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EFFECTS OF *RHAMNUS PRINIODES* FOR BEER BITTERNESS AT DIFFERENT PROCESS CONDITIONS

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ABSTRACT

The aim of this study was to evaluate effects of Gesho (Rhamnus priniodes) solvent extract and herkule bitterness power at different boiling conditions for production of beer in order to find possible sources for future novel bittering agent in brewing industries. In this study, different hop ratios were controlled as constant during each brewing process. The influence of process variables on bitterness of beer was analyzed with a 2^3 Full-Factorial experiment with the aid of design expert 7.0.0 software package has been employed. The study showed the presence of bittering agent in large amounts in R. priniodes crude extract. The results revealed that R. priniodes fraction up to fifty percent exhibited the highest bittering value (24.02±0.295Bu) at P^H =5.4, temp. =80°C, and time=45min. and good beer quality as compared to the other fractions and herkule. The various crude extracts and fractions of R. priniodes significantly had bittering power and reduced the fractions of herkule. Leaf of R. priniodes can be used as source of natural bittering agent and a potential alternative raw material for brewing industries.

KEYWORDS: *Bittering agent, Gesho, herkule, Rhamnus priniodes*

I. INTRODUCTION

Total world hop acreage is about 80 thousand hectares and the Czech Republic, with 6 thousand hectares, is one of the most important world exports. Many varieties of hops are used in the world and each hop variety has a characteristic flavour profile, which is used to distinguish them (Krajl et al., 1991; Peacock & McCarty, 1992). In brewery it is inflorescences of the female plant which are used. These contain the bitter resins and ethereal oils which supply bittering and aroma components of the beer. As far as brewing is concerned, hops are dried hop cone of female hop plant and products made from them which contains only components from hops. Hops are grown in the special growing regions where the necessary conditions are exists (W. Kunze*, 2004*). The largest producers of hops are Germany, the United States, China, and the Czech Republic. Other important growing regions include England, New Zealand, and increasingly Argentina. Climate and soil conditions have a major effect on hops. Varieties developed in one region will have different flavor and aroma profiles when grown in another (Kunze, W. 1996).

Hops were not used in most ancient beer production; instead, spices and other plants were used to flavor the beer. In1079, Saint Hildegard of Germany noted the anti spoilage properties of hops, and brewers began to take note (Bamforth, C.W. 2006). However, hops were not common in beer until the early 1800s. Hops work well as a preserving agent in the brewing process because they eliminate undesirable malt proteins, aid clarification, and stabilize beer flavors. An added benefit is their ease of cultivation, and ability to impart characteristic flavor and aroma (Papazain, C. 2003).

Brewers use hops primarily to get bitterness, flavor, and aroma. Hops can be added at several points in the brewing process to enhance one or the other of those things. Two components of hop composition are essential to beer production, the essential oils and resins. The oil portion contributes to the aroma characteristic of the final beer (Bamforth, C.W. 2006). Hop resins contains alpha acids that contribute to bitterness. The level of alpha acid is unique to each variety of hops. Alpha acids are also referred to as humulones and indicate the bitterness imparted to the beer. Hops are added to the beer during the boiling of the wort. This is necessary to promote the isomerization reaction that renders the alpha and beta acid resins water soluble, as humulinic acid and isohexenoic acid. Once water soluble, these compounds are released into the sweet wort where bitterness is imparted to the wort (Papazain, C. 2003).

Increased boil time increases the bitterness imparted to the final beer. The aroma that hops provide to beer is produced by essential oils, which account for 1%-2% of the total dry weight of the cone. These essential oils are volatile and easily lost during the boil .Therefore; hops are added at scheduled intervals during the boil to produce the desired flavor and aroma in the final beer. Hops are evaluated in two ways, first, by alpha acids in Alpha Acid Units (AAUs), which is the weight of hops (in ounces), multiplied by the percentage of alpha acids, determined by chemical extraction, and second, by International Bittering Units (IBUs). The IBUs estimates how much of the alpha acid is isomerized and dissolved into the beer. The IBUs are calculated from the AAUs, the volume of the boil (V), and utilization (U). Utilization takes into account the time and gravity of the boil to describe the efficiency of the isomerization reaction (Palmer, J.J. 2006).

