Development of apple wine from Golden Delicious cultivar using a local yeast isolate

Sukhvir Sukhviri¹ · G. S. Kocher¹

Revised: 19 March 2019 / Accepted: 4 April 2019 / Published online: 11 April 2019
© Association of Food Scientists & Technologists (India) 2019

Abstract The present study was conducted to optimize fermentation parameters for apple wine production using Golden Delicious apples. Physicochemical analysis of the cultivar revealed a °Brix-acid ratio of 24.61 with ample amount of total and reducing sugars (9.6 and 6.03% w/v); making it a suitable substrate to produce ethanol. Microbiological analysis led to isolation of a yeast strain (namely A2) which was molecularly identified and accessed at GenBank as *S. cerevisiae* KY069279. Ethanol fermentation optimization using response surface methodology revealed that a temperature of 20 °C, an inoculum size of 7.08 (%v/v) and diammonium hydrogen phosphate supplementation @ 154.4 mg/100 mL as optimum for apple wine production which led to 10.73% (v/v) ethanol production with a desirability of 86.9%. Fresh wine having malic acid content of 1.87 (mg/100 mL) was subjected to malolactic fermentation (MLF) for 8 days using *Leuconostoc oenos* NCIM 2219 resulting in apple wine having 0.4 (mg/100 mL) malic acid. Sensory analysis of MLF and non-MLF apple wines categorised them as superior quality with average scores of 69.5 and 74.5, respectively. Gas chromatography–mass spectrometric analysis of apple wine revealed the presence of 38 volatile compounds including higher alcohols, acids, esters etc. The study thus revealed a process for apple wine preparation using an indigenous yeast and also optimized and compared malolactic and non-malolactic fermented ciders.

Keywords Apple wine · Ethanol · Fermentation · Malic acid · Response surface methodology · GCMS

Introduction

Apple (*Malus domestica*) is a fruit known for its pleasant, sub acid and aromatic nature. Its nutritive esteem is notable and is a good resource of sugars, vitamin C, minerals, proteins, pectin, calcium and phosphorus. About 2.47 MT of apples are grown in India (NHB 2015) with Himachal Pradesh producing 83% of ‘Golden Delicious’ apples. These fruits are very perishable and susceptible to bacterial and fungal contamination and hence are difficult to keep for extended period of time (Oyeleke 2007). High rate wastage of these fruits during peak season induces the requirement for post harvest processing innovations towards their quality supplementation as in spite of 6235 (‘000 ha) under fruits cultivation with a production of 91,443 (‘000MT) (NHB 2015), yet the number of food processing wine industries are still lagging behind. Hence, the production of ethanolic beverages from these fruits can help reduce the level of post-harvest problems. Also, wines in general are nutrient rich as they carry the richness of the fruits from which these are prepared. In India, wine industry is still growing with wineries (grape based in general) restricted to Maharashtra (Joshi and Attri 2005) and further fruit wines are produced by a very few breweries restricted to Himachal Pradesh or the one namely Rhythm winery in Pune and the recent years are witnessing a growth in fruit based wines.

Among fruit wines, fermented beverage obtained from apple is a popular drink in many western countries known as apple wine or cider (Wang et al. 2004). Apple wine and cider differ in terms of their ethanol content. While apple

✉ G. S. Kocher
gskocher@pau.edu

¹ Department of Microbiology, Punjab Agricultural University, Ludhiana 141001, India

wine has usually more than 7% (v/v) ethanol content, apple cider contains less than 7% (v/v) ethanol. Biochemically, apple juice contains many sugars, including fructose, glucose, and sucrose along with other carbohydrates, in varying concentrations. Due to the high fructose content in apple juice, the evaluation and selection of a fructophilic yeast strain could be significant to the apple wine industry (Wang et al. 2004).

Besides the type of yeast, fermentation parameters greatly influence ethanol fermentation by modulating the yeast metabolism. It has also been observed that temperature has a vital influence on fermentation rate, production of secondary metabolites and on the levels of certain volatile compounds in apple cider fermentation (Herrero et al. 2006). Yeast growth rate and ethanol production increase with temperature in line with specific growth rate. The yeast strains for production of wine are therefore selected by taking into consideration a number of characteristics such as alcohol tolerance, volatile compound production and fermentation rate (Cabranes et al. 1997). Among these characteristics, organic acids are vital component of fruit flavor, aroma, and add to the overall organoleptic quality e.g. malolactic fermentation (MLF) is an optional fermentation in the vinification procedure and is portrayed by conversion of L-malic acid to L-lactic acid and carbon dioxide also results in decrease in acidity, increased microbial stability and sensorial complexity. These improvements made by MLF, are desirable and contribute to the flavor profile including astringency, sweetness, bitterness and acidity (Jolicoeur 2013). Furthermore, Indian winery is dependent on imported yeast strains for ethanolic fermentation. However, fruits themselves are the rich depositors of efficient non-*Saccharomyces* and *Saccharomyces cerevisiae* showing that they can act as a natural source of the microflora that can help in successful ethanolic fermentation. Therefore, there is a need to isolate microflora specially yeasts to develop local wine strains.

