73	10.30	0.891	2.3
20	40.00	120.00	6.50	15.00	44.51	5.12	0.969	2
21	55.00	180.00	5.50	22.50	71.1	8.4	1.066	3.18
22	40.00	120.00	6.50	30.00	61.95	7.12	0.847	1.5
23	55.00	180.00	5.50	15.00	59.76	6.87	2.578	2.65
24	55.00	240.00	5.50	30.00	79.03	8.84	1.016	3.05
25	55.00	180.00	4.50	22.50	78.86	9.08	1.606	2.5
26	40.00	120.00	4.50	15.00	57.57	6.62	1.175	1.8
27	55.00	120.00	5.50	22.50	68.35	7.86	1.9531	2.63
28	70.00	120.00	6.50	15.00	66.24	7.61	1.61	3.4
29	70.00	240.00	4.50	15.00	76.93	8.84	1.053	3.7
30	40.00	180.00	6.50	15.00	66.24	7.61	0.628	2.15

Table 3.1 presents the main experimental result for mashing process. In this table run number 7 and 11 were conducted at different time and temperature combination, and there were give similar amount % malt extract of 83.33 % and sugar content of 9.57oBx, but different values for protein and viscosity.

This is due to diastatic and proteyoltic enzymes are work optimally at different conditions.

Diastatic enzymes are used to break down / hydrolysis starch material into simple sugar at a temperature range of 45 -70 oC .This means that diastatic enzymes are not functional below or above the given range. Similarly proteyoltic enzymes are used to breakdown proteins and works optimally below temperature of 40 oC.

It was observed (Figure 3.1 (a)) that, the enzyme exhibited its maximum activity at 120 min of reaction time. [8] Reported that enzyme obtained from Bacillus sp. AB68 was active in a broad temperature range between 20 and 90°C, with an optimum of 50°C. But in case of malt enzymes the optimum temperature observed was 70°C. This higher temperature indicated the thermos-stability of malt enzymes and working at greater than 50°C without deactivating. As shown in figure 3.1 (a) after 120 min the activity of enzymes was decreased drastically and enzyme was completely inactivated when heated further of 70°C. Thus, the results concluded that the malt enzyme is moderately temperature stable. The % malt extract is one of the most important quality parameter. It measures the soluble materials from the malt when hydrolysis enzymes in the malt acted optimally.

A pH ranges from 4.5-6.5 was used to study the effect of pH on diastatic enzyme activity (Figure 3.1 (b)) and optimum pH was found at 4.5.