There is a new breed variety developed in Hulls Germany, Herkules, with high alpha acid content of hop With a yield of about 2 300 kg/ha and a good resistance to diseases Herkules shows good agronomical attributes. The new variety has an alpha content of 12 -17 % and a high cohumulone amount of 32- 38 % of alpha acids .In the past the sensory impact of high cohumulone contents on beer taste and quality of bitterness was discussed with opposed opinions(Kusche, Stettner et al. 2007).

The composition of the hops is extremely important for the quality of the beer produced from them. The main composition of dried hop is: Bitter substances (Hop resins), Hop oils, Polyphenols (tannins), Protein and Inorganic. The rest consists of cellulose and other materials which are not importance in beer Production(Kunze, W. 1996).

Rhamnus prinoides L. herit. Amharic name: Gesho, family Rhamnaccea, is a plant which grows up to 6 meter. It is known to occur outside Ethiopia in Cameroon, Sudan, throughout East Africa to South Africa and Angola and also in Arabian (Thulin, M; 1988). It is not yet clear the exact role of Gesho in tella and tej production however Shale and Gashe (1991) have speculated that the role of Gesho in tella should be similar to that hops in beer. Besides its role to suppress bacteria during the fermentation process, Gesho is certainly the main agent that imparts the desirable bitter taste in to tella. It has also received positive assessment as a commercial hopping agent for beer (Alemayehu D .T, 1994). Recently naphthalenic glycoside named Geshoidin has been identified as the substance responsible for the bitter attributes of the plant (Abegaz, B. and Kebede, T. 1995). Hop is one of the ingredients used for bittering, flavoring and aroma imparting agent for beer brewing. In Ethiopia, Gesho (R. prinoides) is particularly used to provide a special aroma and flavor (Shale and Gashe, 1991; Berhanu Abegaz et al., 1999).

Organoleptically, the most important substance is the naphthalenic glucoside, Geshoidin, which is responsible for the characteristic bitter, favour of the beverages derived from this plant (Abegaz et al, 1999). Although Gesho may have antibacterial effect against some groups of bacteria, its main purpose in the process is believed to impart the typical bitter taste to tella (Ashenafi, M. 2006).

Bitterness from hops comes from alpha acids found in the lupulin glands of the hop flowers. The main alpha acids are humulone and cohumulone and adhumulone. In order to become bitter these acids must be chemically altered, isomerized by boiling. Whereas hop flavor and aroma are derived from essential oils found in the lupulin glands. These oils include humulone, myrcene, geraniol, and limonene, among others. The flavors are released as these oils become dissolved into the wort during the boil. However, these oils are highly volatile and are to a large degree lost to evaporation. For this reason flavor hops are added with twenty to forty minutes remaining in the boil. This provides a compromise between isomerization of the alpha acids and loss of essential oils.

Therefore, the rationale of this research work is to investigate the potential of Gesho (R.priniodes) resource which could be a good source of bittering agent in Ethiopia in beer brewing sector.

II. MATERIALS AND METHODS

A) Sampling method and Sample preparation

The cooled wort sample before pitching was taken as representative, aspectic sampling is recommended to prevent contamination and Store at refrigerator at 5-10^{o}c until use. A sample must be degassed without loss of foam by adding 2-4 drops of octanol into the bottle and pouring 5-20 times from one beaker to another or use maginetic stirrer at least for five minutes. If Yeast contained in beer it must be clarified by centrifuging for 15minutes at 3000rpm.

