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RESEARCH PAPER

Optimization of low alcoholic bitter gourd apple beverage by applying Response surface methodology (RSM)

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Abstract

Bitter gourd (*Momordica charantia*)—a member of the cucurbitaceae family, and a popular vegetable among gourds is known as bitter melon, bitter gourd, balsam pear and *karela*. It has bitter taste and is known to possess many medicinal properties including those for the cure of diabetes caused by insulin deficiency or resistance. Attempts were made to prepare a low alcoholic beverage from bitter gourd and apple by standardizing the concentration of apple juice, DAHP and inoculum size by applying central composite design (CCD) of RSM. On the basis of physico-chemical and sensory characteristics, a run having 40% apple juice, 0.15 % DAHP and 2.5% inoculum level were rated as the best. It has the highest TSS, rate of fermentation, ethanol, reducing and, total sugars and total phenols. With the addition of 0.15% DAHP as a nitrogen sources, bitter gourd based wine had the highest total soluble solid (TSS), titratable acidity, ethanol, total and reducing sugar content and lowest volatile acidity and higher alcohols. Different concentrations of yest innoculum used did not significantly influenced most of the physic-chemical characteristics. Must inoculated with 2.5% *Saccharomyces cerevisiae* var *ellipsoideus* used for the preparation of bitter gourd based wine had the highest fermentability and ethanol content. However, the product being low alcoholic, need pasteurization at 62°C for 20 min. keeping a head-space of 2.5 cm in a glass bottle. Bitter gourd beverage so prepared holds promise as a medicinal drink as bitter gourd is known to be antidiabetic.

Keywords: Bitter gourd, ethanol, alcoholic, pasteurization, medicinal drink, diabetes

Bitter gourd is known to possess many medicinal properties such as a tonic, stomachic, stimulant, laxative and is used in cure of rheumatism, gout, disease of spleen and liver, and is considered as a blood purifier besides controlling diabetes (Kalra *et al.* 1988). Besides, several medicinal uses in the traditional methods of medicine especially hypoglycaemic property of bitter gourd fruit have also been reported (Raman and Lau, 1996).

Fruits and seeds of bitter gourd are traditionally used as a medicinal herb as anti- HIV, anti-ulcer, anti-inflammatory, antileukemic, anti-microbial, anti-diabetic and anti-tumer. Recently, the antidiabetic properties of the fruit are being intensively investigated (Marderosiam, 2001). A product normally consumed, if it also possess medicinal property, it would be highly advantageous as advocated by Hippocrates that 'Let food by thy medicine and medicine by thy food'. Alcoholic beverage such as wine would fit in this category.

The fermentation of fruit to prepare wine is an age old technique recorded in ancient Egyptians and Greek writings. Although production of wine had been largely based on the fermentation of grape juice yet it has also been practiced widely from fruits such as apples, cherries, currants, peaches, plums, strawberries and some other wild fruits (Joshi *et al.* 1991; Chung *et al.* 2003, Reddy *et al.* 2010 and Joshi *et al.* 2011). Preparation of alcoholic beverages including wine is one of the options available but less alcoholic and with medicinal properties would meet the objective.

Wine as a beverage with low alcoholic content is gaining popularity among the consumers, as the health benefits of low alcoholic beverages are being recognised (Cuenet *et al.* 1985; Schobinger, 1986). Wine can also be prepared from bitter gourd but not documented widely. So detailed technology of such beverage and its characteristics is lacking. Study on these aspects has become even more relevant as there are a large number of diabetic patients in the developing country. Bitter gourd does not have sufficient sugar level to conduct the alcoholic fermentation and the fruit is pulpy thus, there is need to develop a method to extract the juice that can be combined with other sugar rich juices. Neither there is documentation of preparation of bitter gourd in combination with other fruit juices. The problem is further compounded as the bitter gourd fruit is bitter in taste so the wine is also expected to be bitter. Therefore, a combination of bitter gourd with suitable fruit holds promise to make a palatable beverage yet retaining medicinal properties. The study was therefore conducted with the aim of preparation of bitter gourd apple wine its optimization using Response surface methodology.

