IJFANS
International Journal of Food And Nutritional Sciences

Volume 6, Issue 4, October 2017, www.ijfans.com e-ISSN: 2320-7876

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

IJFANS International Journal of Food and Nutritional Science

e-ISSN 2320 -7876 www.ijfans.com
Vol. 6, No. 4, October 2017

Research Paper ***Open Access***

OPTIMIZATION OF MANGO VINEGAR PRODUCTION BY USING RESPONSE SURFACE METHODOLOGY (RSM)

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Received on: 4th July, 2017 Accepted on: 25th September, 2017

Fruit vinegar is one of the fermented products that combine beneficial properties of a fruit and vinegar, which is gaining importance as a functional nutraceutical food. The present study is aimed at optimizing the acetic acid fermentation conditions for mango (*Mangifera indica Linn*.) vinegar production by Response Surface Methodology (RSM). The juice was extracted from mango fruits by enzymatic treatment and adjusted to 25°Brix. It was used as a substrate for vinegar production, first by alcoholic fermentation using *Saccharomyces cerevisiae* strain (CFTRI 101) that gave 11% (v/v) ethanol, followed by acetic acid fermentation using Acetobacter aceti• (MTCC No: 2945) that produced >4.5% acidity. The ethanol and acetic acid levels produced were analyzed by Spectrophotometric method and titrimetric method, respectively. The optima of temperature, pH, time, ethanol content and inoculum volume were found to be 30 °C, 4.5, 113 h, 8.0% and 10.5%, respectively for the highest yield of acetic acid (65.12 g/L) by the RSM. This has a dual advantage of effective use of surplus mango fruit as well as a value-added new vinegar product.

Keywords: Fermentation, Acetic acid, Mango vinegar, RSM

INTRODUCTION

Vinegar is an important food preservative and condiment which has a long history starting from ca. 3000 BC (Li *et al*., 2014). It is produced through the action of acetic acid bacteria on dilute solutions of ethyl alcohol derived from yeast fermentation, which has versatile and distinct organoleptic properties. It is a liquid fit for human consumption produced from a suitable raw material having starch and/or sugars of agricultural origin, containing a specified amount of acetic acid (Codex Alimentarius, 1987). Currently, the demand for fruit vinegar consumed as a health food product is growing (Ou *et al*., 2009) as it is rich in amino and organic acids, vitamins, and aroma compounds (Chang *et al*., 2004). Fruit vinegar is known for its accelerative buffering effect during the digestion and the acetic acid, which is the main component of vinegar, significantly reduces both blood pressure and rennin activity and the subsequent decrease in angiotensin II, a typical blood pressure regulatory system (Kondo *et al*., 2001). In addition, it has been reported that regular consumption of vinegar contributes to some beneficial effects in terms of anti-oxidative properties and lowering lipid content (Chou *et al*., 2015). Reducing effects of diabetes and prevention of cardiovascular disease are the described functional therapeutic properties of vinegar (Shimoji *et al*., 2002). These health benefits were lead researchers to consider the production of natural vinegars from different raw materials including cane vinegar made from fermented sugarcane juice (Kocher *et al*., 2014), pineapple vinegar (Silva *et al*., 2007), tomato vinegar (Lee *et al*., 2013), blackberry vinegar (Antonio *et al*., 2016), persimmon vinegar (Hidalgo *et al*., 2010), onion vinegar (Horiuchi *et al*., 1999), water melon

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vinegar (Chen *et al*., 2017), orange vinegar (Cristina *et al*., 2016) and so on. However, there are few studies made on the mango fruit vinegar.

The mango (*Mangifera indica* L.) has gained great popularity worldwide among the tropical fruits as a commercially significant fruit, because of its high nutritive-medicinal value, unique flavors and taste. It is known as an excellent source of β-carotene (provitamin A carotenoid), vitamin C and polyphenolic compounds with traces of vitamins E, K and B. It is the 2nd largest tropical crop and India accounts for 52% of the world's production of mango, which is nearly 12.75 million tons per annum, but hardly 1% of the total mango produced is processed in to various products. In spite of some alternatives to direct consumption have already been implemented, a large amount of fruits are left un-utilized in the fields creating both ecologic and economic problems. As the consumption of fruit vinegars are significantly increasing over synthetic vinegars, this study is aimed at the production of mango vinegar and optimized by Response Surface Methodology (RSM).

Optimization of parameters by the conventional method involves changing one independent variable while unchanging all others at a fixed level. This is extremely time-consuming and expensive for a large number of variables and also may result in wrong conclusions (Adinarayana *et al*., 2003). RSM is a combination of mathematical and statistical techniques that is useful for analyzing the effects of several independent variables on the system response (Oh *et al*., 1995). Therefore, RSM technique was applied in the present study and results obtained are discussed in this paper.

MATERIALS AND METHODS

Processing of Mango Fruits

Totapuri variety of mango fruits were obtained from the local market of Tirupati (India), during the months of May and June. The fruits were processed according to the method of Varakumar *et al*. (2012) and the samples were stored at -20 °C until further use. Completely ripened mango fruits were selected randomly, peeled and stones were separated manually from the pulp. The pulp thus obtained was ground in a mixer. The homogenate was treated with pectinase (Bio-Tropicase, Biocon, India) enzyme then filtered through two layered cheese cloth. The juice thus obtained was centrifuged at 10,000 g for 10 min at room temperature to obtain clear supernatant.