B) EXPERIMENTAL PROCEDURES

i) Procedure for optical density

Ten milliliters of degassed test sample was pipetted into conical flask with glass stopper which was acidified with 1ml of 3N HCl and was filled with 20ml of isooctane (2,2,4-trimethylpentane), Stopper the flask tightly and place it in mechanical shaker ,Shake vigorously for 15min. at room temperature and then allow decanting for 10 minute until the supernatant organic phase will create. Then the absorbance of isooctane at 275nm in a1.ocm quarz cell or cuvette was measured using Isooctane from the batch as the blank. The optical density is expressed in terms of European brewing convention bitterness units as follows:

Bitterness (Bu) = 50*ABS@275nm

Where, Bu= Bitterness unit

ABS= Absorbance

ii) Determination of polyphenol in a beer

A sample was degassed 5times and ten mililiters of degassed test sample was pipetted into volumetric flask (25ml) with glass stopper which was basified with 0.5ml of 25% ammonium solution, 0.5% ferric reagent and 8ml of CMC/EDTA were added to the solution. The blank was run in the parallel except 0.5% ferric reagent to determine polyphenol amount in the beer. Then the reaction allowed for 10min and measures the absorbance of the blank at 600nm in a1.ocm quarz cell or cuvette using sample except ferric as a blank. The optical density is expressed in terms of European brewing convention bitterness units as follows.

Polyphenol (pphl) = 120*ABS@600nm

iii) Determination of VDK

One hundred milliliters of beer was added into distillation flask and start distillation. Control the heating rate carefully to prevent over foaming and the available period under gentle heating should be at least 6min until the first drop is collected into the receiving cylinder. Then 25ml of the distillate was collected within 8-10min and mixed thoroughly, 10ml of the distillate was pipette into 50ml flask with glass stopper and then 0.5ml O-phenylediamine was added with mixing and the flask was placed for 20-30minutes in the darkness. After adding 2ml of 4N HCl, the sample was mixed thoroughly and measured within 20min against the blank. Finally, the absorbance of the blank at 275nm in a1.ocm quarz cell or cuvette was measured using isooctane as a blank. The optical density is expressed in terms of European brewing convention ppm.

VDK, ppm= 2.7*ABS335.

Figure 1: Bitterness shaker.

C) Experimental Design and Statistical Analysis

Experimental design selected for this study was a 2^3 Full-Factorial analysis with two level of treatment for comparative experiment and the main response was beer bitterness gained. The experiment was analyzed by the Design Expert software 10.0.4.0 and the study conducted to develop and evaluate *Rhamnus priniodes* in beer production by varying ratios with hops.

It was also used to identify the relationship existing between the dependent responses (beer bitterness) and independent process variables as in the brewing process. The three independent variables or factors studied were: brewing temperature (80^{o}c and 90 ^{o}c), time (45min and 90 min), P^{H} (5.2 and 5.4) for actual variable levels. For each factor, an experimental range was adjusted based on the results of literature data and on the performance of preliminary experimental trials. These three factors: temperature, P^{H} and time were selected as independent variables, because of their influence on brewing process. In this study, hop ratio was controlled as constant during each brewing process. Therefore, in this work, the influence of process variables on bitterness of beer was analyzed with a 2^3 Full-Factorial experiment. And to do these, design expert 10.0.4.0 software package has been employed.

III. RESULTS AND DISCUSSION

a) Effects of boiling conditions on *R. priniodes* and Herkule Beer bitterness

The effect of PH, boiling temperature and time on beer bitter taste value was studied and evaluated for good value of beer bitterness for production of beer in the pilot plant scale. The experimental values of different ratio of local Gesho (*R. priniodes)* to imported hops (Herkule) beer bitterness at different boiling conditions are presented in Table 1.