Materials and Methods

To prepare the wine, juice was ameliorated with fermentable sugars (apple) as in case of bitter gourd juice the TSS was around 2.5 to 3.0^{o} Brix i.e. sugar source need to be added to produce some amount of ethanol to prepare alcohol. The whole experiment was designed by using RSM (Response surface methodology).

Raw materials

Extraction of bitter gourd and apple juice: The bitter gourd juice was extracted by addition of water. Fruit was cut into two halves and the juice was extracted with the help of screw type juicer extractor by adding simultaneously different proportions of water into it (Kumar, 2014). The juice was collected in a container just placed below the juice extractor and was taken for the wine preparation. Apple juice was also extracted from apple fruits as per the conventional method (Lal *et al.* 1986). The fruits were grated and then, the juice was extracted by using hydraulic press followed by hot filling, bottling and pasteurization.

Preparation of bitter gourd apple juice must: Must was prepared by blending bitter gourd juice with apple juice at different concentrations as per the design (Table 2). To this mixture, diammonium hydrogen phosphate (DAHP) as nitrogen source and 0.5% pectinase enzyme for clarification were added and sulphur dioxide (100 ppm) was added to kill the wild microorganisms.

Addition of nitrogen source, inoculum concentration and apple juice concentration: The higher and lower concentrations of nitrogen source, inoculum concentration and apple juice used for the wine preparation, were chosen as treatments as detailed in Table 1. These were designed by RSM (Response Surface Methodology).

Table 1: Range of values for the RSM

Variables	-1	0	+1
Apple juice concentration (%)	20	30	40
DAHP (%)	0.05	0.1	0.15
Inoculum size (%)	2.5	5.0	7.5

The different combinations were made as per the expert RSM design version 7.0 (Stat Ease, Inc, Minneapolis, USA). The details are given in Table 2.

Table: 2 Experimental plan of bitter gourd apple wine as per the design

Treatments	DAHP (%)	Inoculation (%)	Apple juice (%)
T_1	0.05	7.5	20
T_2	0.1	5	30
T3	0.1	5	30
T_4	0.05	2.5	40

T_5	0.1	5	46.82
T_6	0.15	2.5	20
T_7	0.05	7.5	40
T_8	0.1	5	30
T_9	0.1	5	30
T_{10}	0.15	2.5	40
T_{11}	0.1	9.2	30
T_{12}	0.1	5	30
T_{13}	0.02	5	30
T_{14}	0.1	0.8	30
T_{15}	0.05	2.5	20
T_{16}	0.18	5	30
T_{17}	0.1	5	13.18
T_{18}	0.15	7.5	20
T_{19}	0.1	5	30
T_{20}	0.15	7.5	40

Best treatment from the design was selected on the basis of physico-chemical characteristics of wine.

Fermentation

The respective must was inoculated with activated culture of *Saccharomyces cerevisiae* var. *ellipsoideus*. Fermentation was carried out at room temperature (20-22^{o}C). When a stable TSS was reached, the fermentation was considered complete. Air locks were fitted in the mouth of glass bottles near the end of fermentation. Siphoning was done and the wine was kept for maturation. The wines were analyzed for different physico-chemical and sensory characteristics as such and during maturation period.

Analysis of wine

Physico-chemical analysis

Bitter gourd apple wine was analysed for various physico-chemical characterstics as per the standard methods. TSS was measured using Erma hand refractrometer (0-32^{o}B). Titrable acidity was estimated by titrating a known aliquot of the sample against N/10 NaOH solution using phenolphthalein as an indicator. pH was taken with ELTOP-3030 pH meter. The total and reducing sugars of bitter gourd juice and bitter gourd wine were estimated by Lane and Eynon volumetric method (A.O.A.C., 1980). Volatile acidity of bitter gourd apple wine was determined by the standard method (Amerine *et al.* 1980). The distillate was titrated with 0.025 N NaOH and the volatile acidity was expressed as acetic acid (g/100 ml) and ethanol content was determined by spectrophotometric method (Caputi *et.al.* 1968). The quantity of total phenols in bitter gourd wine and bitter gourd juice were determined by Folin Ciocalteu procedure given by Singleton and Rossi (1965). The work was estimated by taking optical density at 440 nm.

