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RESEARCH PAPER

Effect of Nutrients and Growth Stimulators on Acetic Acid Fermentation using Natural Consortia

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Abstract

Acetic acid bacteria comprise a group of Gram negative, rod-shaped with aerobic metabolism using oxygen. From the natural inoculum, bacteria were isolated and characterized. Two main genera were determined as *Acetobacter* and *Gluconobacter*. Optimization of concentrations of alcohol, nitrogen source, phosphate source and magnesium sulphate was carried out by applying central composite design (CCD) of RSM for preparation of apple cider vinegar. Apple wine and cider vinegar was prepared by using optimized conditions from response surface methodology (RSM) experiment. Acetic acid fermentations were carried out with different cultures and growth regulators. The treatment having maximum titratable acidity (5.07%) was achieved in run number 2 having 5% initial alcohol concentration, 0.5% K_2HPO_4 and 0.25% ammonium sulphate with natural consortia. It was found that the maximum acidity of 4.56% was achieved in run number 23 having 5% alcohol concentration, 1.25% yeast extract, 0.25% NH_2HPO_4 and 0.55% $MgSO_4$ with natural consortia. It is concluded that to have optimum acetic acid production for cider vinegar production, the optimized concentration of nutrients and growth regulators was suitable, using natural consortia in the form of pellicle.

Keywords: Acetic acid, bacteria, CCD, RSM, *Acetobacter, Gluconobacter,* acetic acid, natural consortia

Vinegar is an ancient fermented food consumed by man since Babylons period (Lea, 1989). It is a condiment, made from various sugary and starchy materials by alcoholic and subsequently, acetic acid fermentation (Okafor, 1987; Downing, 1989; Rosma *et al.*, 2016; Joshi and Sharma 2009). Acetic acid is the predominant flavoring and antimicrobial component in vinegar. The importance of acetic acid as a direct food additive or more recently as a food processing aid, to decontaminate food prior to distribution and consumption has also been reviewed (Marshall *et al.*, 2000). Preparation of vinegar involves two fermentations viz., alcoholic and acetic acid fermentation; the alcoholic fermentation is carried out by *Saccharomyces cerevisiae,* while the acetic acid fermentation is performed by acetic acid bacteria (AAB). The acetic acid bacteria include the genus of *Acetobacter* and *Gluconobacter*, are characterized by their ability to convert ethyl alcohol (C_2H_5OH) into acetic acid (CH_3COOH). Vinegar production ranges from traditional methods employing wood casks and surface culture to submerged fermentation in acetators at industrial scale (Morales *et al.*, 2001).

Most of the acetic acid bacterial strains are derived from vinegar factories that are able to oxidize ethanol into acetic acid and some strains over oxidize acetic acid into CO_2 and H_2O (over-oxidation) and are classified in the genus *Acetobacter* (De Ley *et al.*, 1984). Many techniques have been developed to improve industrial production of vinegar. Most try to increase

the speed of the transformation of ethanol into acetic acid in the presence of the acetic acid bacteria (Tesfaye *et al.,* 2002). In the production of vinegar, the submerged culture is being employed extensively (Mendonca *et al*., 2002; Kocher *et al*., 2003; Kocher *et al.,* 2007) with diverse technical modifications to improve the general fermentation conditions (aeration, stirring, heating, etc.). Venegar from tea has also been prepared in batch and semi-continuous fermentation (Kaur *et al.,* 2011).

In acetic acid fermentation, the important physical parameters that affect the growth of *A. aceti* are, temperature, aeration and pH. It is believed that at the lower pH of wine, the growth of *A. aceti* is inhibited. It has been found that cell numbers of *A. aceti* decreased faster at pH 3.4 than at pH 3.8, under strict anaerobic conditions (Joyeux *et al.,* 1984). The optimum pH for the growth of *A. aceti* varied from 5.5 -6.3. A temperature range of 25 – 30°C is considered optimum for the growth of *A. Aceti* (Holt *et al*., 1994). However, thermotolerant strains of *A. aceti* are also able to grow at 37 – 40°C (Saski *et al.,* 1997).

Production of vinegar at home scale/cot age industry is mostly carried out using natural fermentation. With respect to cider vinegar, the vinegar made from apple, the acetification is carried out naturally but is a slow process. The acetic acid bacteria in pure culture have also been employed but the performance is always lower and most of the fermentations employ consortia or natural culture or mixed acetic acid bacteria. So, there is a need to standardize the culture of bacteria. There is also need for optimizing the acetic acid fermentation conditions viz., the use of stimulators, initial alcohol concentrations, etc. so as to increase the yield and reduce the time of fermentation. For the acetic acid fermentation such factors have not been documented so far, though for alcoholic fermentation some of the factors have been optimized and documented (Joshi *et al*., 2014). The results have been reported here af er conducting the studies on these aspects.

Materials and Methods

Preparation of apple juice

Juice was extracted from apple fruits as per the method described earlier (Joshi and Sharma, 2009). The fruits were grated and then, the juice was extracted by using hydraulic press, which was used as such, bot led and pasteurized as described under experimental section. It was used for alcoholic fermentation.

Yeast culture

The yeast culture viz. *Saccharomyces cerevisiae* var. *ellipsoideus*, was used in the study. It was maintained on yeast malt extract agar (YMEA) medium and re-cultured af er every three months or whenever needed from the stock yeast culture. It was used for ethanolic fermentation.

Cultures

Two cultures of *acetic* acid bacteria *Acetobacter Aceti* and *Gluconobacter oxydan* and natural consortia were used. Different nitrogen sources, phosphate sources, initial alcohol concentration and magnesium sulphate were employed in the acetic acid fermentation. The pellicle on the surface of vinegar fermentation of apple juice was used as a source of natural consortia.

To optimize the concentrations of additives for producing apple cider vinegar, the whole experiment was designed by using RSM (Response surface methodology). Ethanolic product was used for conversion into acetic acid in vinegar production. Different range of values used for RSM are given in Table 1.

Table 1: Range of values for the RSM

Independent variables	-1	0	+1
Initial Alcohol (%)	4	5	6
Yeast extract (%)	1	1.25	1.5
NH_2HPO_4 (%)	0.2	0.25	0.3
$MgSO_4$ (%)	0.3	0.55	0.8
Ammonium sulphate (%)	0.1	0.15	0.2
K_2HPO_4	0.2	0.35	0.5

Fermentation

Alcoholic fermentation for all the treatments was carried out at room temperature (20-22°C).