Table 1: Experimental results for 100%, 50:50%, 25:75 and 75:25% *R. priniodes* and Herkule

Run	Factors			Beer bitterness [Bu]				
	Temp [^{0}C]	PH [PH]	Time [Min]	100% Herkule	100% *R. Priniodes*	50:50% *R. Priniodes* to Herkule	75:25% *R. Priniodes* to Herkule	25:75% *R. Priniodes* to Herkule
1	**90**	**5.2**	**45**	18.073±0.015	18.997±0.026	22.57±0.105	22.07±0.075	22.48±0.485
2	**80**	**5.4**	**45**	18.31±0.040	16.85±0.130	24.02±0.295	21.68±0.315	24.47±0.515
3	**80**	**5.2**	**90**	21.64±0.320	17.73± 0.095	22.08±0.325	21.80±0.115	24.42±0.895
4	**90**	**5.2**	**90**	20.89±0.315	18.36±0.060	21.82±0.550	21.58±0.250	21.83±0.715
5	**80**	**5.4**	**90**	19.19±0.120	16.85±0.130	22.55±0.175	22.39±0.670	23.85±0.235
6	**90**	**5.4**	**45**	19.82±0.210	18.91±030	23.61±1.410	21.69±0.165	23.46±0.265
7	**90**	**5.4**	**90**	21.32±0.435	17.93±1.60	22.01±0.415	21.56±0.235	23.85±0.250
8	**80**	**5.2**	**45**	21.62±0.180	18.45±0.453	23.42±0.393	21.87±0.090	24.19±0.504

The resulted data (Table 1) were analyzed using Design expert® 10.0.4.0 software to determine the effects of PH, boiling temperature and time on the value of beer bitterness for production of beer at different ratio of local to imported hops. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors.

b) Adequacy check for the developed models

The adequacy of the model was checked by analysis of variance (ANOVA) and some diagnostic plots. The model was found to be highly significant with the correlation coefficients of R-squared, adjusted R-squared and predicted R-squared having a value of 0.9547, 0.9207and 0.8188, respectively. Table 2 showed the summary of the analysis of variance (ANOVA) of the response (beer bitterness). The quality of the model developed could be evaluated from their coefficients of correlation. Value of R-squared for the developed correlation is 0.9547. It implies that 95.47% of the total variation in the degree of bitterness is attributed to experimental variables studied. The adequacy of the model was further checked with analysis of variance (ANOVA) as shown in Table 2. Based on the 95% confidence level, F-value is a test for comparing model variance with residual (error) variance. If the variance close to the same, the reaction will be close to one and it is likely that any of the factors have the significant effect on the response with the P-value less than 0.05. It is calculated by model mean square divided by residual mean square; here the model F-value of 28.10 implies the model is significant. There is only a 0.38% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant.

In this study it was realised that the individual factors and their interaction effects were significant model terms. Values greater than 0.1000 indicate the model terms are not significant. Analysis of variance (ANOVA) is employed to test the siginificance of the developed models.

Table 1: Analysis of variance (ANOVA) for 50:50% *R. priniodes* to Herkule Beer bitterness

Source	Sum of Squares	Df	Mean Squares	F-value	P-value Prob > F	
Model	4.52	3	1.51	28.10	0.0038	Significant
A	0.53	1	0.53	9.89	0.0347	
B	3.33	1	3.33	60.62	0.0014	
C	o.66	1	0.66	12.33	0.0246	
Residual	0.21	4	0.054			
Cor Total	4.73	7				

The graph of predicted value obtained using the developed correlation versus actual values is shown in the figure 2. The results in the figure 2 demonstrated that the regression model equation provided a very accurate description of the experimental data, in which all the points are very close to the line of perfect fit. This result indicates that it was successful in the capturing the correlation between the three wort boiling process variables to the design of Beer bitterness.