Sensory analysis

The sensory analysis of bitter gourd apple wine was conducted by semi-trained panel of judges by using composite scoring and hedonic rating test (Joshi, 2006). In composite scoring, coded samples were given to 10 judges. They were asked to rinse their mouth before or in between testing the given sample. Each sample was evaluated for overall acceptability on hedonic scale or composite scores using various quality attributes viz. colour and appearance, aroma and bouquet, volatile acidity, total acidity, sweetness, body, flavour, bitterness, astringency and overall impression (Amerine *et al.* 1980) on a prescribed proforma.

Statistical analysis

Statistical analysis of the quantitative data of chemical parameters obtained from the experiments was carried out by Response surface methodology (RSM). The statistical analysis of the data obtained from sensory evaluation of the bitter gourd apple wine was done by Response surface methodology (RSM).

Results and Discussions

Physico-chemical and sensory evaluation of bitter gourd apple wine

For standardization of fermentation parameters such as, concentration of nitrogen source (DAHP

%), inoculum size and apple juice proportion, response surface methodologywas applied on these factors. Second-order experimental design, *i.e.* Central Composite Design (CCD) with three factors at three levels was employed to investigate the first and higher-order main effects of each factor and interactions among them. The design involved 8 central design points with 'α' value being ± 2. The three coded levels investigated in the current study were -1, 0, and 1. The results so obtained for different physico-chemical and sensory characteristics of apple bitter gourd wine are presented in table 3.

Effect on TSS, rate of fermentation and ethanol content

Figure 1 depicts the expected response of TSS and correlation between the independent variables in three dimensional plots, with DAHP concentration at X- axis and inoculum concentration at Y- axis while keeping all other variables at their 'O' central levels. Lower and higher levels of inoculum size did not influence TSS. DAHP and inoculum concentration affected the TSS level independently. While with increase in DAHP concentration there was a significant decrease in TSS level. Maximum TSS of 3.1°B was observed at 46.82% apple juice, 0.10% of DAHP and at 5.0% inoculum, and minimum TSS of 2.4°B was observed at 0.05% DAHP, 2.5% inoculum level and 20% apple juice concentration.

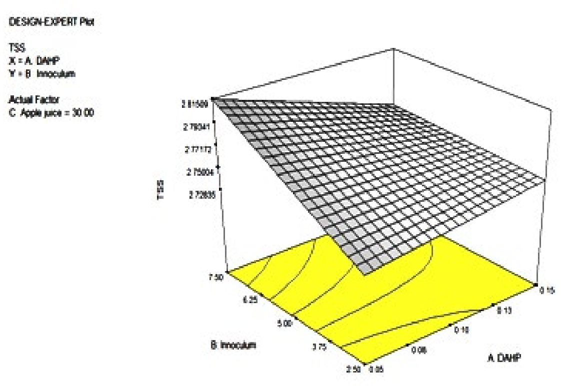

Fig. 1: Effect of DAHP concentration and inoculum size on the TSS (°B) of bitter gourd apple wine

Figure 2 depicts the expected responses for rate of fermentation and correlation between the independent variables in three dimensional plots, with DAHP at the X-axis and Inoculation concentration at Y-axis while keeping all other variables at their 'O' central levels. None of the pairs of factors interacted effectively to increase the rate of fermentation. With increase in DAHP and Inoculum concentration, a decrease in rate of fermentation was recorded. Maximum rate of fermentation of 0.66 was observed at 46.87% apple juice concentration, DAHP 0.10% and at 5.0 % inoculum.

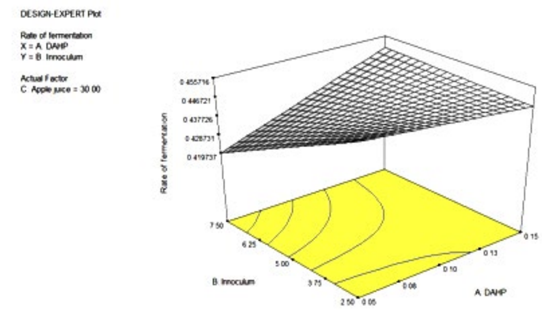

Fig. 2: Effect of DAHP concentration and inoculum size on the Rate of fermentation of bitter gourd apple wine

The expected response of ethanol and correlation between the independent variables in three dimensional plots, with DAHP at the X-axis and inoculum concentration at Y- axis while keeping all other variables at their 'an O'central level is shown in Fig. 3. The graph shows the significant effect of the factors and their interactions. With increase in DAHP and Inoculum concentrations, increase in ethanol content took place. Maximum ethanol i.e. 2.92 % was observed at 40% apple juice concentration, 0.15% of DAHP, and at 2.5 % inoculum.