Microorganisms and Preparation of Inoculum

The *Saccharomyces cerevisiae* strain (CFTRI 101) was maintained on MPYDA slants containing (g/L): Malt extract, 3; Peptone, 5; Yeast extract, 3; Dextrose, 10; and Agar, 20 (pH 5.0) and stored at 4 ºC. The yeast cells were grown by inoculating the slant culture into 25 mL of the sterile MPYD liquid medium in 100 mL Erlenmeyer conical flask and incubating on a rotary shaker (100 rpm) for 24 h. This inoculum (3×10^6 cells/mL) was transferred to 250 mL conical flask having 100 mL sterile mango juice and incubated at 28 °C, on a rotary shaker (100 rpm) for 24 h. This was used as inoculum in the fermentation of mango juice to mango wine (Kumar *et al*., 2009).

For acetification, the inoculum was prepared by inoculating a standard culture of *Acetobater aceti* (MTCC No. 2945) from YPMA slants containing (g/L): Yeast extract, 5.0; Peptone, 3.0; Mannitol, 2.5 and Agar 20.0, in to a 150 mL Erlenmeyer flask containing 50 mL sterile liquid YPM medium and incubating the flask on a rotary shaker at 200 rpm and 30 °C for 24 h.

Alcoholic Fermentation

The alcoholic fermentation was carried out by adding aseptically 5% inoculum of the actively growing *Saccharomyces cerevisiae* strain (CFTRI 101) in to a 5.0 L stainless steel fermenter (BioSpin-05i, BIO-AGE, Mohali, India) having working volume of 3.0 L of sterile (sterilized at 121 °C for 20 min.) mango juice as fermentation medium. The fermentation was carried out at 28 °C under agitation of 100 rpm for 5 days. After fermentation, the broth was centrifuged at 5000 rpm for 20 min to separate out the cells, and then the supernatant wine was pasteurized (using a water bath at 65 °C for 30 min). This wine was stored at 8 °C in amber colored airtight glass container to avoid oxygen contact as described by Naresh *et al.* (2014).

Acetic Acid Fermentation

The acetic acid fermentation of the above wine was carried out by setting pH to the desired value (3.5 to 5.5) by means of calcium carbonate or citric acid addition, and inoculating with *A. aceti* (MTCC No: 2945) inoculum (5 to 15%) and incubating on a rotary shaker incubator at 160 rpm at different temperatures (26 to 34 °C) and time (48 to 144 h). All the runs were carried out, according to the Central Composite Design (CCD). The fermented broth was centrifuged at 10,000 rpm, and the supernatant vinegar was pasteurized by using a

water bath at 65 °C for 30 min. The samples were analyzed for various parameters.

ANALYTICAL PROTOCOLS

Determination of Soluble Solids and Ethanol of Mango Wine and Vinegar

The soluble solid content was determined at 20 °C with a refractometer (0-30) (Erma, Japan). All measurements were conducted in triplicate and reported as °Brix. The percentage of ethanol produced during alcoholic fermentation and acetic acid fermentation were determined by Spectrophotometric method (Caputi *et al*., 1968). According to this method, diluted sample was distilled at 40 °C and the distillate containing ethanol was reacted with chromic acid and incubated at 50 °C for 30 min. The colour developed was read using spectrophotometer at 600 nm.

Determination of pH and Titratable Acidity of Mango Wine and Vinegar

Samples were centrifuged (5 min at 350 × g) and the pH was measured at 20 °C using a hand digital pH meter (Eutech, Japan) which was pre-calibrated with buffers of pH 4.0 and 7.0. Titratable acidity was determined by titrating with 0.1 N NaOH previously standardized using standard oxalic acid and the values were expressed as tartaric acid equivalents.

High-Performance Liquid Chromatography (HPLC)

The content of organic acids was detected through HPLC (Shimadzu) using a Supelco gel C-610H column (Supelco, Bellefonto, PA, USA) connected to a UV detector. Mango vinegar sample (1 mL) and distilled water (9 mL) were mixed for 2 min in a 10 mL flask using a vortex mixer. The supernatants were filtered twice using a 0.2 μm filter membrane. The column temperature was set at 40 °C with a degassed aqueous mobile phase containing 8mM H_2SO_4 in Milli Q H_2O (isocratic mode). The injection volume was 10 μL, and the detection wavelength was at 210 nm. The flow rate was 0.7 mL min^{-1}. All organic acids were recorded on a computer-based data system. Each compound was quantified by comparing its peak area against the standard curve obtained specifically for the reference solutions containing that compound.

Measurement of Colour

The colour of the mango vinegar sample produced was measured using a Hunter's Lab color measurement device (Hunter Color Ultra Scan PRO, Hunter Associates Laboratory, Reston, VA, USA). The colour values were expressed as L* (whiteness or brightness/darkness), a* (redness/greenness), b* (yellowness/blueness), chroma (saturation, C*) and hue angle (h°) at any time, respectively.