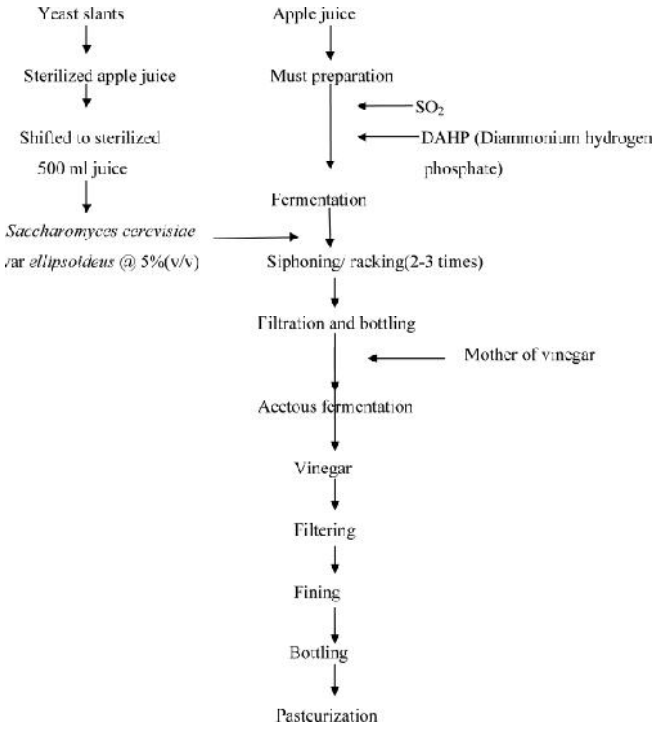

Fig. 1: Process flow diagram for preparation of apple cider vinegar

When a stable TSS was reached, the fermentation was considered complete. The ethanolic liquid or cider from alcoholic fermentation was used for the acetic acid fermentation.

It was carried out using the natural Consortia, *Acetobacter Aceti* and *Gluconobacter oxydans*. The fermentation was carried out in glass containers with cot on plug so as to provide maximum aerobic conditions. During the fermentation, samples were drawn for monitoring the fermentation viz., TSS, acidity, pH, ethanol at various intervals of time for the ethanolic fermentation and acidity, pH, for acetic acid fermentation. After the completion of fermentation, these observations were used to calculate the rate of fermentation and other analytical parameters. The samples were stored at low temp. in refrigerator till used.

Analyses

The titrable acidity was measured by titration method (AOAC, 1980). The TSS was determined by a hand refractometer. The ethanol was measured by spectrophotometer method (Caputi *et al.* 1968). The pH was measured by a pH meter. The rate of fermentation was calculated as per the routine methods.

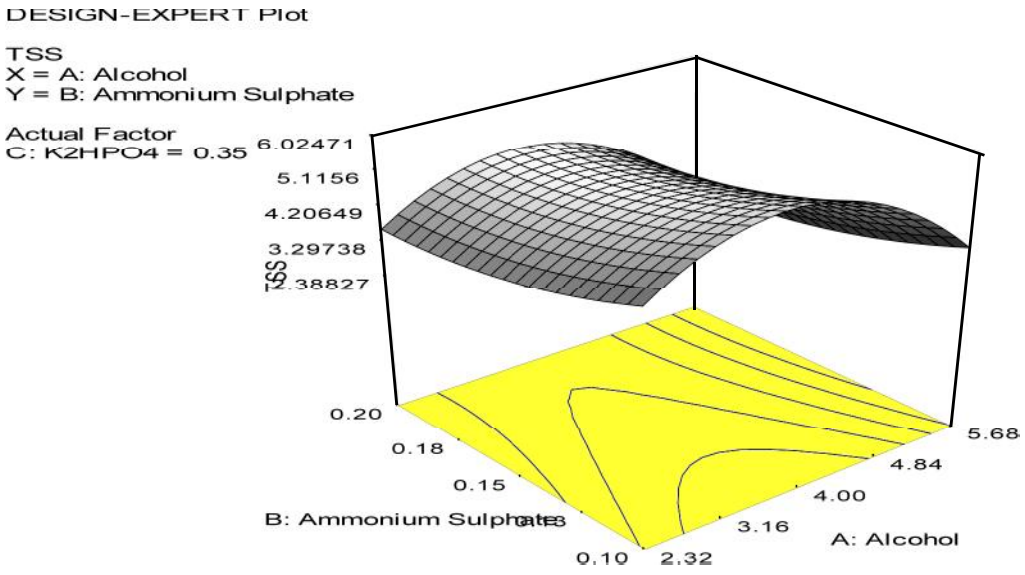

Fig. 2: Effect of ammonium sulphate, K_2HPO_4, alcohol on TSS

Results and Discussion

Effect of ammonium sulphate, K_2HPO_4 and natural consortia on acetic acid fermentation of cider vinegar

Effect of various factors such as nitrogen source (ammonium sulphate, yeast extract %), phosphate source (K_2HPO_4, NH_2HPO_4) and Magnesium sulphate was investigated using the response surface methodology (RSM). The design involved 8 centre design point with 'α' value being ±2. The three coded levels investigated in the current study were -1, 0 and 1. The results are discussed here.

TSS

Maximum TSS of 5.9°B was observed at 0.07 per cent ammonium sulphate and 0.35 per cent K_2HPO_4. Minimum TSS of 2.8°B was observed at 0.20 per cent ammonium sulphate and 0.50 per cent K_2HPO_4 (Table 2).

With increase in initial alcohol concentration TSS first increased and then decreased. With increase in ammonium sulphate concentration there was no effect on TSS.

Rate of fermentation

Maximum rate of fermentation of 0.528 was observed at 0.15 per cent ammonium sulphate, 0.35 per cent K_2HPO_4. Minimum rate of fermentation of 0.157 was observed at 0.20 per cent ammonium sulphate, 0.20per cent K_2HPO_4 (Table 2).

With increase in initial alcohol and ammonium sulphate concentration, there was a non-significant effect on rate of fermentation.

Ethanol

Minimum ethanol of 0.6 per cent was observed at 0.15 per cent ammonium sulphate, 0.60 per cent K_2HPO_4. Maximum ethanol of 3.5 per cent was observed at 0.10 per cent ammonium sulphate, 0.20 per cent K_2HPO_4 (Table 2).

With increase in initial alcohol concentration, residual ethanol increased and with increase in ammonium sulphate concentration, there was slight decrease in residual ethanol.

Titratable acidity

Maximum titratable acidity of 5.07 per cent was observed at 0.15 per cent ammonium sulphate and 0.35 K_2HPO_4 (Table 2). With increase in alcohol and ammonium sulphate concentration, acidity increased.