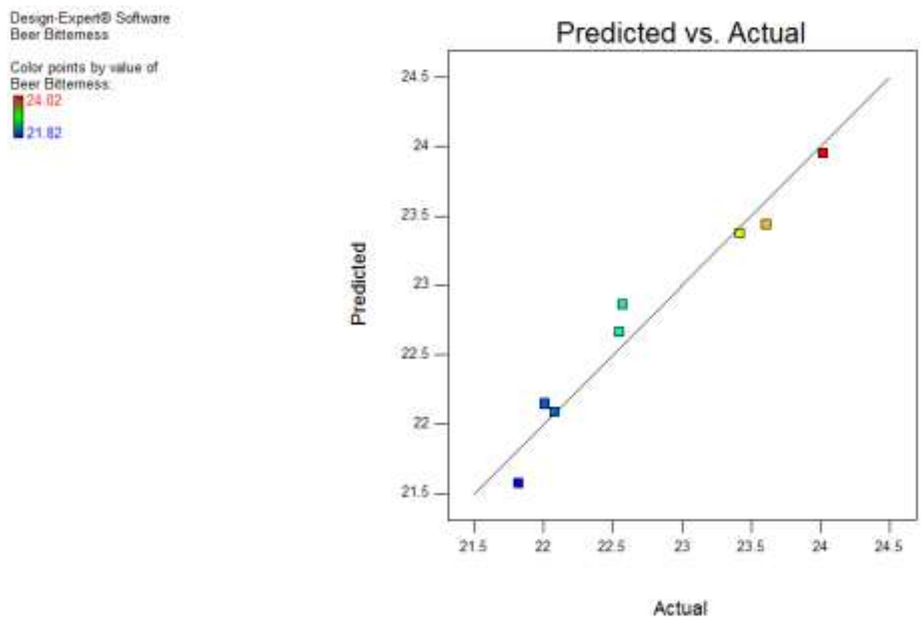


Figure 2: Predicted versus Actual degree for Beer bitterness.

The quality of the model developed coude be evaluated from their coefficients of correlation, R^2 (coefficent of determination) as showed in Table 3 was high for both responses, a value > 0.75 indicates the aptness of the model. For a good statistical mode, the R^2 value should be close to one. Results ensured a satisfactory adjustment of the 2FI model to the experimental data and indicated that approximately 95.47% of the variability in the dependent variables of Beer bitterness could be explained by these models and only 4.53% of the total variance could not explained by these models for Beer bitterness. The value of R^2 for the response was very high and close to one which indicates a good agreement between experimental and predicted values. The predicted R^2 was in a reasonable agreement with the adjusted R^2 for both responses.

Table 3: Model adequacy measures for Beer bitterness

Std. Dev.	0.23	R-Squared	0.9547
Mean	22.76	Adj R-Squared	0.9207
C.V. %	1.02	Pred R-Squared	0.8188
PRESS	0.86	Adeq Precision	14.535

In this case model for Beer bitterness the "Pred R-Squared" of 0.8188 was in reasonable agreement with the "Adj R-Squared" of 0.9207. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. In this case, ratio of 14.535 indicates an adequate signal as shown in Table 3 for Beer bitterness. So, this model can be used to navigate the design space.

Final Equation in Terms of Coded Factors:

Beer bitterness= +22.76-0.26 * A-0.65 * B+0.29* C

Where, A - Tempreture

B - Time

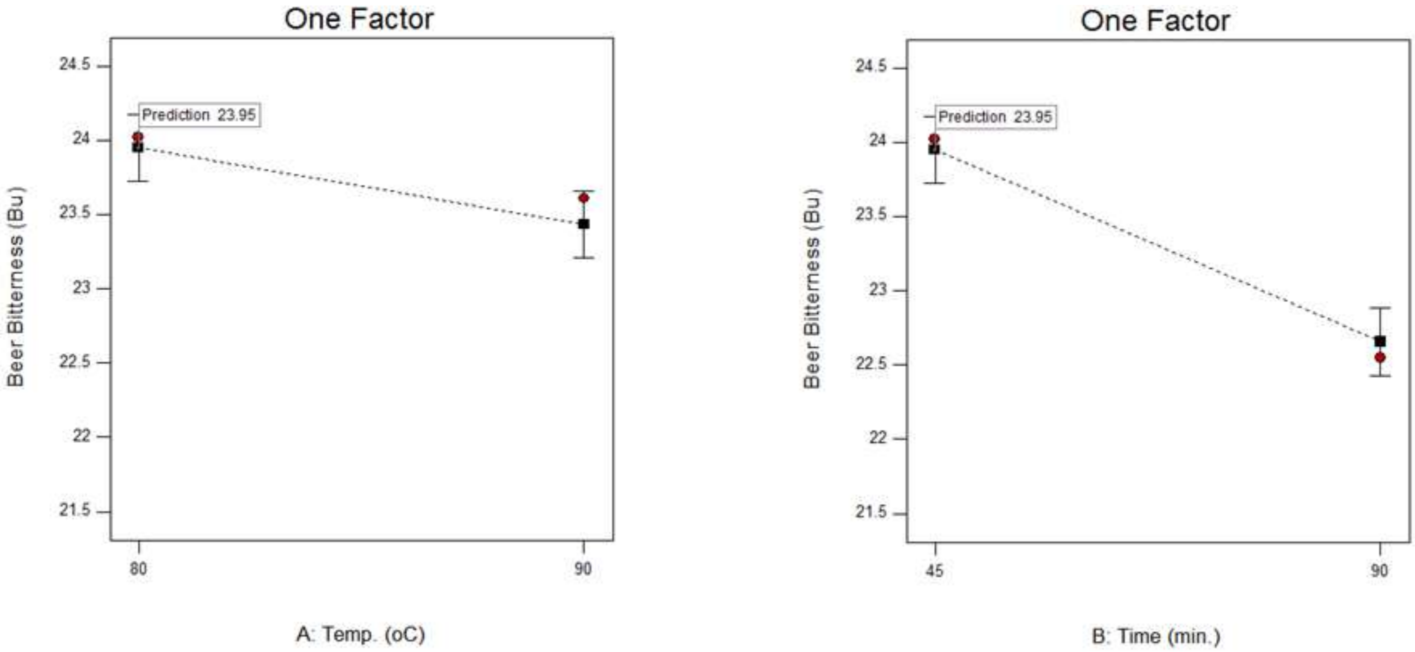
C - PH

c) Effects of Boiling Process Variables on Beer bitterness

The overall screened hop ratio for beer bittering conducted in this study. The effects of boiling time, temperature and PH on beer bitterness, flavour and aroma were studied and evaluated for best boiling conditions. Based on the analysis of variance, beer bitterness was significantly affected by various interactions between the boiling process variables. In addition to the interaction effect, significant individual process variables that affect the beer bitterness were boiling temperature, time, and P^H. There was a general decrease in the beer bitterness due to an increase in the boiling temperature and time whereas increasing beer bitterness as boiling P^H increase for 50:50% *R. priniodes* to Herkule ratio.

i) Effect of Individual Process Variables

As shown in Figure 3 (a) and (b) below the beer bitterness was significantly affected by both boiling temperature and time. It can be seen from the figure that with increasing boiling temperature and time from 80°C to 90°C and 45min. to 90 min. respectively, the beer bitterness was generally decreased.



(a) (b)

Figure 3: Effect of temperature and time on beer bitterness (a) at time = 45min. and PH = 5.4 (b) at temperature = 80^{o}C and PH = 5.4, respectively.

Figure 4 showed that the effects of boiling PH on beer bitterness for 50:50% R. priniodes to Herkule ratio. Increasing the amount of boiling PH increased the beer bitterness significantly.