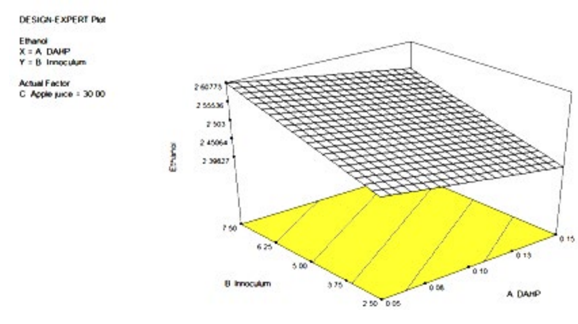

Fig. 3: Effect of DAHP concentration and inoculum size on the Ethanol content (%) of bitter gourd apple wine

Table 3. Experimental results for CCD of RSM

Run	DAHP (%)	Inoculum (%)	Apple juice (%)	TSS⁰B	Titrable acidity (%)	Rate of fermen-tation oB/24 hr	pH	Ethanol (%v/v)	Total sugars (%)	Reducing sugars (mg/100ml)	Total phenols (mg/l)	Colour (OD at 440nm)	Higher alcohols (mg/l)	Methanol (mg/l)	Volatile acidity (%acetic acid)	Overall acceptability (Hedonic scale)
1	0.05	7.5	20	2.6	0.352	0.33	4.12	2.30	0.216	31.87	156.60	0.419	41.275	127.20	0.025	7.5
2	0.1	5	30	2.8	0.352	0.44	4.24	2.62	0.325	35.23	162.40	0.394	42.510	111.30	0.025	7.5
3	0.1	5	30	2.9	0.384	0.42	4.19	2.61	0.345	32.63	172.55	0.392	39.580	111.30	0.026	7.5
4	0.05	2.5	40	3.0	0.352	0.56	4.11	2.85	0.382	43.13	255.20	0.403	40.950	95.40	0.029	8.0
5	0.1	5	46.82	3.1	0.406	0.66	4.16	2.86	0.397	56.34	165.40	0.375	50.180	84.55	0.028	8.0
6	0.15	2.5	20	2.5	0.384	0.33	4.28	2.50	0.205	32.49	165.30	0.408	43.745	127.20	0.025	7.0
7	0.05	7.5	40	3.0	0.416	0.56	4.06	2.92	0.385	45.40	175.40	0.373	46.605	95.40	0.028	8.0
8	0.1	5	30	2.8	0.384	0.44	4.13	2.63	0.365	37.94	182.70	0.379	44.785	111.30	0.028	7.0
9	0.1	5	30	2.7	0.384	0.46	4.29	2.48	0.338	34.48	168.20	0.377	45.435	111.30	0.028	7.0
10	0.15	2.5	40	3.0	0.384	0.56	4.22	2.92	0.384	44.90	155.54	0.378	46.800	95.40	0.027	8.5
11	0.1	9.2	30	2.8	0.384	0.44	4.24	2.83	0.364	26.08	174.00	0.375	41.990	111.30	0.026	7.0
12	0.1	5	30	2.8	0.384	0.44	4.2	2.55	0.268	41.83	263.90	0.381	57.850	111.30	0.026	7.0
13	0.02	5	30	2.8	0.416	0.44	4.1	2.36	0.374	45.61	220.40	0.376	38.675	111.30	0.025	7.0
14	0.1	0.8	30	2.7	0.384	0.46	4.16	2.03	0.314	32.95	162.40	0.464	40.495	111.30	0.028	7.0
15	0.05	2.5	20	2.4	0.352	0.35	4.21	2.25	0.236	48.07	150.80	0.413	38.935	127.20	0.027	6.5
16	0.18	5	30	2.7	0.384	0.46	4.28	2.07	0.283	49.77	137.80	0.472	39.650	111.30	0.027	7.0
17	0.1	5	13.18	2.5	0.38	0.22	4.25	1.98	0.187	28.47	226.20	0.463	76.245	138.04	0.024	7.5
18	0.15	7.5	20	2.6	0.352	0.31	4.16	2.01	0.204	32.00	153.70	0.419	83.720	127.20	0.025	6.0
19	0.1	5	30	2.7	0.406	0.46	4.23	2.46	0.275	32.51	162.40	0.486	68.510	111.30	0.027	7.5
20	0.15	7.5	40	2.9	0.384	0.58	4.21	2.83	0.351	35.09	281.30	0.363	59.150	95.40	0.028	8.0