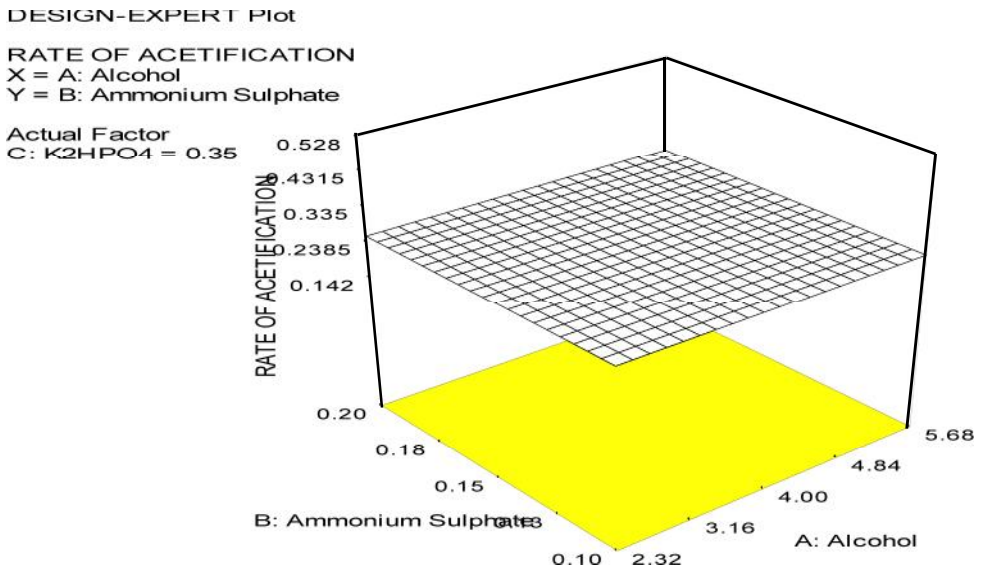

Fig. 3: Effect of ammonium sulphate, K_2HPO_4, alcohol on rate of fermentation

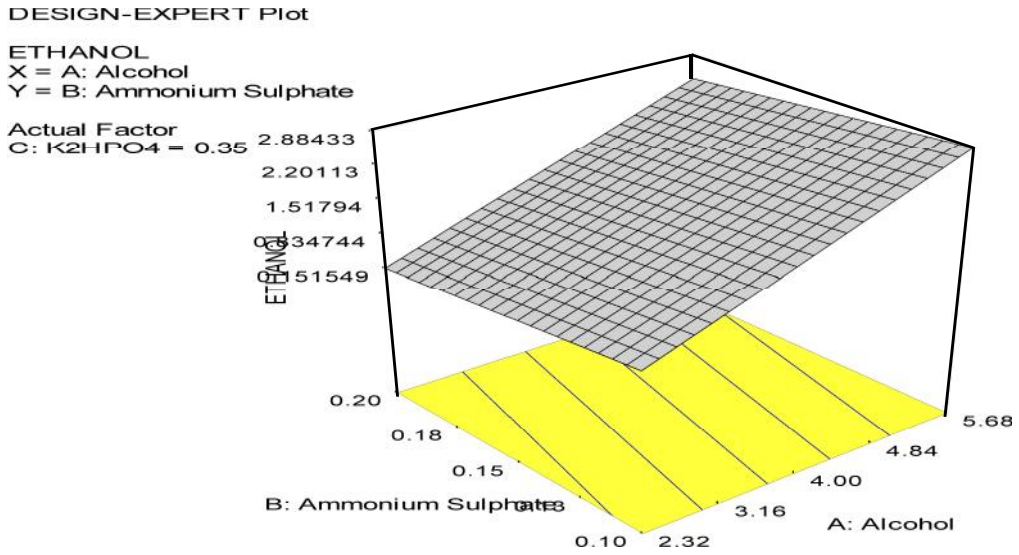

Fig. 4: Effect of ammonium sulphate, K_2HPO_4, alcohol on ethanol

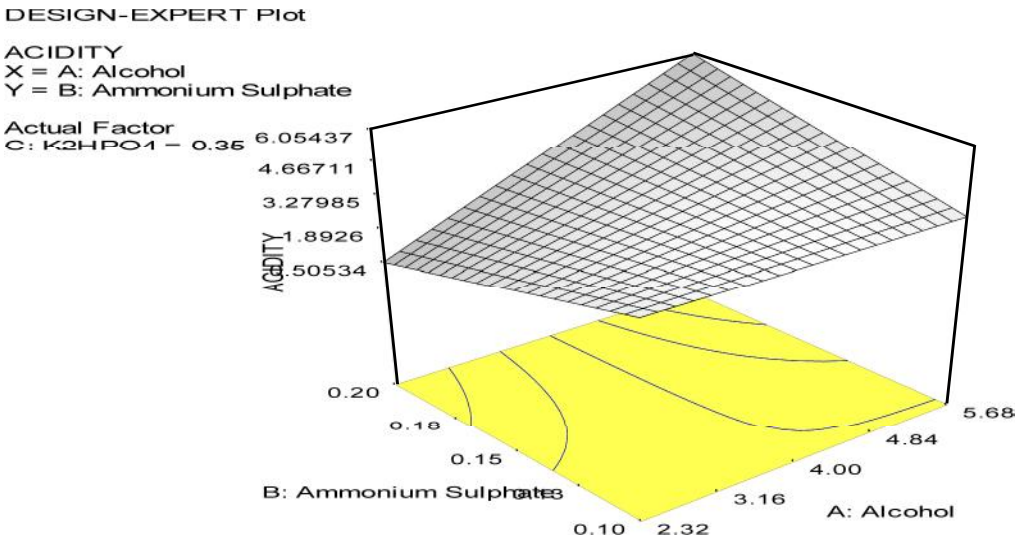

Fig. 5: Effect of ammonium sulphate, K_2HPO_4, alcohol on titratable acidity

pH

Minimum pH of 2.34 was observed at 0.20 per cent ammonium sulphate and 0.50 per cent K_2HPO_4. Maximum pH of 3.71 per cent was observed at 0.10 per cent ammonium sulphate and 0.20 per cent K_2HPO_4 (Table 2). With increase in alcohol and ammonium sulphate concentration there was non significant effect on pH.

Reducing sugars

Maximum reducing sugars of 28.02 mg/100g was observed at 0.15 per cent ammonium sulphate and 0.10 per cent K_2HPO_4 and at 0.55 per cent $MgSO_4$ (Table 2). With increase in alcohol and ammonium sulphate concentration there was non-significant effect on reducing sugars.