Apple juice generally contains less nitrogen (0.35%) and thus needs supplementation of nitrogen so as to have 140 mg/L of free amino nitrogen represented by amino acids and NH_4^+ (Novak et al. 2013). DAHP supplementation has also been shown to improve ethanol content and overall acceptability of apple tea wine (Joshi and Kumar 2017) and guava wine (Shankar et al. 2006; Nikhanj et al. 2017). Although being a suitable substrate for the value addition yet very less work has been done towards the processing of Golden Delicious apple cultivar. This paper provides a production technology to process Golden Delicious apple cultivar to apple wine using a local yeast isolate from apple.

Materials and methods

Apple juice extracted from apples (cultivar Golden Delicious) having brix of 9.6 ± 1.5 °B and pH of 3.5 ± 0.2 was procured from local market during 2015 and 2016. The culture used in study was apple isolate A2 (isolated from apple skin) and later identified by 18sDNA analysis.

Physiochemical analysis of apple juice

The physico-chemical analysis of apples included the estimation of Total soluble solids (TSS) (digital refractometer, ATAGO), total sugars (Dubois et al. 1956), reducing sugars (Miller 1959), pH (Hanna HI96170), titratable acidity (Amerine and Roessler 1976), malic acid (Goodban and Stark 1957), total phenols (Malik and Singh 1991) and tannins (Polshettiwar and Ganjiwale 2007).

Microbiological analysis of apples

Isolation and identification of yeast

Apples were peeled and their skin was transferred to peptone water blanks. Samples (diluted/undiluted) were directly spread onto glucose yeast extract (GYE) for isolation of yeasts, using serial dilution method. The colonies grown on GYE media were randomly selected on the basis of their different morphologies and color characteristics. Selected colonies with different morphology were sub-cultured on GYE at 25 ± 2 °C for 48 h to obtain pure cultures and were maintained at 4 °C, till use. The isolate was chosen on the basis of high ethanol production; fermentation efficiency and sugar catabolic rate on apple juice for further studies. Molecular identification of selected yeast isolate was outsourced from Eurofins Genomics India Pvt Ltd, Bangalore using 18 s rDNA gene sequencing.

Growth studies and optimization of ethanolic fermentation parameters

Saccharomyces cerevisiae KY069279 was studied for its growth profile using apple juice (150 mL, ameliorated with cane sugar i.e. 20 °B) using di-ammonium hydrogen orthophosphate (DAHP) @ 100 mg/100 mL. The yeast inoculum was inoculated @ 5×10^6 cells/mL and flasks were incubated under shaking (120 rpm) at 28 °C for different periods of time. Samples were taken periodically and analyzed for viable haemocytometer count which was 10^8 cells/mL at 24 h (Sukhvair 2016). The data was used for calculation of growth rate and generation time.

Optimization of ethanolic fermentation parameters for apple wine production was carried out using 150 mL cane

sugar chaptalized apple juice (20 °B) and later validated at 5 L and 50 L scale. Fermentation conditions viz. temperature (°C), inoculum size (%v/v) and Di-ammonium hydrogen orthophosphate (DAHP) supplementation (mg/100 mL) were statistically optimized by response surface methodology (RSM) using Design expert 9.0. A set of 20 experiments were carried out for three variables [A: Temperature (15–25 °C), B: Inoculum size (5–10% v/v) and C: DAHP supplementation (100–300 mg/100 mL)]. The experimental design matrix in actual form of variables is given in Table 2. The second degree polynomial equation (Eq. 1) was calculated with the statistical software (Design Expert software 9.0) to estimate the response of the dependent variable. The response function (y) was related to the coded variables (xi, i = 1, 2, and 3) by a second degree polynomial equation as given below:

$$y = b_0 + b_1 \times 1 + b_2 \times 2 + b_3 \times 3 + b_{12} \times 1 \times 2 + b_{13} \times 1 \times 3 + b_{23} \times 2 \times 3 + b_{11} \times 1^2 + b_{22} \times 2^2 + b_{33} \times 3^2 + \varepsilon \quad (1)$$