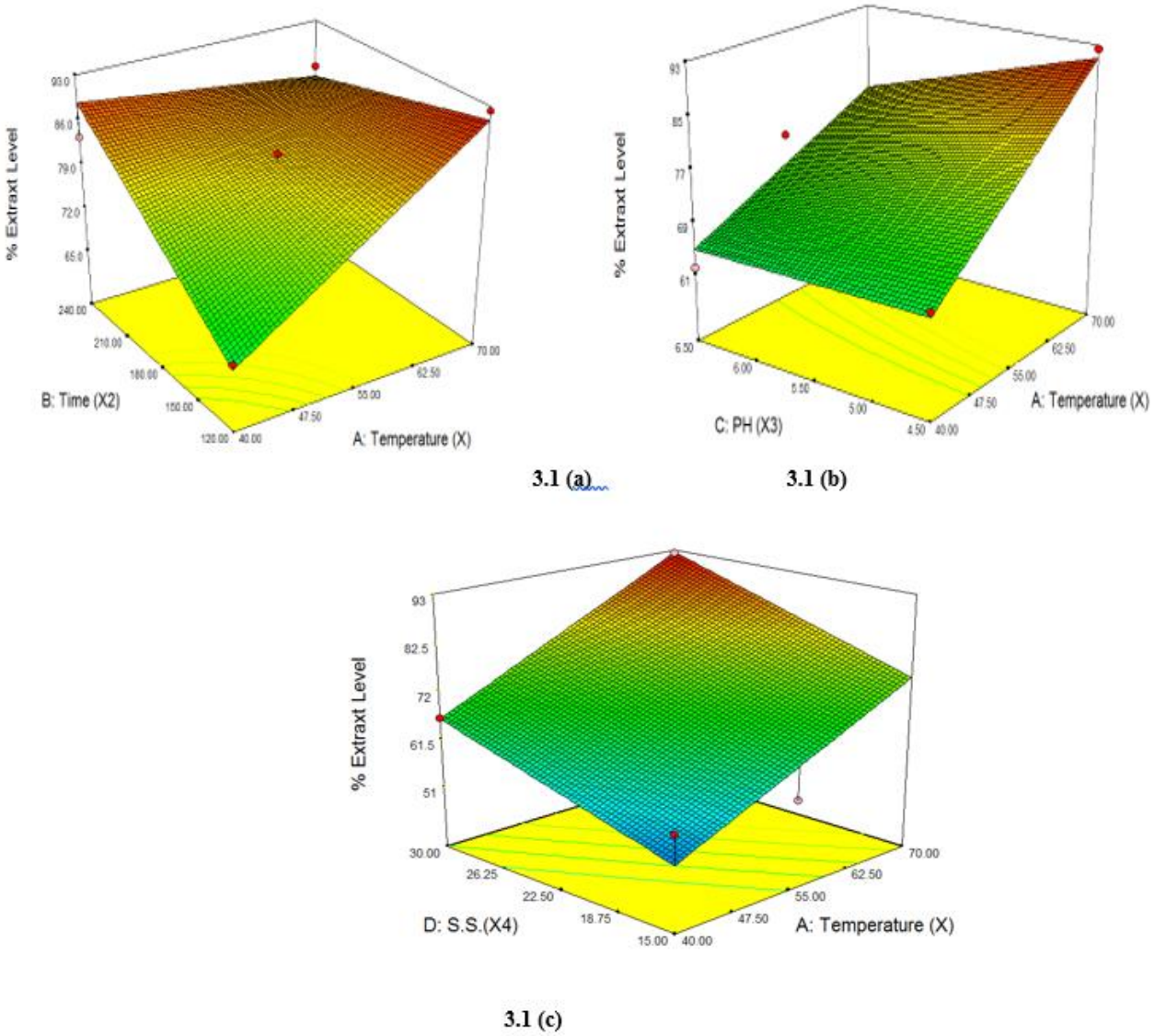

Figure 3.1 (a-c): 3-D % malt extract graphs for two varying parameters

Higher enzyme activity was observed in the acidic pH range, but when the pH range proceeds to alkaline, the activity was lower. The effect of temperature on enzyme activity was assayed at different temperatures ranging from 40-70°Cat optimum pH, time and S.S.C. The results showed that enzyme activity was increased with temperature and it showed highest enzymes activity at temperature 70°C (Figure 3.1 (a & b)). At this temperature the highest % malt extract value of 92.36 % was observed. Above 70°C temperature enzyme activity was also decreased. For determination of optimum reaction time, enzyme assay was carried out at different reaction time ranging from 120 –240 minutes at constant temperature pH and S.S.C. The relation between temperature with respect to substrate source is presented in figure 3.1 (c). The maximum response value is observed at maximal of temperature and maximal of S.S.A. At maximal of the temperature with pH and temperature with S.S.A. interaction parameters, we can observe that % malt extract was minimum. This is due to, during enzyme substrate reaction, the initial reaction velocity gradually increases with increasing both substrate concentration and temperature. Increasing temperature increases the kenetic energy that molecules possess.

Fig. 3.2 (a) describe the combined effect of temperature and time on the fermentable sugar content, with pH and S.S.C. held constant at its lower and upper value. The color key on the green, yellow and red surface becomes “hot" at higher response levels, this means that when the interaction factors (temperature and time) increase the sugar content also increase to a higher value and the maximum fermentable sugar was observed at the right edge of x-axis with the value 10.53.