Sensory Analysis

The mango vinegar produced with the optimized conditions is subjected to sensory analysis using ten-point Hedonic scale with trained panel experts to evaluate its quality by comparing with the original vinegar produced with normal conditions. A structure scale was used to score all the attributes, with 9-10 representing outstanding vinegar, 7-8.9 representing standard vinegar 5-6.9 representing commercial vinegar, 3-4.9 representing below commercial vinegar acceptability and 1-2.9 spoiled vinegar. Coded samples identified by three-digit random numbers were presented to panelists in random order. The analysis was carried out in triplicate.

Experimental Design of the RSM

It is an empirical mathematical modeling technique which was employed in this study to understand the interaction influence of temperature, pH, incubation time, alcohol content and inoculum volume on acetic acid production by *A. aceti*. A 45-runs central composite design was employed using a Central Composite Design (CCD) according to RSM using STATISTICA version 8.0 software (Stat-soft Inc., Minneapolis, MN, USA) for this study. All selected variables were studied in 5 levels. Table 3 depicts the selected variables and their levels and Table 4 depicts the experimental plan along with the results obtained. All real values were coded in to the -2 to + 2 according to Hymavathi *et al.* (2009). The obtained results were subjected to multiple regression analysis. The empirical formula of the regression equation is as follows:

$$Y_i = {}_0 + \sum_{i=1}^{k} {}_i x_i + \sum_{i=1}^{k} {}_{ii} x_i^{\,2} + \sum_{i}^{i\langle} \sum_{j}^{j} {}_{ij} x_i x_j + e \quad ---(1)$$

where Y_i is the predicted acetic acid production, ${}_0$ is the offset term, ${}_i$ is the i^{th} linear coefficient, ${}_{ii}$ is the i^{th} quadratic coefficient, ${}_{ij}$ is the interaction coefficient x_i and x_j are input variables that influence the response variable, and e is the error. The regression analysis was performed with coded values. The correlation coefficient (R^2) value was used to determine the percentage of the variability of the optimization parameter that is explained by the model. The statistical analysis of the model was performed in the form

of an analysis of variance (ANOVA) which is depicted in Table 5. The surface (3D) and contour (2D) plots were used to determine the interactive effect of selected variables on acetic acid production.

Statistical Analysis

All experiments were performed in triplicate. Results were expressed as the mean values with standard error. Analysis of Variance (One-way ANOVA) followed by Duncan's multiple range test was performed to identify differences between means, using SPSS Software version 20.0 (SPSS-IBM Chicago, IL, USA). Differences were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

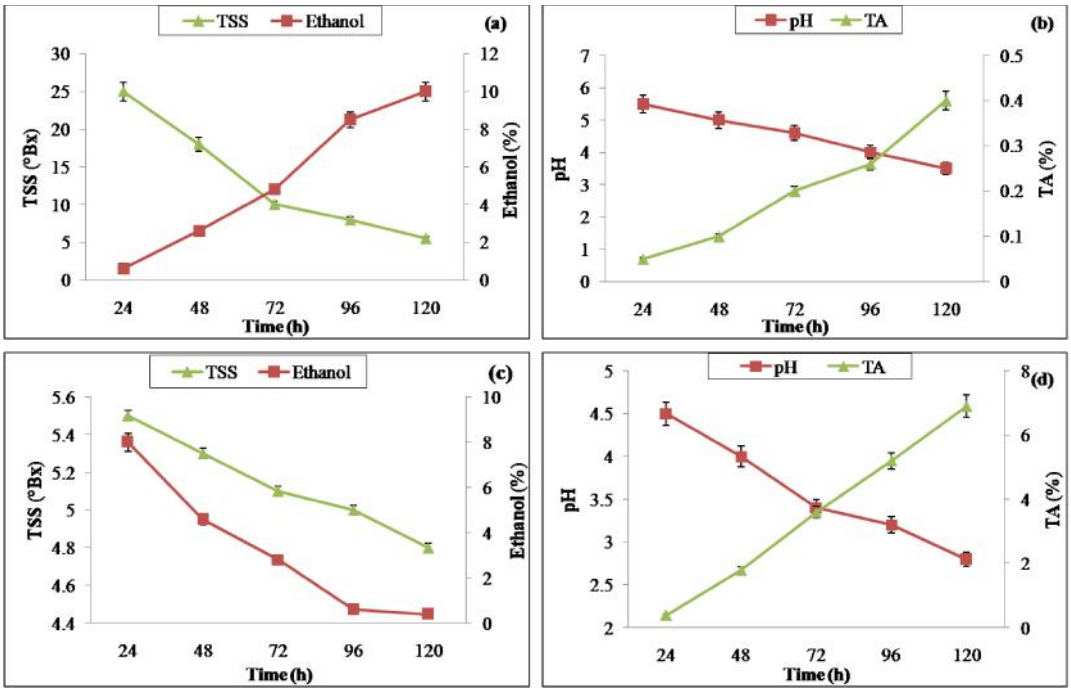
Determination of Soluble Solid Content, Ethanol Content, pH and Acidity of Mango Wine

Changes in soluble solid content and ethanol content of mango wine during alcoholic fermentation are shown in Figure 1a. The soluble solid content decreased gradually during alcoholic fermentation while the ethanol content increased gradually and reached 5.5 °Brix and 10%, respectively after 5 days of alcoholic fermentation. Changes in pH and titratable acidity during alcoholic fermentation of mango wine are shown in Figure 1b. The pH decreased from 5.5 to 3.5 over 5 days of alcoholic fermentation. The acidity increased gradually during alcoholic fermentation and after 5 days it reached 0.40%.