The variance for each factor was assessed and partitioned into linear, quadratic and interactive components. The coefficients of the polynomial were represented by b_0 (constant), b_1 , b_2 , b_3 (linear effects); b_{12} , b_{13} , b_{23} (interaction effects); b_{11} , b_{22} , b_{33} (quadratic effects); and (random error). The significance of all the terms in the polynomial functions was assessed statistically using F-value at probability (p) of 0.05. The statistical analysis of the data and three-dimensional (3D) plotting were performed using Design Expert software 'DE-9'.

For each of the 20 runs, 150 mL of chaptalized apple juice was taken in 250 mL capacity flasks and inoculated with apple isolate A2 and incubated as per the conditions of RSM (Table 2) for ethanolic fermentation. Samples were periodically withdrawn and analyzed for ethanol, total sugars and reducing sugars till the completion of the fermentation.

Malolactic fermentation

Fresh apple wine produced from apple juice using apple isolate A2 (*S.cerevisiae* KY069279) was subjected to malolactic fermentation at a scale of 150 mL in 250 mL conical flasks. Apple wine was inoculated with *Leuconostoc oenos* NCIM 2219 at already optimized conditions i.e. temperature 28 °C, inoculum size 7.1% (v/v) and yeast nitrogen base supplementation 243.2 mg/L (Pooja 2016). Malic acid degradation was observed for 16 days till constant malic acid value was obtained.

Post-fermentative treatments

Siphoning

The flasks/bottles containing raw apple wine were stored at 15 °C and debris was allowed to settle down. No settling or fining agent was added. The siphoning was repeated after every 15 days till there was no further settling of debris. The stored wine was supplemented with KMS @ 0.01% and sparged with nitrogen at every racking (@ 1.5 bar pressure for 3–5 s).

Bottling and storage

Cleared apple wine was stored in glass bottles (washed earlier with boiling water and capped) for up to 6 months (with decantation treatment every 4 weeks) under mild refrigeration conditions at 10–15 °C. Glass bottles were supplemented with KMS and sparged with nitrogen as described earlier.

Sensory analysis

Sensory analysis of apple wine was carried out by a panel of 10 semi trained panelists. For the purpose, 80 point modified Davis score card was used (Amerine and Roessler 1976). The panelists were served with refrigerated (10–15 °C) apple wine samples in 30 mL wine glasses and 3–4 samples were evaluated in one sitting. The final sensory scores were statistically analysed by student's t test.

Gas chromatography mass spectrometry analysis

GCMS analysis of prepared apple wine was outsourced from SAIF-Lab, PU, Chandigarh. Volatile compounds in the wine were analysed using a Thermo Trace 1300 GC system coupled to a Thermo TSQ 8000 MS. The column employed was TG 5MS (30 m × 0.25 mm, 0.25 µm). Chromatographic conditions were as follows, the initial temperature 40 °C; program rate of 10 °C/min, final temperature 280 °C, and 250 °C for the injector temperature and 280 °C for the detector temperature. MS conditions were: interface temperature 280 °C, Helium flow 1 mL/min, EI source temperature 230 °C, and injection volume 1 µL. The qualitative and quantitative identification of volatile substances was based on the comparison of retention times and peak surface area read from the chromatogram and matching with the library of the compounds available with the analytical laboratory.

Statistical analysis

The experiments on optimization of apple juice fermentation for apple wine were carried out using RSM design expert 9.0. For all other experiments involving physico-chemical analysis of juice, malolactic fermentation, fermentation scale up and sensory analyses, statistical analysis of data was carried out by CPCS1 software developed by Department of Maths, Stat and Physics, Punjab Agricultural University, Ludhiana (Cheema and Singh 1991).

Results and discussion

Physicochemical analysis of Apple juice

Fresh and stored (4 °C) Golden Delicious apple juice was analysed for physico-chemical parameters i.e. initial °Brix, total sugars, reducing sugars, tannins, polyphenols, acidity, pH and juice recovery.