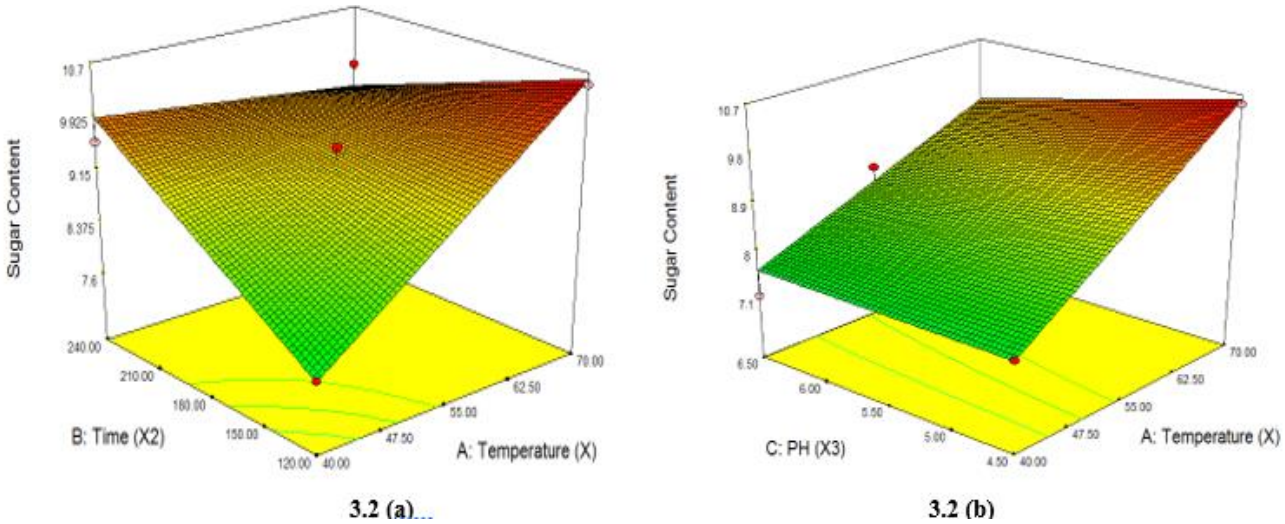

Figure 3.2 (a-b): 3-D plot of sugar content graphs for two varying parameters

This is due to the starch hydrolyzing enzymes was worked at the optimum value of 70 ^{O}C and time of 120 min. Fig.3.2 (b) shows the interaction effect of temperature and pH keeping the actual factors constant at (pH, 4.5 and S.S.A., 30 gm). In this case this fig. presented a plot of sugar content as a function of temperature and pH. As clearly shown the fermentable sugar content was increase as increasing the red color key. It showed that the maximum value was recorded at 70 ^{O}C, and 4.5, temperature and pH respectively. In the other hand the sugar content was decreased as the pH increased, but is observed increase as temperature increase.

3.2 Effect of Fermentation Parameters on distilled beverage quality

Since the research work is originated from the Ethiopian indigenous ethanol ("Areki") production

Technology, the fermentation process was done without the presence of any external bio-catalyst (Yeast strain) as shown in table 3.2. In order to optimize the distillery beer in the fermentation process, the optimized wort (i.e wort with maximum % extract and fermentable sugar content) was taken from the previous mashing experiment.

Table 3.2: Result of degree of attenuation for distillery beer in fermentation process

Run	*Temperatur (^oC)*	*eTime (Hr)*	*PH*	*S.S.S (gm)*	*OG*	*FG*	*AAL (%)*
1	40.00	96.00	4.00	75	1.0546	1.028	49
2	30.00	96.00	4.40	75	1.055	1.0175	68
3	40.00	72.00	4.40	75	1.0557	1.0218	60
4	20.00	96.00	4.80	75	1.055	1.008	86
5	30.00	96.00	4.40	50	1.0549	1.0185	66
6	30.00	72.00	4.40	75	1.055	1.016	71
7	20.00	48.00	4.80	100	1.054	1.016	70
8	30.00	72.00	3.60	50	1.055	1.0178	67
9	40.00	96.00	4.00	100	1.055	1.025	54
10	20.00	48.00	4.80	75	1.0548	1.015	73
11	20.00	48.00	4.00	50	1..055	1.0155	72
12	30.00	72.00	4.40	100	1.0551	1.0186	66
13	40.00	72.00	4.80	100	1.055	1.0258	52
14	20.00	72.00	4.00	100	1.0545	1.014	74
15	30.00	48.00	4.40	50	1.055	1.021	62
16	40.00	48.00	4.80	50	1.0551	1.0268	51
17	20.00	72.00	4.00	50	1.055	1.013	76
18	30.00	48.00	5.20	100	1.055	1.020	64
19	40.00	48.00	4.80	100	1.055	1.0248	55
20	20.00	72.00	4.80	75	1.0548	1.012	78
21	40.00	96.00	4.80	50	1.0549	1.026	53
22	30.00	96.00	4.40	100	1.0556	1.0195	64
23	30.00	48.00	4.40	75	1.0548	1.0175	68
24	40.00	72.00	4.40	75	1.0551	1.022	60
25	20.00	96.00	4.00	100	1.055	1.0115	79
26	40.00	48.00	4.00	50	1.055	1.024	56
27	20.00	96.00	4.40	50	1.0545	1.010	82
28	20.00	96.00	4.40	75	1.055	1.0085	85
29	40.00	72.00	4.00	50	1.0551	1.0245	56
30	30.00	48.00	4.40	75	1.055	1.018	67