Determination of Soluble Solid Content, Ethanol Content, pH and Acidity of Mango Vinegar

Changes in soluble solid content and ethanol content of mango vinegar during acetic acid fermentation are shown in Figure 1c. The soluble solid content as well as ethanol content decreased gradually, during acetic acid fermentation and reached 4.8 °Brix and 0.4%, respectively after 5 days of acetic acid fermentation. Changes in pH and titratable acidity during acetic acid fermentation of mango vinegar are shown in Figure 1d. The pH decreased from 4.5 to 2.8 over 5 days of acetic acid fermentation. The acidity increased gradually during acetic acid fermentation and after 5 days it reached 6.9%. The acidity differences in various vinegars could be attributed to variations in the raw materials, inoculum volume of acetic acid bacteria added, as well as fermentation time

Figure 1: Changes in TSS and Ethanol (a), pH and Titratable Acidity (b) (During Alcoholic Fermentation) Followed by Changes in TSS and Ethanol (c), pH and Titratable Acidity (d) During Acetic Acid Fermentation



and dilution (Li *et al*., 2014). The acidity obtained was in the same range as strawberry and persimmon vinegar (Hidalgo *et al*., 2010). The final acidity achieved in the present study was higher than that achieved in ginger vinegar (4.82%), garlic vinegar (4.9%) and tomato vinegar (5.6%) (Ko *et al*., 1998; Lee *et al*., 2013; and Leonel *et al.*, 2015).

Organic Acids in Mango Vinegar

The level and nature of the organic acids present in vinegars may provide information concerning the origin of the raw material, microbiological growth, and even processing techniques. Although many organic acids are present in vinegars, the titratable acidity of vinegars is typically expressed as acetic acid, which is the major organic acid in vinegars (Morales *et al*., 1998). In the present study, oxalic acid, tartaric acid, malic acid, acetic acid, citric acid, succinic acid and propanoic acid were found in mango vinegar (Table 1). The major organic acid in this fermentation was acetic acid and its concentration reached to a level of 65.12 g/L after acetic acid fermentation. The amount of acetic acid in mango vinegar was higher than that of onion vinegar (29.4 g/L) (Horiuchi *et al*., 1999) and watermelon vinegar (52.95 g/L) (Yang Chen *et al*., 2017). A similar observation had previously been made in white wine vinegar, red wine vinegar, alcohol vinegar, malt vinegar, and cider vinegar (Morales *et al*., 1998; and Saiz-Abajo *et al*., 2005). Hence the mango wine fermentation to vinegar yields better levels of acetic acid and other organic acids than the other sources.

Determination of Colour of Mango Vinegar

The colour or pigment in the fruit vinegars represents the presence of certain biologically active phyto-chemical compounds that have been reported to promote good health as well as the quality of the product. Since, colour is an important factor related to the sensorial properties of vinegar (Lopez *et al*., 2005), the colour of mango vinegar was measured and the results are given in Table 2.

Table 1: The Main Organic Acids Content of the Mango Vinegar (g/L)

Organic Acids	Mango Vinegar
Oxalic acid	0.85±0.17
Tartaric acid	1.07±0.01
Malic acid	1.16±0.02
Acetic acid	65.12±0.08
Citric acid	0.49±0.02
Succinic acid	0.57±0.01
Propanoic acid	0.61±0.05

Table 2: Hunter's Color Value of Mango Vinegar

Hunter Parameters	Mango Vinegar
L* value	15.56±0.07
a* value	2.92±0.05
b* value	11.81±0.04
Chroma (C*)	12.17±0.05
Hue angle (h*)	76.13.±0.03

Note: Mean ± standard deviation (n=3).The values are given as mean ± standard deviation of triplicate determinations.

Model Prediction and Optimization

The single-factor experiment showed that the temperature, pH, time, alcohol content and inoculum volume significantly affected the quality of mango vinegar fermentation. To improve further acetic acid production and to determine the interaction influence of these parameters on acetic acid yield, a CCD method of optimization was employed. The full-factorial CCD with 45 experiments runs along with obtained experimental results are shown in Table 4. Through the experimentation, it was shown that the acetic acid production varied from 2.5 to 6.5% indicating the influence of selected parameters and their levels on *A. aceti* metabolism and subsequent effect on the acetic acid yield. To understand the relationship between selected variable and acetic acid production, a regression analysis was performed. In regression analysis, the acetic acid yield was taken as a depended variables and selected parameters as independent variables. The analysis of data yielded the following second-order polynomial equation (Equation 2).