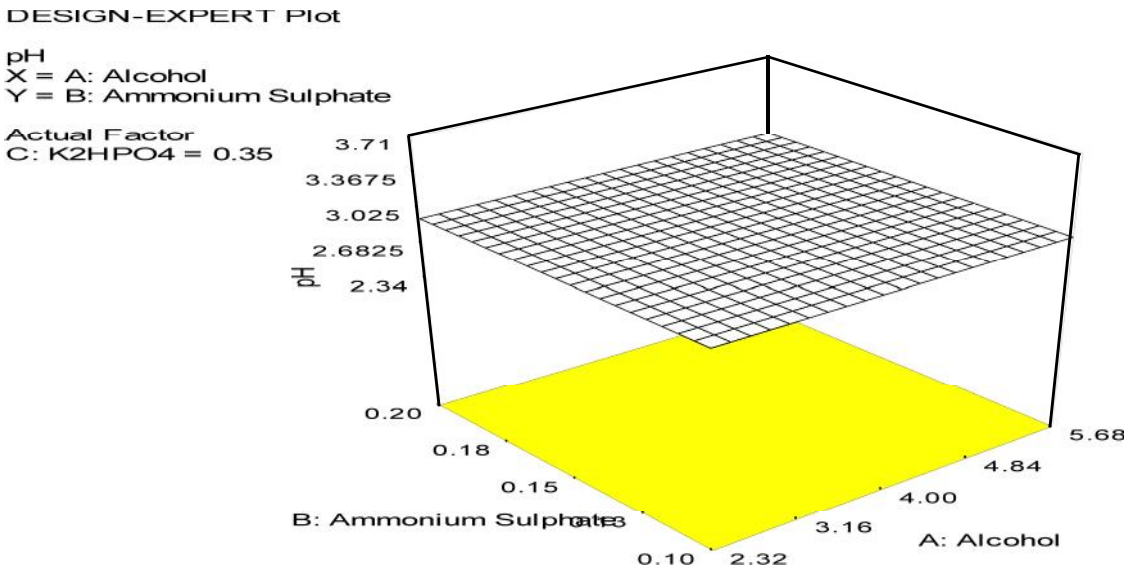

Fig. 6: Effect of ammonium sulphate, K_2HPO_4, alcohol on pH

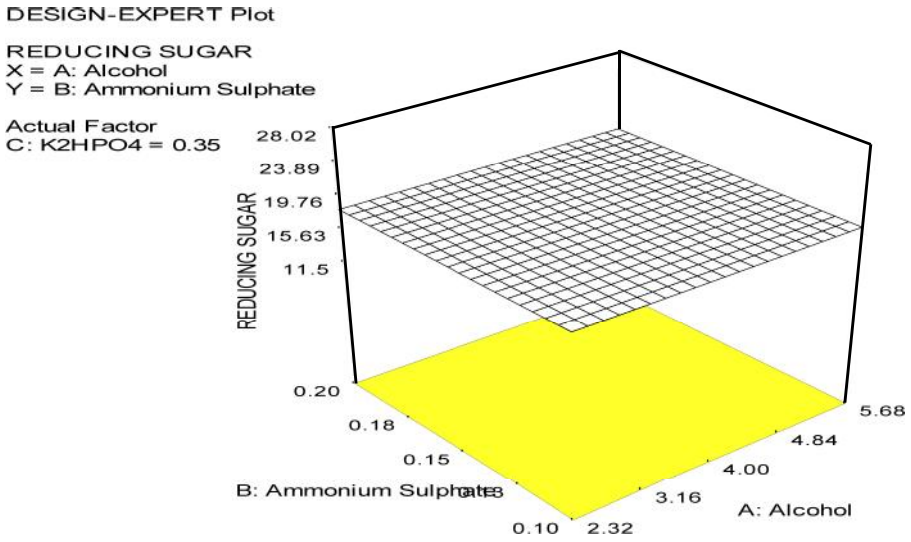

Fig. 7: Effect of ammonium sulphate, K_2HPO_4, alcohol on reducing sugars

Total sugars

Minimum total sugars of 2 per cent was observed at 0.15 per cent ammonium sulphate, 0.35 per cent K_2HPO_4. Maximum Total sugars of 2.65 per cent was observed at 0.15 per cent ammonium sulphate, 0.10 per cent K_2HPO_4 (Table 2). With increase in alcohol and ammonium sulphate concentration there was non significant effect on total sugars.

Antioxidant activity

Maximum oxidant of 0.171 per cent was observed at 0.15 per cent ammonium sulphate and 0.35 per cent K_2HPO_4 (Table 2).

Antimicrobial activity

Maximum antimicrobial activity of 45 mm was observed at 0.15 per cent ammonium sulphate and

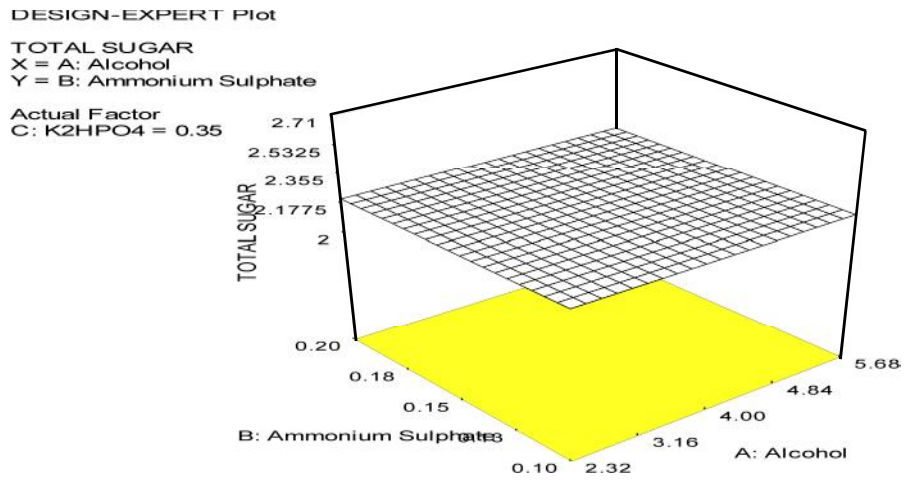

Fig. 8: Effect of ammonium sulphate, K_2HPO_4, alcohol on total sugars

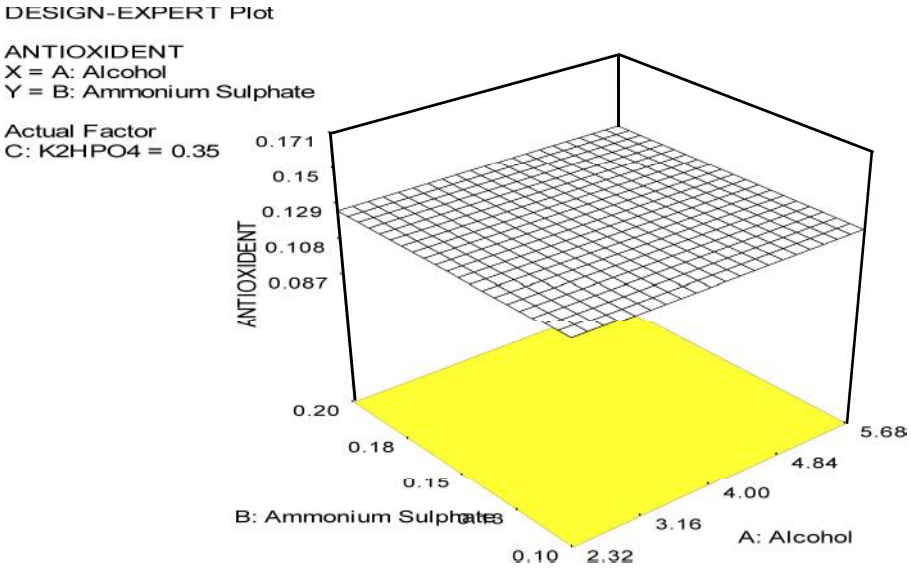

Fig. 9: Effect of ammonium sulphate, K_2HPO_4, alcohol on antioxidant activity

0.35 per cent K_2HPO_4 (Table 2). From the table 2, showed that maximum acidity (5.07) was achieved in Run 2 having 5 per cent initial alcohol concentration, 0.5 per cent K_2HPO_4 and 0.25 per cent ammonium sulphate and the highest antimicrobial activity was achieved.