With Saccharomyces it has long been known that the rate of alcohol production increases with temperature up to 40 ^oC, [3]**.** [6] and [4] showed an increase in ethyl acetate production with increases in incubation temperature. There are many reports that the syntheses of enzymes are affected by the growth temperature [5]**.**

The optimized mash bill from the mashing experiment was used as fermentation feed in order to optimize the distillery beer by controlling four factors as described in section 2.5. The

interaction and single effect of controlling variables on degree of attenuation is presented in fig. 3.2 (a-c).

The maximal AAL, which is 86 % was observed at minimum fermentation temperature and maximum fermentation time. The metabolic activity of the unspecific yeast strain which, is used as a catalyst for the fermentation process was very high at 20 ^{o}C, and lower at higher temperature of 40 ^{o}C. This result also indicated that the unknown yeast strain was breakdown/convert much of the fermentable sugar to alcohol and carbon dioxide at 20 ^{o}C.

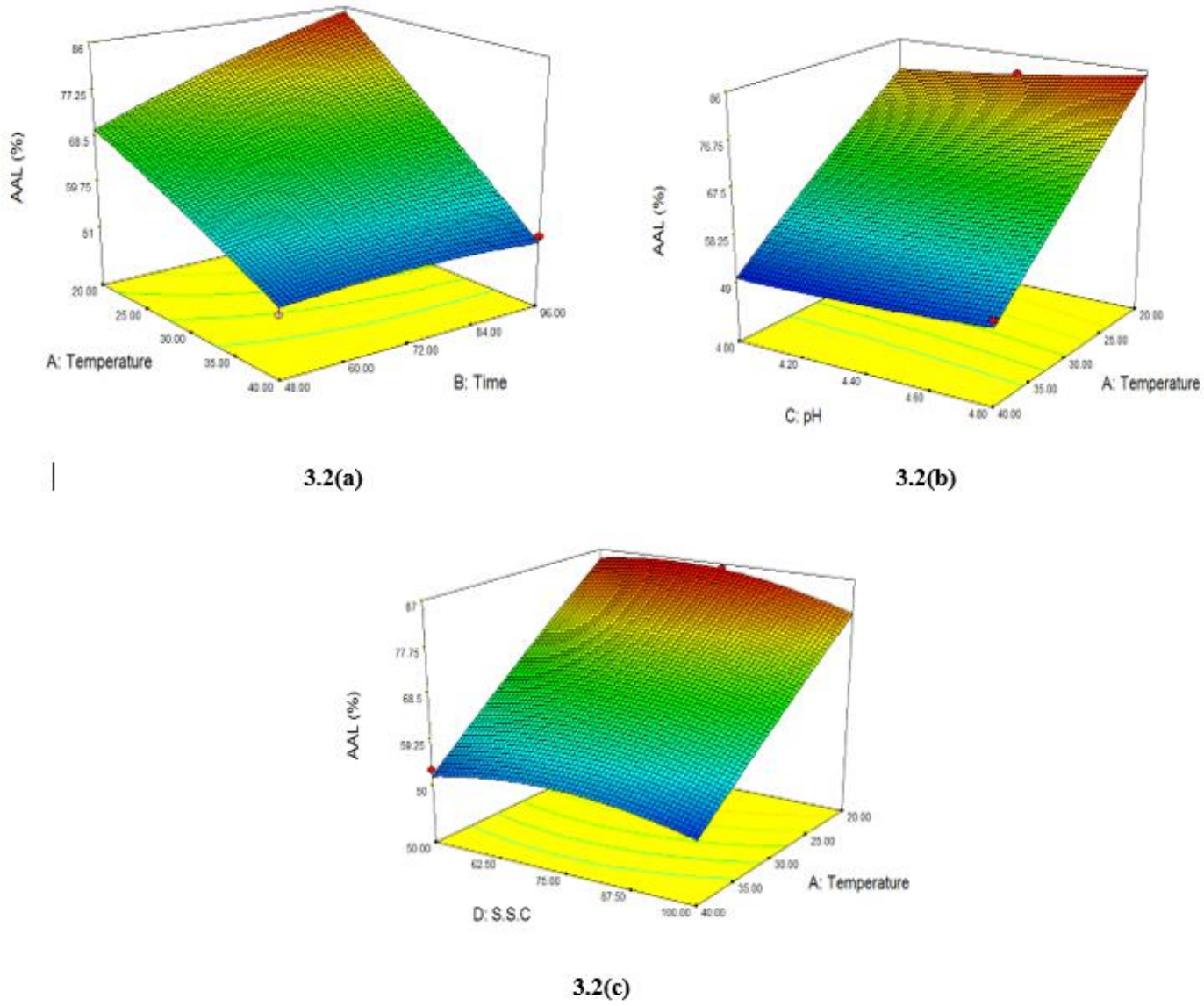

Figure 3.2 (a-c):3-D plot of effect of time, temperature, pH and S.S.A. on AAL (%)

This indicated that the rate of fermentable sugar consumption by unspecified yeast and the production of ethanol (EtOH) and carbon dioxide (CO_2) increased as the fermentation temperature decreased and fermentation time increased. As indicated by the color key in figs. 3.2(a), the surface becomes **‘’**hot**"** at higher response levels, yellow in the "70"s and red above 80 for AAL. The effect of single factor (temperature and time) can also discussed from fig.3.2 (a). The degree of attenuation (AAL) was reducing as increase of fermentation temperature and increase as the fermentation time increase. This indicated that the