Acetic acid% (Y) =6.47625 +0.12750* X1 +0.11250* X2 +0.63750* X3 +0.15750* X4 +0.11250* X5 -0.96016* X1 *X1 -0.63516 * X2* X2 -0.48516 * X3* X3 -0.44766 * X4* X4-0.29766 * X5* X5 +0.090625 * X1 * X2 +0.12187* X1 * X3 +3.12500E-003 * X1 * X4 +0.071875* X1 * X5 +0.14062* X2 * X3 -0.015625* X2 * X4 +0.028125* X2 * X5 -0.021875* X3 * X4 +9.37500E-003* X3 * X5 +0.028125* X4 * X5 ...(2)

The model's goodness of fit was checked by determination coefficient (R^2). The R^2which can be defined

Table 3: Experimental Range of Alpha Values of the Independent Variables for the Mango Vinegar Production

Variables		Coded Levels					Step Change (ΔS)
Real	Coded	-2	-1	0	1	2	
Temperature, (°C)	X1	26	28	30	32	34	2
pH	X2	3.5	4	4.5	5	5.5	0.5
Time, (h)	X3	48	72	96	120	144	12
Alcohol content, (%)	X4	6	7	8	9	10	1
Inoculum volume, (%)	X5	5	7.5	10	12.5	15	2.5

Table 4: Central Composite Experimental Design for Optimization Along with Experimental and Predicted Acetic Acid %

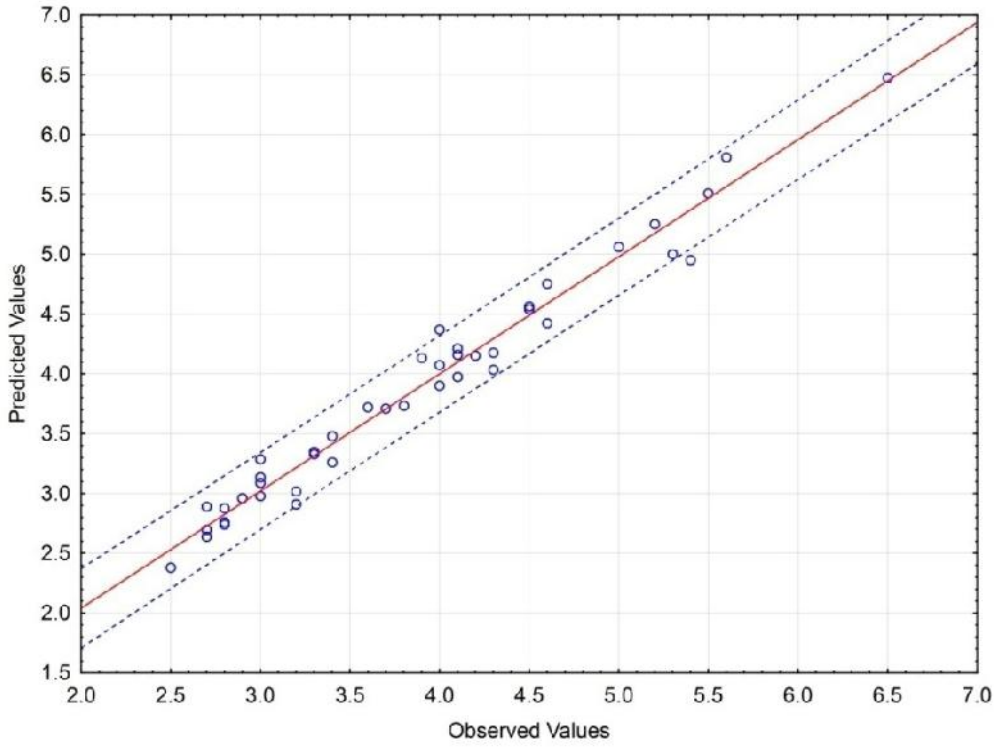
S. No.	Temperature (°C) (X1)	pH (X2)	Time (h) (X3)	Alcohol Content (%) (X4)	Inoculum Volume (%) (X5)	% of Acetic Acid		
						Experimental	Predicted	Error
1	28	4	72	7	7.5	2.9	2.96	-0.06
2	28	4	72	7	12.5	3.2	2.91	0.29
3	28	4	72	9	7.5	3	3.29	-0.29
4	28	4	72	9	12.5	3.3	3.35	-0.05
5	28	4	120	7	7.5	3.8	3.73	0.07
6	28	4	120	7	12.5	3.6	3.72	-0.12
7	28	4	120	9	7.5	4.1	3.97	0.13
8	28	4	120	9	12.5	4	4.07	-0.07
9	28	5	72	7	7.5	2.7	2.7	0
10	28	5	72	7	12.5	2.8	2.76	0.04
11	28	5	72	9	7.5	2.9	2.96	-0.06
12	28	5	72	9	12.5	3	3.14	-0.14
13	28	5	120	7	7.5	4.3	4.03	0.27
14	28	5	120	7	12.5	3.9	4.13	-0.23
15	28	5	120	9	7.5	4.1	4.21	-0.11
16	28	5	120	9	12.5	4.6	4.42	0.18
17	32	4	72	7	7.5	2.7	2.64	0.06
18	32	4	72	7	12.5	2.8	2.88	-0.08
19	32	4	72	9	7.5	3	2.98	0.02
20	32	4	72	9	12.5	3.3	3.33	-0.03

Table 4 (Cont.)