(DAHP=Diammonium hydrogen orthophosphate)

Effect on pH, titratable acidity and volatile acidity

Figure 4 depicts the expected response for pH and correlation between the independent variables in three dimensional plots, with DAHP at the X-axis and inoculum concentration at Y- axis while keeping all other variables at their 'O' central levels. Observation on graphs showed that there was an increase in pH value with increase in DAHP and inoculum concentration. Maximum pH (4.29) was observed at 30% juice concentration, 0.10% of DAHP and at 5.0 % inoculum.

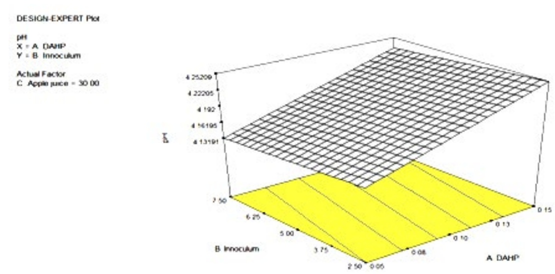

Fig. 4: Effect of DAHP concentration and inoculum size on the pH of bitter gourd apple wine

Figure 5 depicts the expected response of titratable acidity and correlation between the independent variables in three dimensional plots, with DAHP at the X-axis and inoculum concentration at Y-axis while keeping all other variables at their 'O'central levels. DAHP concentration and inoculum concentration has a non-significant effect on titratable acidity. Maximum titratable acidity i.e. 0.41 % was documented at 30% apple juice concentration, 0.2% DAHP and at 5.0 % Inoculum.

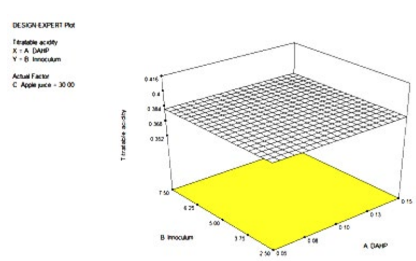

Fig. 5: Effect of DAHP concentration and inoculum size on the titratable acidity (%) of bitter gourd apple wine

It is clear from the Figure 6 that the higher levels of DAHP and inoculum size, increased volatile acidity. Maximum volatile acidity i.e. 0.029 % was observed at 0.05% DAHP concentration, 2.5% inoculum level and 40% apple juice concentration.

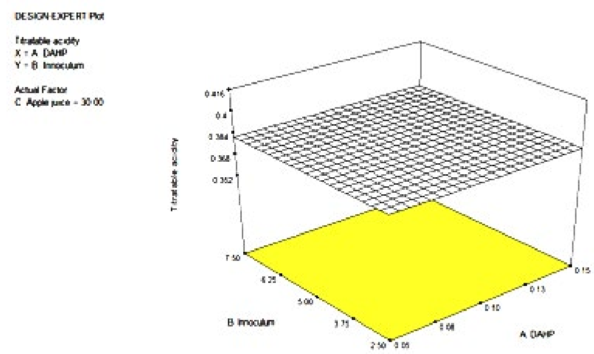

Fig. 6: Effect of DAHP concentration and inoculum size on the Volatile acidity (% acetic acid) of bitter gourd apple wine

Effect on higher alcohol and total phenols

From the figure 7 it can be seen that with respect to DAHP there was an increase in level of higher alcohol upto 0.13% after that there was a slight increase in higher alcohol while with the increase in concentration there was a constant increase in higher alcohols. Maximum higher alcohol (59.01 mg/l) was observed at 13.18% apple juice concentration, 0.10% of DAHP and at 5.0 % inoculum.