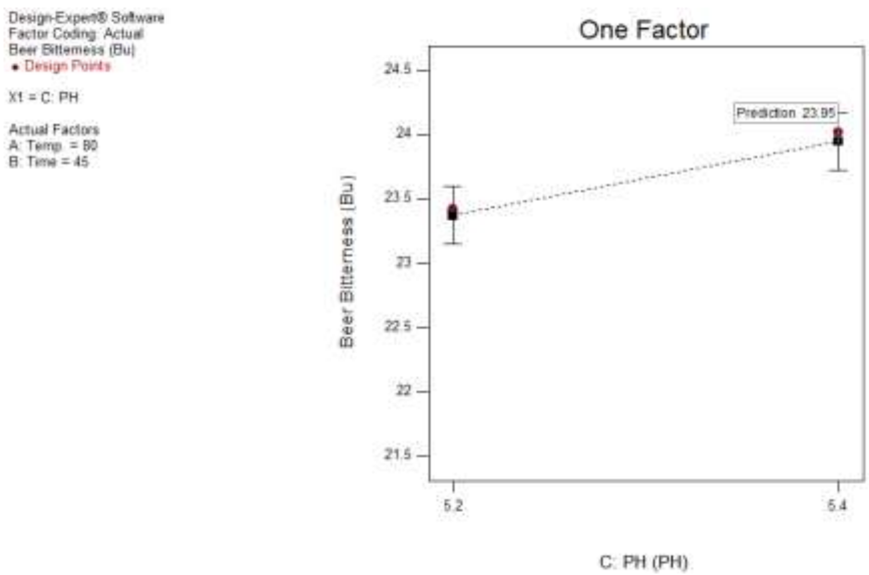


Figure 4: Effect of PH on Beer bitterness at temp. = 80 and time = 45min.

ii) Interaction Effect between Boiling conditions on Beer bitterness

Interaction between boiling conditions can affect the performance of *R. priniodes* and Herkule beer bitterness. But, as it already seen, the interaction effects of the process variables are significant. The interactions are between boiling temperature, time and PH on beer bitterness.

Generally, an increase in reaction temperature was found to decrease the beer bitterness. This was due to similar explanation given in the previous section. From the three interaction effects shown in the figures, there was a general decrease in the beer bitterness due to an increase in the boiling temperature and time linearly at 50:50 *R. priniodes* to Herkule ratio. As observed from this study, higher beer bitterness was resulted at high boiling PH due to the interaction effect.