21	32	4	120	7	7.5	4	3.9	0.1
22	32	4	120	7	12.5	4.3	4.18	0.12
23	32	4	120	9	7.5	4.2	4.15	0.05
24	32	4	120	9	12.5	4.5	4.54	-0.04
25	32	5	72	7	7.5	2.8	2.74	0.06
26	32	5	72	7	12.5	3	3.09	-0.09
27	32	5	72	9	7.5	3.2	3.02	0.18
28	32	5	72	9	12.5	3.4	3.48	-0.08
29	32	5	120	7	7.5	4.5	4.56	-0.06
30	32	5	120	7	12.5	5.4	4.95	0.45
31	32	5	120	9	7.5	4.6	4.75	-0.15
32	32	5	120	9	12.5	5.2	5.25	-0.05
33	26	4.5	96	8	10	2.5	2.38	0.12
34	34	4.5	96	8	10	2.7	2.89	-0.19
35	30	3.5	96	8	10	3.7	3.71	-0.01
36	30	5.5	96	8	10	4.1	4.16	-0.06
37	30	4.5	48	8	10	3.4	3.26	0.14
38	30	4.5	144	8	10	5.6	5.81	-0.21
39	30	4.5	96	6	10	4	4.37	-0.37
40	30	4.5	96	10	10	5.3	5	0.3
41	30	4.5	96	8	5	5	5.06	-0.06
42	30	4.5	96	8	15	5.5	5.51	-0.01
43(C)	30	4.5	96	8	10	6.5	6.48	0.02
44(C)	30	4.5	96	8	10	6.5	6.48	0.02
45(C)	30	4.5	96	8	10	6.5	6.48	0.02

as the ratio of the explained variation to the total variation was a measure of the degree of fit. The closer the R^2 value to 1, the better the empirical model fits the actual data. The value of determination of coefficient R^2 is 0.9794, which indicated that model could explain 97.94% of variability and unable to explain only 2.06% of the total variation. The adjusted R^2 was a corrected value for R^2 after elimination of the unnecessary model terms. If many non-significant terms have been included in the model, the adjusted R^2 would be remarkably smaller than the R^2. The adjusted R^2 was 0.96223, which is more suitable for comparing models with different numbers of independent variables. The observed low difference (0.01717) of the adjusted R^2value (0.96223) and the R^2 value (0.9794) further confirm the data accuracy. The predicted values of acetic acid are presented in the Table 4. It was noticed that majority runs have the percent error below 5. The low percentage of variation between the observed and predicted values indicates the accuracy of the experiments done. Figure 2 depicts the correlation between the experimental and predicted values. In this figure

Figure 2: Predicted vs. Actual Observation Run Values for Acetic Acid Yield



all the data points nearer to the regression line indicate the good correlation of obtained and predicted acetic acid yield values.

The analysis of the variance (ANOVA) of the quadratic regression model demonstrated that Equation 2 is statistically significant model of acetic acid content response in the vinegar samples, as was evident from higher F-value (51.32) and lower p-value ($p<0.0001$). Further the adequate to precision value of 28.266 and coefficient of variation value of 5.35 indicates that this model can be used to navigate the design space. Table 5 shows the effects, regression coefficients and ANOVA. From the effects in the table, it was noticed that among selected parameters square term of temperature has highest effect followed by square term of pH and linear term of time. The interaction terms have no significant effect, which signifies that there is no strong interaction influence of selected parameters on acetic acid production by *A. aceti.* Temperature, pH, alcohol content and inoculum volume has higher effect values in their square terms compared with linear terms, which indicates that these parameters levels have high influence on the acetic acid production, a small variation could result in a large variation in the production. Coefficients which have a low p-value and high F-value are considered as significant terms. Based on this, all linear and square terms are significant. The interaction terms of temperature with pH and time (X1*X2 and X1*X3) as well as pH and time (X2*X3) were significant, remaining all other terms were insignificant.

The regression Equation (2) developed here was used to generate 3D, 2D surface and contour plots respectively. Using the drawn surface and contour plot interactions, selected parameters at different conditions were evaluated. All contours were circular or elliptical in nature, indicating that all selected parameters were independent of each other. Figure 3a and c depict the interaction of temperature with pH, fermentation time and alcohol content. Temperature at the range of 30-32 °C was optimum for effective acetic acid production by *A. aceti*. The maximum acetic acid yield obtained at higher fermentation time with the temperature at its middle level. The Figure 3d represents the interaction of the pH and time on the acetic acid production. The interactive effect of the variable on the response was found to be significant. A slight change in pH does not affect the production of the acetic acid. Figures 3e and 3f depict the significant interaction of inoculum volume with fermentation time and alcohol content, respectively. Increased or

Table 5: Effects and Analysis of Variance (ANOVA)