TSS

Maximum TSS (4.5°B) was observed at 1.25 per cent yeast extract, 0.25 per cent NH_2HPO_4 and at 0.55 per cent $MgSO_4$, and minimum TSS (1.6°B) was observed at 1.00 per cent yeast extract, 0.25 per cent NH_2HPO_4 and at 0.55 per cent $MgSO_4$ (Table 3). TSS decreased with increase in alcohol concentration and decreased with increase in yeast extract concentration.

Rate of fermentation

Maximum rate of fermentation (0.276) was observed at 1.00 per cent yeast extract concentration, NH_2HPO_4 0.30 per cent and at 0.30 per cent $MgSO_4$

Table 2: Experimental results of CCD of RSM: Effect of different growth stimulators and natural consortia on acetic acid fermentation

Run	A:Alcohol conc.(%)	B:K_2HPO_4 (%)	C:Ammonium Sulphate(%)	TSS (°B)	pH	Titratable Acidity (%)	Antioxidant Activity (%)	Antimicrobial (mm)	Residual ethanol (%)	Reducing Sugar (mg/100ml)	Total Sugar (%)	Rate of Ferm. (°B/24H)
1	3	0.1	0.25	4	2.83	2.25	0.109	20	1.31	17.2	2.65	0.257
2	5	0.5	2.5	4.2	3.65	5.07	0.112	45	1.15	16.5	2.162	0.257
3	4	0.3	3.2	4.8	2.92	2.70	0.168	21	2.0	21.3	2.13	0.314
4	5	0.10	2.5	4	2.81	4.23	0.126	40	1.6	25.4	2.051	0.285
5	5	0.10	0.25	4	2.75	3.96	0.105	25	2.10	18	2.07	0.314
6	3	0.50	0.25	4	2.82	3.15	0.096	27	0.60	19.1	2.120	0.285
7	2.32	0.30	1.38	5.2	3.14	2.37	0.123	17	0.62	18.2	2.15	0.257
8	4	0.30	1.38	5.8	3.19	3.6	0.171	30	1.25	15.02	2.009	0.200
9	4	0.30	1.38	4.9	2.62	3.78	0.162	32	1.10	14.3	2.165	0.157
10	4	0.30	1.38	5	3.34	3.27	0.113	30	1.4	13	2.340	0.142
11	4	0.10	2.50	5.5	2.39	4.2	0.107	38	0.92	11.5	2.110	0.214
12	5	0.50	2.50	5.9	3.11	2.19	0.152	20	3.32	17	2.07	0.157
13	4	0.30	1.38	4.6	2.73	2.67	0.087	19	1.90	27	2.12	0.2
14	4	0.64	1.38	4.7	3.06	3.33	0.165	27	1.31	28.02	2.65	0.257
15	4	0.04	1.38	4.8	3.02	3	0.094	30	1.62	23	2.35	0.171
16	4	0.30	0.52	4.2	2.87	2.85	0.090	22	1.78	14	2.120	0.40
17	4	0.30	1.38	4.8	2.63	2.82	0.137	25	1.72	15.4	2.010	0.2
18	5.68	0.30	1.38	4.6	3.71	2.7	0.141	20	3.5	17.1	2.057	0.228
19	5	0.50	0.25	2.8	2.34	3.6	0.110	30	2.23	13	2.150	0.314
20	4	0.30	1.38	4.8	2.67	4.17	0.145	37	0.91	17.9	2.71	0.528

A: Alcohol conc. **B:** K_2HPO_4 **C:** Ammonium sulphate

Table 3: Effect of yeast extract, NH_2HPO_4, $MGSO_4$ and natural consortia on acetic acid fermentation

Run	Block	Factor 1 A: Alcohol	Factor 2 B:Yeast Extract	Factor 3 C: NH_2HPO_4	Factor 4 D: $MgSO_4$	TSS (°B)	pH	Titratable Acidity (%)	Residual ethanol (%)	Antioxidant activity (%)	Antimi-crobial (mm)	Total Sugar (%)	Reducing Sugar mg/100ml	Rate of Ferm. (°B/24H)
1	Block1	5	0.75	0.25	0.55	4.0	2.93	1.35	3.71	0.1086	18	2.800	16.69	0.150
2	Block1	6	1.50	0.20	0.30	1.6	2.20	3.30	3.50	0.1738	50	2.790	17.07	0.184
3	Block1	5	1.25	0.25	0.55	2.4	2.48	0.57	4.50	0.1146	15	2.790	18.40	0.276
4	Block1	3	1.25	0.25	0.55	2.0	2.80	2.46	1.28	0.1146	30	2.827	10.83	0.150
5	Block1	5	1.25	0.25	0.55	2.0	2.24	2.43	3.00	0.1032	14	2.905	13.73	0.230
6	Block1	5	1.50	0.20	0.80	4.0	2.61	2.70	2.90	0.1460	20	2.780	16.44	0.150
7	Block1	7	1.00	0.30	0.80	2.4	2.21	3.30	3.90	0.1624	19	2.850	13.10	0.276
8	Block1	5	1.00	0.30	0.30	2.0	2.78	0.99	4.00	0.1472	20	2.840	11.65	0.150
9	Block1	6	1.25	0.25	0.55	2.7	2.69	2.55	3.80	0.1410	32	2.752	13.16	0.253
10	Block1	5	1.25	0.25	0.05	3.0	2.24	4.50	1.62	0.1205	38	2.790	19.90	0.230
11	Block1	5	1.25	0.25	0.55	3.7	2.89	1.79	3.60	0.1303	17	2.830	17.01	0.100
12	Block1	4	1.50	0.30	0.30	4.3	3.49	1.43	2.90	0.0950	20	2.820	16.50	0.050
13	Block1	4	1.00	0.20	0.80	2.5	2.40	3.99	1.00	0.1108	45	2.817	17.13	0.269
14	Block1	6	1.25	0.15	0.55	3.0	2.40	3.96	2.90	0.1048	51	2.867	11.06	0.230
15	Block1	5	1.00	0.30	0.30	3.0	3.05	2.34	3.27	0.1156	20	2.855	16.38	0.150
16	Block1	5	1.25	0.25	1.05	2.5	2.7	1.90	3.50	0.1455	16	2.827	19.53	0.192
17	Block1	5	1.50	0.20	0.80	2.3	2.55	3.00	2.72	0.0945	11	2.802	11.46	0.207
18	Block1	4	1.50	0.30	0.30	4.5	3.80	1.71	2.70	0.0990	20	2.815	10.33	0.115
19	Block1	4	1.75	0.25	0.55	2.7	2.80	0.99	3.42	0.1070	20	2.832	13.23	0.176
20	Block1	6	1.00	0.20	0.30	2.7	2.39	4.20	2.72	0.1749	11	2.852	7.870	0.176
21	Block1	4	1.25	0.25	0.55	4.1	3.69	1.00	3.19	0.1021	40	2.817	9.820	0.146
22	Block1	4	1.50	0.20	0.30	3.4	2.89	1.21	3.00	0.1211	12	2.850	11.46	0.123
23	Block1	5	1.25	0.25	0.55	3.1	2.92	4.56	1.82	0.0939	37	2.820	13.90	0.223
24	Block1	4	1.25	0.25	0.55	3.1	3.08	1.00	3.21	0.1080	20	2.755	10.70	0.146
25	Block1	6	1.00	0.20	0.30	3.1	2.32	3.66	3.25	0.1678	40	2.827	12.90	0.223
26	Block1	4	1.00	0.30	0.80	3.2	3.83	1.02	3.10	0.1727	12	2.792	15.80	0.138
27	Block1	4	1.00	0.20	0.80	3.4	3.34	1.30	2.90	0.1450	18	2.785	10.83	0.123
28	Block1	5	1.50	0.30	0.80	3.5	2.54	2.25	3.00	0.1026	22	2.827	13.04	0.192
29	Block1	5	1.50	0.30	0.80	3.4	3.71	1.20	4.02	0.0945	14	2.760	11.27	0.123
30	Block1	4	1.25	0.35	0.55	3.2	3.59	1.40	2.90	0.1470	12	2.790	12.34	0.130