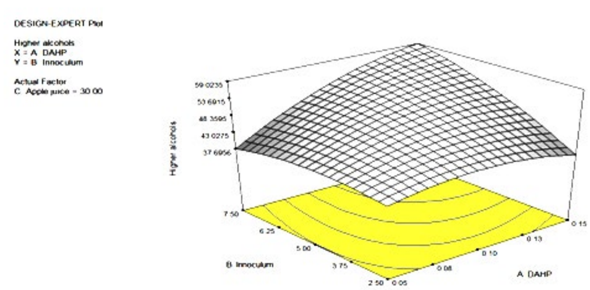

Fig. 7: Effect of DAHP concentration and inoculum size on the higher alcohol (mg/l) of bitter gourd apple wine

From the figure 8 it is seen that there are some interactions among all the factors for total phenols. With respect to DAHP there is increase in total phenols with increase in DAHP level, while with respect to inoculum size there was decrease in total phenol took place as the concentration level of inoculum level increased. Maximum total phenols i.e. 230.65 mg/l was observed at 46.185% apple juice concentration, 0.15% of DAHP and at 2.50 % inoculum.

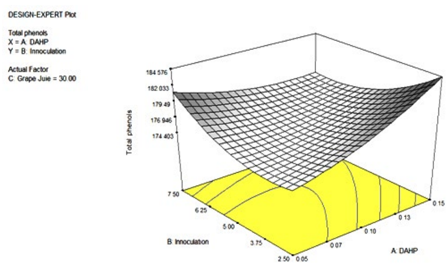

Fig. 8: Effect of DAHP concentration and inoculum size on the total phenol (mg/l) of bitter gourd apple wine

Effect on reducing sugars and total sugars

Reducing sugars

Figure 9 depicts the expected response for reducing sugars and correlation between the independent variables in three dimensional plots, with DAHP at the X-axis and inoculum concentration at Y- axis while keeping all other variables at their 'O'central levels. It is apparent that, with respect to DAHP there was first decrease in reducing sugars up to a level of 0.10% followed by a slight increase in reducing sugars was recorded while in case of inoculum size there was increase in reducing sugars up to 5.00% level and after that decreasing trend was observed. Maximum reducing sugar i.e. 46.34 mg/100g was observed at 46.82 % apple concentration, 0.10% of DAHP and at 5.0 % inoculum and minimum at 0.1% DAHP, 9.2% inoculum level and 30% juice concentration.

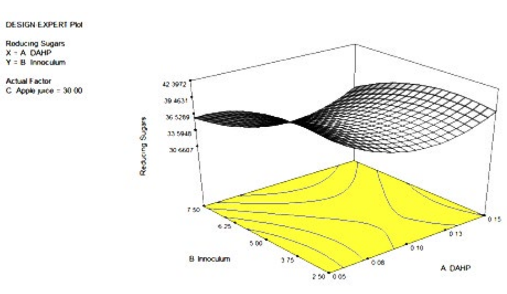

Fig. 9: Effect of DAHP concentration and inoculum size on the Reducing sugars (%) of bitter gourd apple wine

Graph shows that there was a decrease in total sugar content with increase in the concentration of DAHP and inoculum size (Fig.10). Maximum total sugar i.e. 0.374% was observed at 30 % apple juice concentration, 0.02% of DAHP and at 5.0 % inoculum, while minimum at 0.15% DAHP, 7.5% inoculum level and 20% juice concentration was recorded.

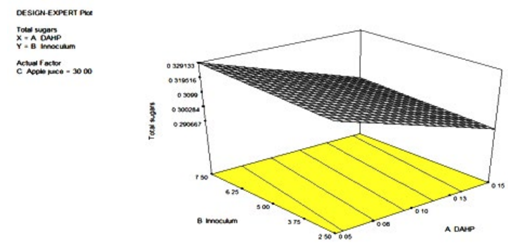

Fig. 10: Effect of DAHP concentration and inoculum size on the Total sugars (%) of bitter gourd apple wine

Effect on colour and overall acceptability

There is no effect of various factors on colour values of different wines. All the factors have acted independently towards colour (Fig.11). With respect to DAHP a constant increase in colour intensity was recorded, while decreasing trend was recorded with respect to inoculum size. Maximum colour intensity of 0.486 was observed at 30% apple juice concentration, 0.10% of DAHP and at 5.0 % inoculum size.