Factor	Effect	Coefficients	SS*	DF*	MS*	t-value	p-value	F-value
Mean or Intercept	6.47625	6.47625				55.7455	0	
X1	0.255	0.1275	0.65025	1	0.65025	3.8018	0.000868	14.4536
X2	0.225	0.1125	0.50625	1	0.50625	3.3545	0.002636	11.2528
X3	1.275	0.6375	16.25625	1	16.25625	19.0089	0	361.3389
X4	0.315	0.1575	0.99225	1	0.99225	4.6963	0.00009	22.0554
X5	0.225	0.1125	0.50625	1	0.50625	3.3545	0.002636	11.2528
X1*X1	-1.92031	-0.96016	23.60064	1	23.60064	-22.9039	0	524.5877
X1*X2	-1.27031	-0.63516	10.32764	1	10.32764	-15.1512	0	229.5596
X3*X3	-0.97031	-0.48516	6.02564	1	6.02564	-11.5731	0	133.9361
X4*X4	-0.89531	-0.44766	5.13014	1	5.13014	-10.6785	0	114.0312
X5*X5	-0.59531	-0.29766	2.26814	1	2.26814	-7.1004	0	50.4155
X1*X2	0.18125	0.090625	0.26281	1	0.26281	2.417	0.023615	5.8417
X1*X3	0.24375	0.121875	0.47531	1	0.47531	3.2504	0.003399	10.5651
X1*X4	0.00625	0.003125	0.00031	1	0.00031	0.0833	0.93427	0.0069
X1*X5	0.14375	0.071875	0.16531	1	0.16531	1.9169	0.067231	3.6745
X2*X3	0.28125	0.140625	0.63281	1	0.63281	3.7505	0.000987	14.066
X2*X4	-0.03125	-0.01563	0.00781	1	0.00781	-0.4167	0.680588	0.1737
X2*X5	0.05625	0.028125	0.02531	1	0.02531	0.7501	0.46049	0.5626
X3*X4	-0.04375	-0.02188	0.01531	1	0.01531	-0.5834	0.565063	0.3404
X3*X5	0.01875	0.009375	0.00281	1	0.00281	0.25	0.804691	0.0625
X4*X5	0.05625	0.028125	0.02531	1	0.02531	0.7501	0.46049	0.5626
Error			1.07973	24	0.04499			
Total SS			52.40311	44				

Note: SS*- sum of squares, DF- degree of freedom, MS- mean square.

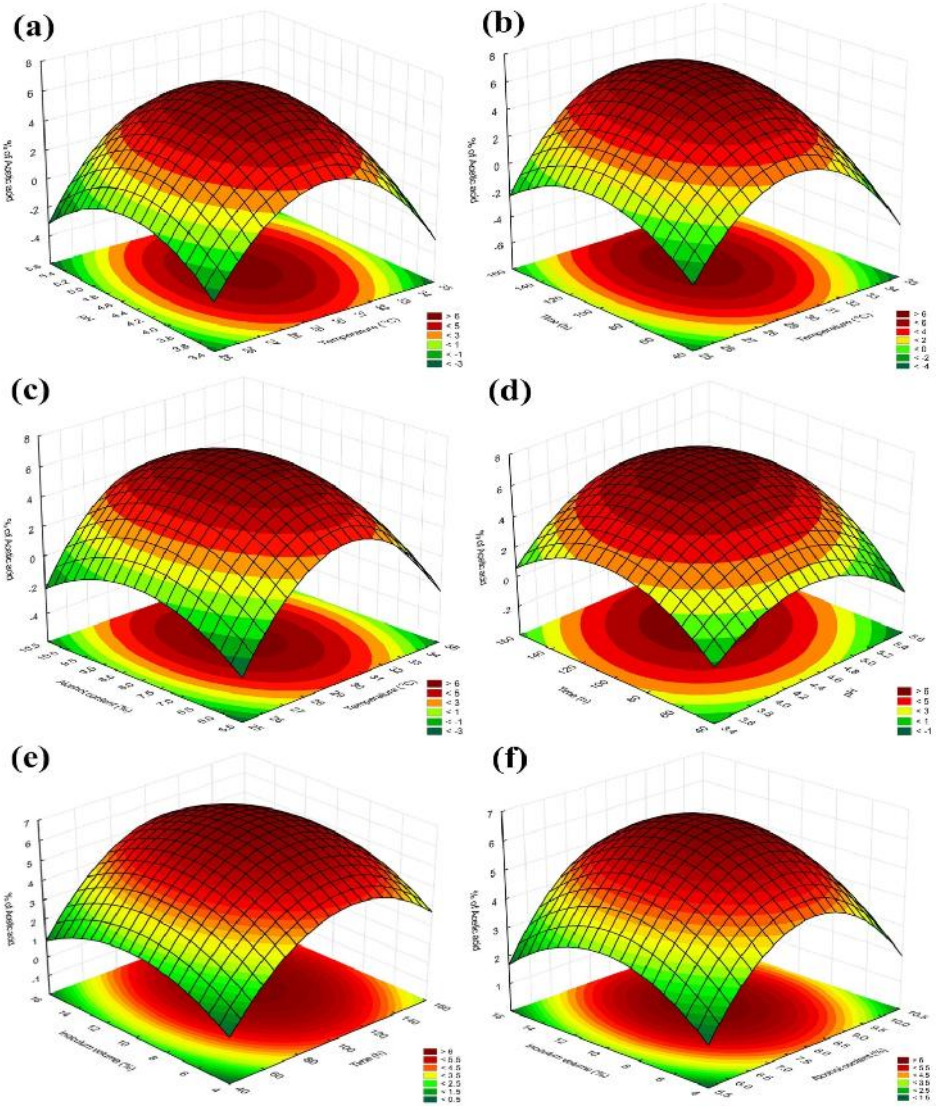
decreased concentration of alcohol content eventually decreases the acetic acid yield at longer fermentation time.