A: Alcohol B: Yeast extract C: NH_2HPO_4 D: $MgSO_4$

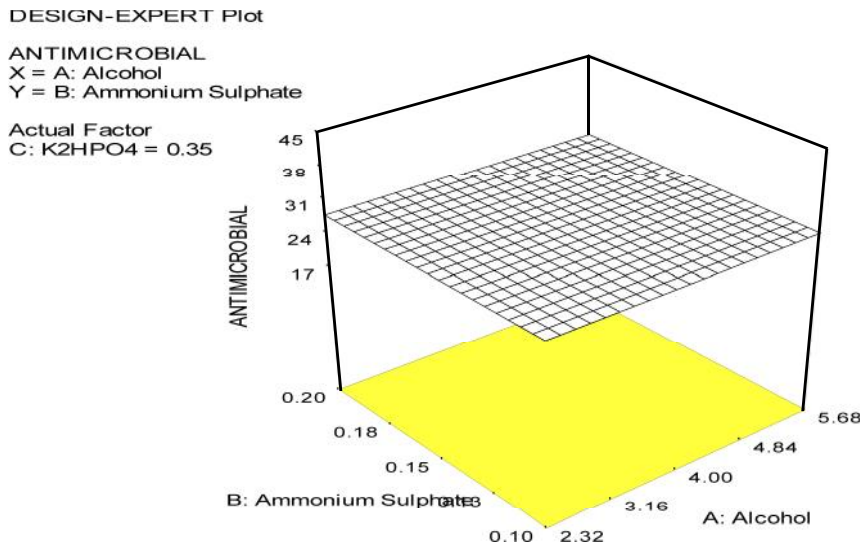

Fig. 10: Effect of ammonium sulphate, K_2HPO_4, alcohol on antimicrobial activity

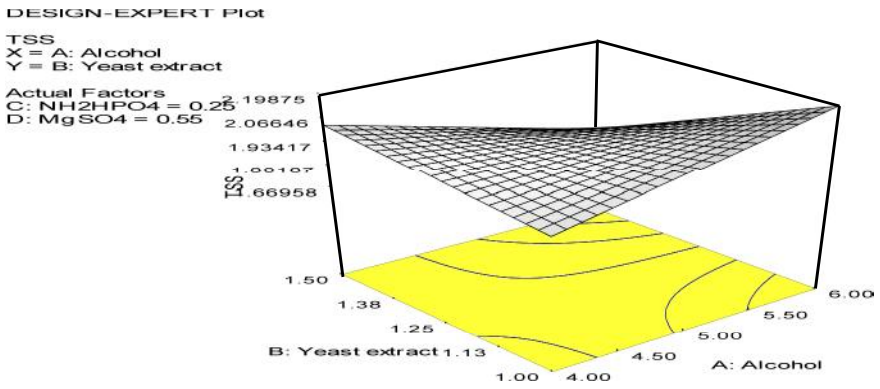

Fig. 11: Effect of yeast extract, alcohol, NH_2HPO_4 on TSS

(Table 3). With increase in alcohol and yeast extract concentration there was non-significant effect on rate of fermentation.

Ethanol

Minimum ethanol (1%) was observed at 1.25 per cent yeast extract, 0.25 per cent NH_2HPO_4 and at 0.05 per cent $MgSO_4$, and maximum ethanol (4.02 %) was observed at 1.50 per cent yeast extract, 0.30 per cent NH_2HPO_4 and at 0.80 per cent $MgSO_4$ (Table 3).

Ethanol first increased and then declined with increase in initial alcohol concentration however, with increase in yeast extract concentration ethanol decreased at point 4.50 and again increased.

Titratable acidity

Maximum titratable acidity (4.56%) was observed at 1.25 per cent yeast extract, 0.25 per cent NH_2HPO_4 and at 1.05 per cent $MgSO_4$ (Table 3).

Acidity increased with increase in alcohol concentration and increased with increase in yeast extract concentration.

pH

Minimum pH (2.2) was observed at 1.00 per cent yeast extract, 0.25 per cent NH_2HPO_4 and 0.55 per cent $MgSO_4$. Maximum pH (3.83 %) was observed at 1.25 per cent yeast extract, 0.25 per cent NH_2HPO_4

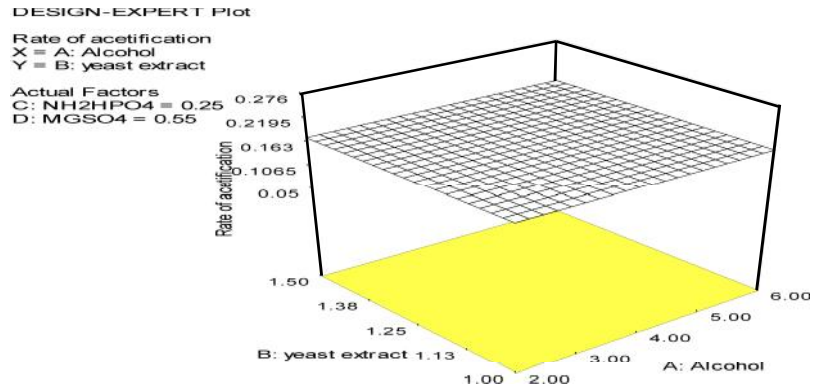

Fig. 12: Effect of yeast extract, alcohol, NH_2HPO_4 on rate of fermentation

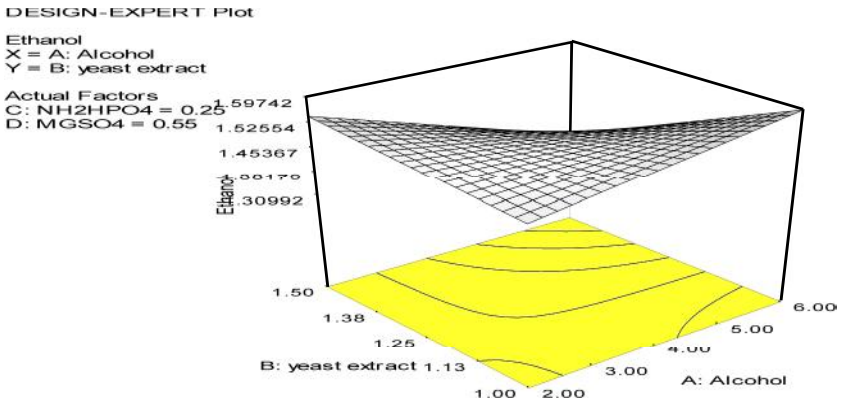

Fig. 13: Effect of yeast extract, alcohol, NH_2HPO_4 on ethanol

and at 1.05 per cent $MgSO_4$ (Table 3). pH sharply decreased with increase in alcohol and yeast extract concentration.