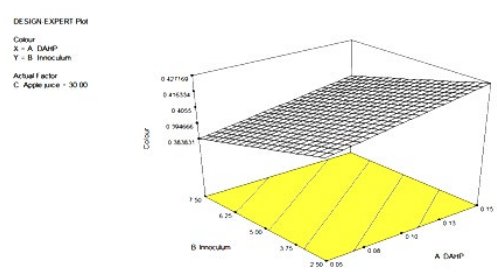

Fig. 11: Effect of DAHP concentration and inoculum size on the Colour (OD at 440 nm) of bitter gourd apple wine

Figure 12 depicts the expected response of Overall acceptability and correlation between the independent variables in three dimensional plots, with DAHP at the X-axis and inoculum concentration at Y- axis while keeping all other variables at their

'O' central levels. DAHP concentration and inoculum concentration has a negative effect on Overall acceptability. A decreasing trend with increase in DAHP and inoculum size was observed. Maximum Overall acceptability i.e. 8.5 % was observed at 40% apple juice concentration, 0.15% of DAHP and at 2.5 % inoculum level.

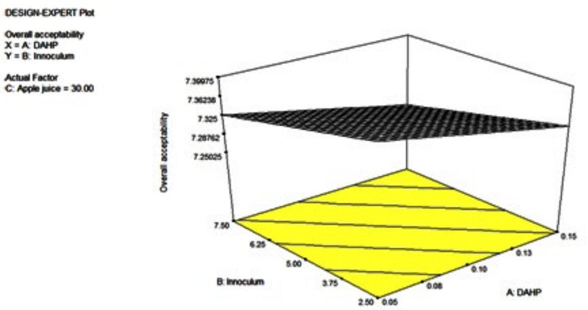

Fig. 12: Effect of DAHP concentration and inoculum size on the Overall acceptability of bitter gourd apple wine

Runs having 40% apple, 0.15 % DAHP, and 2.5 % inoculum level gave the best results for all the parameters (Table 3). Optimization of parameters by the conventional method involves changing one independent variable while keeping all others at a fixed level. This is extremely time-consuming and expensive for a large number of variables (Adinarayana *et al.* 2003) and may also lead to wrong conclusions. Optimized results for initial sugar concentration are in line with the findings of Joshi and Sandhu (1997) and Joshi *et al.* (2011a) who reported that initial sugar concentration (ISC) of the must influences the physico-chemical and sensory characteristics of the wine. The level of 40% juice optimized in our study was considered desirable, as the purpose was to produce a low alcoholic beverage, to serve ultimately as a medicinal product. That is why no additional sugar was added. In our optimization, 0.2 % DAHP gave the best results for all the parameters but these are similar to these findings of Joshi *et al.* (1990a) who reported that addition of DAHP at the rate of 0.1 % enhanced the rate of fermentation considerably in wild apricot fermentation, regardless of dilution levels. The difference could be related with different levels nitrogen content available with must of a specific fruit which determine the addition as not of nitrogen source as is the case of concentration of DAHP.

Conclusion

Based on the results of RSM, among different concentrations of apple juices, bitter gourd based wine having 40% apple juice concentration had the highest TSS, rate of fermentation, ethanol, reducing sugars, total sugar and total phenols. With 0.15% DAHP as a nitrogen sources, bitter gourd based wine had the highest total soluble solid (TSS), titratable acidity, ethanol, total and reducing sugar content and lowest volatile acidity and higher alcohols. Among different concentrations of innoculum used, for most of the parameters, there were non-significant differences. Must inoculated with 2.5% *Saccharomyces cerevisiae* var *ellipsoideus* for the preparation of bitter gourd based wine had the highest fermentability and ethanol content. Bitter gourd based wine having 40% apple juice concentration as a sugar source, 0.15% DAHP as a nitrogen source and fermentation with 2.5% *Saccharomyces cerevisiae* var. *ellipsoideus* scored the highest score for overall acceptability. On the basis of physico-chemical and sensory characteristics, run having 40% apple juice concentration, 0.15 % DAHP and 2.5 % inoculum level was rated as the best and hence optimized.

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