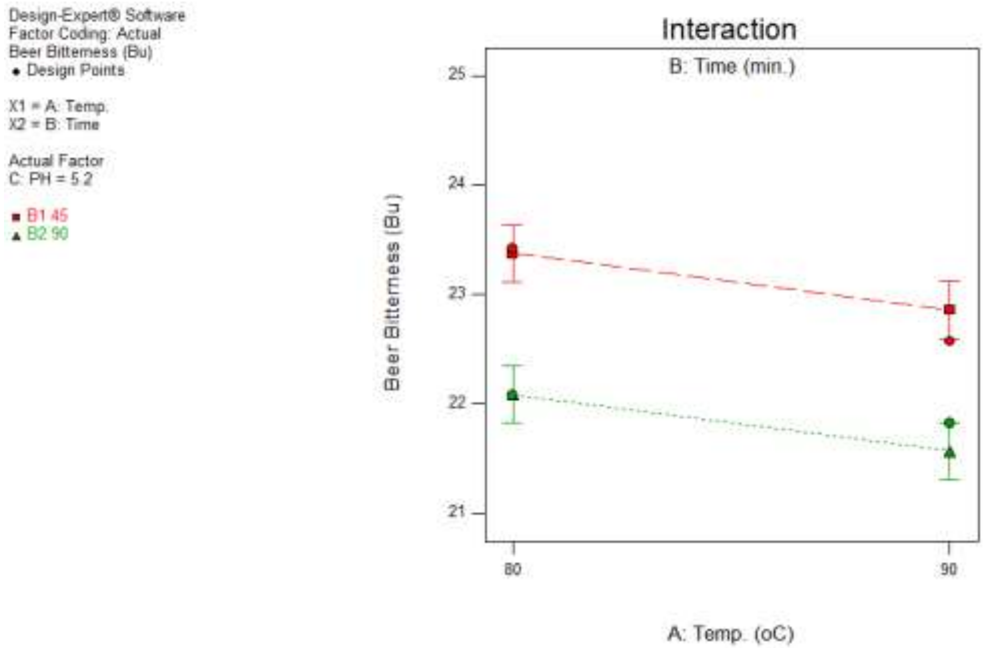


Figure 5: Highest Predicted value for the interaction of temperature and time on beer bitterness at PH= 5.4

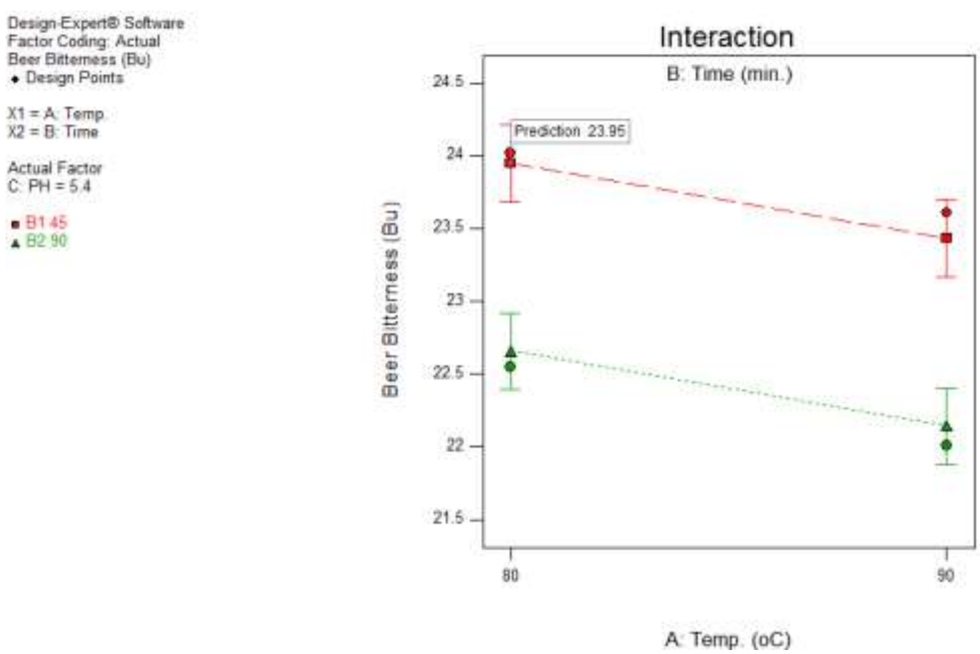


Figure 6: Lowest Predicted value for the interaction of temperature and time on beer bitterness at PH= 5.4

IV. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, in this study the bitter effects derived from *R. priniodes* and Herkule at different hop ratio and boiling conditions were tested. The results has demonstrated that bitter taste properties of 25% and 50% *R. priniodes* was screened for their suitability for beer production. In order to pronounce the bitter taste of *R. priniodes* in the beer production 50% of *R. priniodes* at temperature of 80^{O}C, time of 45min. and PH of 5.4 was selected. The beer was found to have a PH of 4.48, an alcohol content of 4.87%, colour of 7.95EBC and bitterness of the beer were 15.6Bu. Similarly, the commercial brand of beer (imported hop beer) was also tested for the above mentioned parameters which did not differ much with that of 50% R. priniodes beer. From the sensory quality evaluation, it was found that R. priniodes beer was comparable with that of commercial beer except differing slightly in colour which can be further improved. These results show that ethanolic extract of this plant could be considered as a natural alternative source of a good bittering agent in beer brewery sectors.

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