fermentation yeast strain (unspecified) was more active at minimal temperature and maximal time. The indigenous ethanol ("areki") production technology is operating at atmospheric (average 23.5 ^{o}C) temperature and at maximum fermentation time (four day). Fig. 3.2(b) described the impact of temperature and pH on fermentability. The interaction effect of temperature and pH on degree of attenuation is similar with the effect of temperature and time which is presented in this figure. As observed in this figure 3.2(b) the AAL become increases as the pH increase and the temperature decreases. The third interaction which is presented in fig. 3.2 (c) is the effect temperature and SSC on AAL. The fermentability recorded at the interaction effect of temperature and SSC was increased as both temperature and SSC decreased by keeping the other factors at stationary. The maximal AAL value, which is 86 %, was observed at minimal temperature, 20 ^{o}C and SSC, 50 gm. The temperature still has negative impact on fermentability, this means that when the temperature increasing the degree of attenuation decreases. This is due to the high temperatures can also leads to excessive levels of diacetyl.

4. Conclusion

The research was intended to optimize the processing conditions (dependent variables or factors that affect the quality of wort and fermented beer at mashing and fermentation processes) by conducting experimental investigations. And as we observed from the experimental work, the maximum wort quality was achieved at 70 ^{o}C mashing temperature, 120 min mashing time, 4.5 mashing pH and 30 gm supplementary substrate addition. Similarly the better fermented beer was obtained at the condition of 20 ^{o}C fermentation temperature, 4.4 pH, 96 hour fermentation time and 75 gm substrate concentration. We finally conclude that we can apply the above optimum conditions to produce quality wort and fermented beer from mashing and fermentation processes respectively.

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Conflict interest

There is no conflict interest among the authors

Data Availability statement

No data used to support the findings of this study.

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