Reducing sugars

Maximum reducing sugars (19.53 mg/100g) was observed at 1.25 per cent yeast extract, 0.25 per cent NH_2HPO_4 and at 0.55 per cent $MgSO_4$ (Table 3). There was sharp increase in reducing sugars with increase in alcohol and yeast extract concentration.

Total sugars

Minimum total sugars (2.75%) was observed at 1.50 per cent yeast extract, 0.30 per cent NH_2HPO_4 and at 0.80 per cent $MgSO_4$. Maximum Total sugars (2.905%) was observed at 1.50 per cent yeast extract, 0.20 per cent NH_2HPO_4 and at 0.80 per cent $MgSO_4$ (Table 3).

Total sugars slightly decreased with alcohol concentration and with increase in yeast extract concentration it neither increased nor decreased.

Antioxidant activity

Maximum oxidant (1.74%) was observed at 1.75 per cent yeast extract, 0.25 per cent NH_2HPO_4 and at 0.55 per cent $MgSO_4$ (Table 3)

Antioxidant activity increased with increase in alcohol concentration and with increase in yeast extract concentration antimicrobial activity also increased.

Antimicrobial activity

Maximum antimicrobial activity (50 mm) was observed at 1.00 per cent yeast extract, 0.25 per cent NH_2HPO_4 and at 0.55 per cent $MgSO_4$ (Table 3).