By using the numerical methods from Equation 2 the optimum conditions were determined. The optimum combination of factors was as follows: temperature (30.26 °C), pH (4.5), time (113 h), alcohol content (8.16 %) and inoculum volume (10.57%). At these conditions the acetic acid predicted from the mode was 6.48%. Repeated experiments were performed at optimum conditions to verify the predicted model. The actual acetic acid obtained was 6.5%. Validation experiments indicate that the developed model was precise for acetic acid production by *A. aceti*.

Optimized Fermentation Conditions

The independent variables, namely fermentation temperature, pH, time, alcohol content and inoculum volume

Figure 3: Response Surface Plot Show the Interactive Effect of Selected Parameters on Acetic Acid Yield, a) Temperature and pH, b) Temperature and Fermentation Time, c) Temperature and Alcohol Content, d) pH and Fermentation Time, e) Inoculum Volume and Fermentation Time, f) Alcohol Content and Inoculum Volume

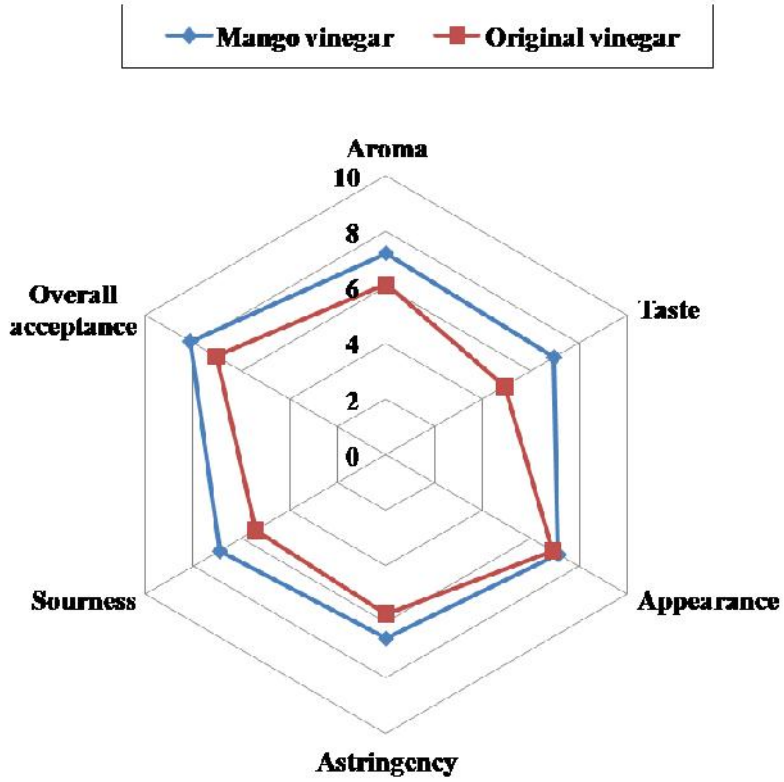


were optimized. For convenient practical operation, the optimum combination of factors was set as follows: temperature (30 °C), pH (4.5), time (113 h), alcohol content (8%) and inoculum volume (10.5%) gives 65.12 g/L of acetic acid production. In comparison, the production of acetic acid by *Acetobacter pasteuriem* AS.1.41 is 52.45 g/L which is relatively lesser than present result, and also reported that the inoculum volume, alcohol content and temperature influence the acetic acid production (Qui *et al.*, 2015). Ghosh *et al.* (2012) reported that the highest yield of acetic acid was 68.12 g /L by *Acetobacter aceti* (NCIM 2251) where pH, temperature and time were regarded to have significant influence.

Sensory Evaluation

From the sensory analysis of the mango vinegar, it was found that the overall acceptance (8.12 ± 0.23) of optimized vinegar in terms of aroma (7.21 ± 0.86), taste (6.97 ± 0.32), appearance (7.14 ± 1.6), astringency (6.57 ± 0.02) and sourness (6.89 ± 0.15), was better than the sensory analysis of the original vinegar which has shown the overall acceptance (7.05 ± 0.15) with respect to aroma (6.1 ± 0.02),

Figure 4: Sensory Evaluation of Original and Mango Vinegar



taste (4.9 ± 0.15), appearance (6.9 ± 0.26) astringency (5.72 ± 0.02) and sourness (5.42 ± 0.05). Thus, the mango vinegar obtained with the optimized fermentation conditions has been found to possess better sensory characteristics (Figure 4).

CONCLUSION

Optimization of vinegar fermentation using mango juice/ wine by CCD and RSM has been found to an ideal and time saving approach that overcomes the problems faced in conventional method of optimization**.** Optimal levels of process variables such as temperature, pH, fermentation time, alcohol content and inoculum volume were determined for maximum acetic acid production of 65.12 g/L, and proved to be well suited for evaluating the main and interactive effects of the process variables on vinegar production from mango wine. The present studies have revealed that mango fruit could be successfully utilized for the production of vinegar. However, in order to commercialize the process, the pilot scale trials are to be conducted to evaluate the technology. This will have a positive economic impact on both the mango processing and vinegar industries.

ACKNOWLEDGMENT

Ms.A.Harika, acknowledges the grant of Research Fellowship from DST-INSPIRE Programme. Special thanks to Dr S.C. Basappa, Former Deputy Director and Scientist, Central Food Technological Research Institute (CFTRI), Mysore, for his encouragement and critical comments on the manuscript.

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