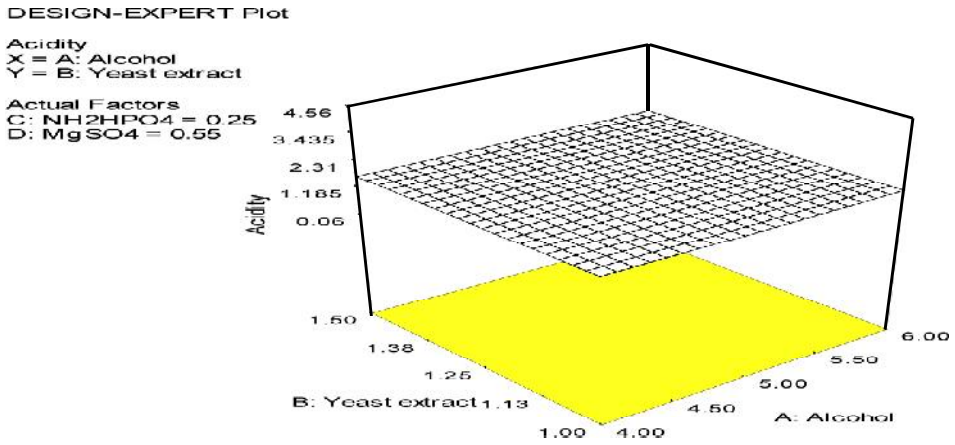

Fig. 14: Effect of yeast extract, alcohol, NH_2HPO_4 on titratable acidity

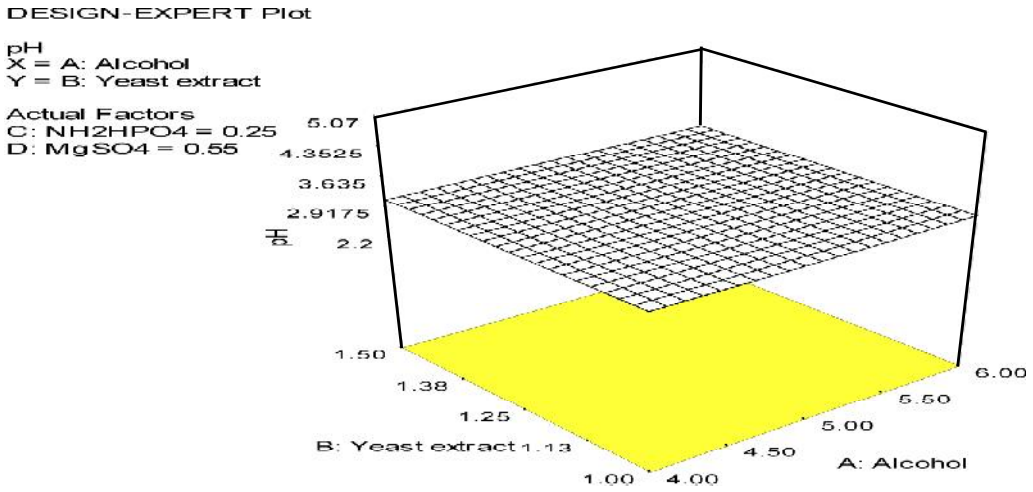

Fig. 15: Effect of yeast extract, alcohol, NH_2HPO_4 on pH

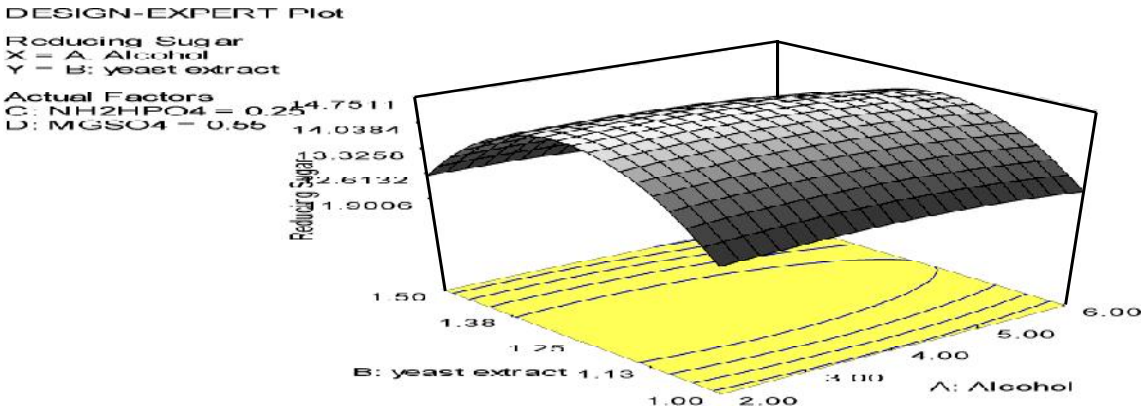

Fig. 16: Effect of yeast extract, alcohol, NH_2HPO_4 on reducing sugars

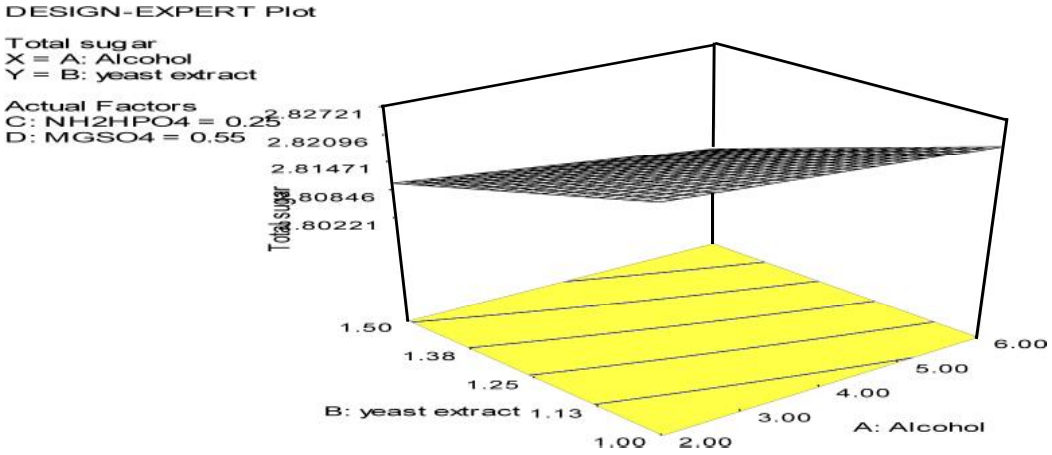

Fig. 17: Effect of yeast extract, alcohol, NH_2HPO_4 on total sugars

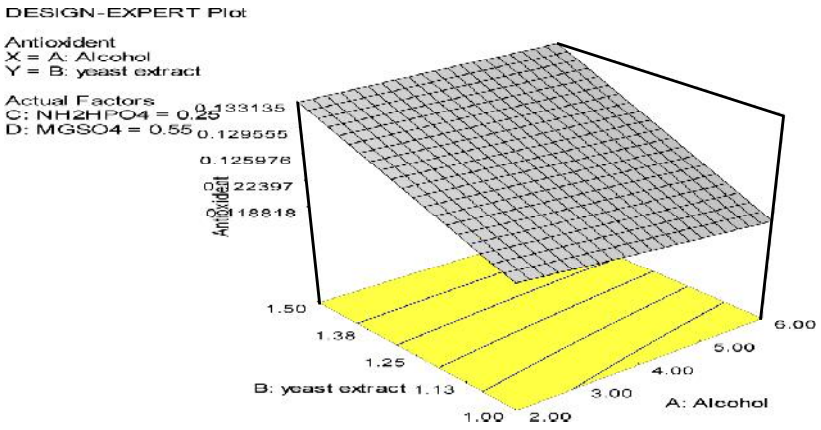

Fig. 18: Effect of yeast extract, alcohol, NH_2HPO_4 on antioxidant activity

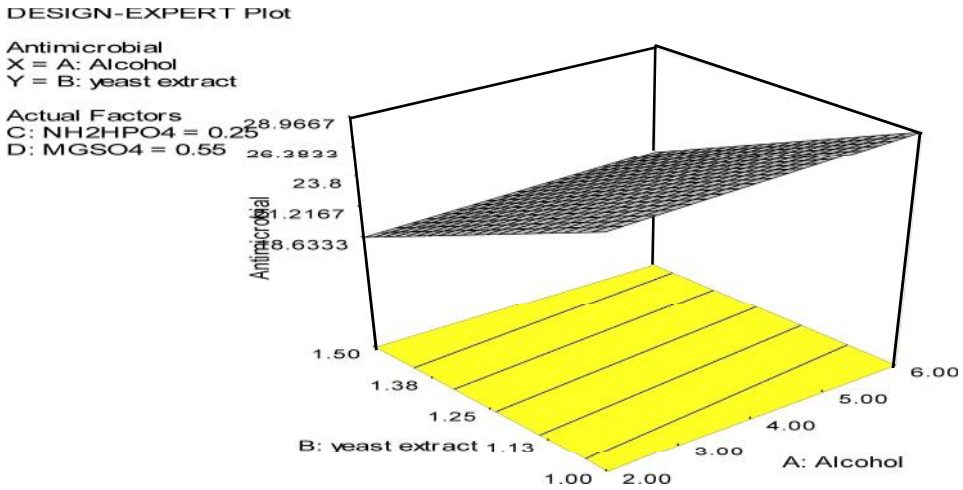

Fig. 19: Effect of yeast extract, alcohol, NH_2HPO_4 on antimicrobial activity

Antimicrobial activity was stable at point (3.75) and increased with increase in alcohol concentration. Whereas, with increase in yeast extract concentration antimicrobial activity increased.

Table 3 showed that, the maximum acidity of 4.56 per cent was achieved in run 23 having 5 per cent initial alcohol concentration, 1.25 per cent yeast extract, 0.25 per cent NH_2HPO_4 and 0.55 per cent $MgSO_4$ followed by run 10 of acidity 4.5 per cent having 5 per cent alcohol conc., 1.25 per cent yeast extract, 0.25 per cent NH_2HPO_4 and 0.05 per cent $MgSO_4$.

The maximum titratable acidity (5.07 %) was achieved in run 2 having 5 per cent initial alcohol concentration, 0.5 per cent K_2HPO_4 and 0.25 per cent ammonium sulphate with natural consortia. Similarly, it was found that, the maximum acidity of 4.56 per cent was achieved in run 23 having 5 per cent alcohol concentration, 1.25 per cent yeast extract, 0.25% NH_2HPO_4 and 0.55% $MgSO_4$ with natural consortia. Out of 2 treatments, the fermentation carried out with 5 per cent initial alcohol concentration, 0.5% K_2HPO_4 and 0.25% ammonium sulphate with natural consortia gave the higher acetic acid in acetic acid production.

Application of natural consortia of acetic acid fermentation has been found to be the best in earlier study also (Downing, 1989; Joshi and Thakur, 2000; Joshi and Somesh, 2009). Further, its bet er performance could also be correlated with adaption of acetic acid bacteria as reported earlier (Hubert *et al.*, 1976).

In our study, we had achieved an acetic acid conc. of more than 4 per cent which is as per the standard of vinegar level of 4% acetic acid and is mandatory also (Lea, 1989; Rangana, 1994). So, from this point of view it is desirable. However, where the standard of acetic acid in vinegar is more than 4 per cent, efforts to increase the same would be required.

Conclusion

It is concluded from the study that the maximum titratable acidity (5.07%) was achieved in run 2 having 5% initial alcohol concentration, 0.5% K_2HPO_4 and 0.25% ammonium sulphate with natural consortia. It was found that the maximum acidity of 4.56% was achieved in run 23 having 5% alcohol concentration, 1.25% yeast extract, 0.25% NH_2HPO_4 and 0.55% $MgSO_4$ with natural consortia. Thus, the fermentation carried out with 5% initial alcohol concentration, 0.5% K_2HPO_4 and 0.25% ammonium sulphate with natural consortia gave the highest acetic acid production in cider vinegar production.

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