The overall range in respect of °Brix (6.2–9.6), total sugars (5.87–9.31 g/L), reducing sugars (3.74–6.03 g/L), tannins (0.59–0.98 g/L), polyphenols (172.3–320.8 GAE/L), total acidity (0.382–0.390 g/100 mL), pH (3.5–3.9) and juice recovery (22.9–43.2%) was observed among stored and fresh apple juice (Table 1). Upon storage, all the parameters i.e. °Brix, total sugars, reducing sugars, tannins, polyphenols, brix-acid ratio and juice recovery showed significant decrease except acidity and pH. Further, the results indicated that fresh Golden Delicious apple juice with a brix-acid ratio of 24.61, was optimum for fermentative processing which was not so with stored apples whereby Brix-acid ratio was decreased to 16.31. As the juice parameters for fermentation i.e. juice recovery was more in fresh cultivar, the latter was selected for further processing.

Cabranes et al. (1997) studied that Spanish apple juice contains 100–150 g/L of total fermentable sugars, including fructose, glucose, sucrose as well as other carbohydrates, in varying concentrations. On the other hand, Indian

apple cultivar Golden Delicious showed total sugars of 96 g/L which is in comparison to the sugar concentration of Spanish apple juice. Kahle et al. (2005) reported a phenol content of 154–178 mg/L in dessert apples (Red Delicious, Golden Delicious, Granny Smith, and Fuji) and 261–970 mg/L in cider apples (Kaiser Alexander, KaiserWilhelm, Bittenfelder, Winterrambur, Bohnapfel, Bretacher, and Boskoop). Among these apple cultivars Boskoop showed the highest (970 mg/L) and Granny Smith the lowest (154 mg/L) of polyphenol content. According to Downing (1989), a sharp and bittersweet apple has acidity above 0.45 g malic acid/100 mL, whereas sweet or bittersweet apple has content below this amount. They also reported that the tannin content is below 0.2 g/100 mL in sweet and sharp apple or above 0.2 g/100 mL in bittersweet and bittersweet apple.

Identification of yeast strain

Isolate (from apple skin) resembling the characteristics of a yeast morphologically and microscopically was named as A2 and it was purified by repeated streaking on the GYE medium. After 48 h, white to cream colored colonies appeared on agar plates. The colonies were smooth, shiny and raised with entire margin. Observation of the colonies under microscope also showed budding of the cells, a typical characteristic of yeasts. The yeast isolate A2 was identified on the basis of 18 s rDNA sequencing. 500 bp nucleotide sequence was generated which was directly submitted to GenBank and accession number KY069279 was provided for respective sequence. Hence, A2 isolate was identified as a strain of *S. cerevisiae* and named as *S. cerevisiae* KY069279 (GenBank 2016).

Growth of *S. cerevisiae* KY069279 and optimization of fermentation parameters for apple wine production

The growth studies of *S. cerevisiae* KY069279 on apple juice revealed maximum growth at 24 h with a specific

Table 1 Physico-chemical analysis of fresh and stored Golden Delicious apples

Parameters	Brix (°B)	Total sugars (g/100 mL)	Reducing sugars (g/100 mL)	Tannins (g/L)	Polyphenols (mg GAE/L)	Total acidity % (g/100 mL)	pH	Brix-Acid ratio	Juice recovery ^a %
Fresh	9.6	9.31	6.03	0.98	320.8	0.390	3.5	24.61	43.2
Stored	6.2	5.87	3.74	0.59	172.3	0.382	3.9	16.31	22.9
CD (5%)	1.01	0.97	0.62	0.10	32.10	NS	NS	2.60	4.31

^aValues presented are mean values of triplicates for the years 2015 and 2016

Juice recovery (%) = $\frac{\text{Volume of extracted juice (ml)}}{\text{Weight of apples taken (kg)}} \times 100$

growth rate of 0.335/h and a generation time of 25 min. (Sukhvir 2016). The corresponding count of yeast cells was 10^8 cells/mL and the later was used for optimization experiments. Statistical optimization of fermentation conditions [temperature ($^{\circ}\text{C}$), inoculum size (%v/v) and Diammonium hydrogen orthophosphate (DAHP) supplementation (mg/100 mL)] was carried out using RSM plan under design expert 9.0 software with yeast strain *S. cerevisiae* KY069279. Table 2 revealed the 20 combinations of three fermentation factors along with their responses for *S. cerevisiae* KY069279. By keeping constraints at a ‘target’ temperature of 20 $^{\circ}\text{C}$, inoculum size ‘in range’, DAHP supplementation, total sugars and reducing sugars to ‘minimum’ and ethanol content to ‘maximum’; a solution was provided by RSM as demonstrated in Table 3. Results indicated that when 7.08% (v/v) inoculum size was supplemented with 154.41 mg/100 mL DAHP and fermented at 20 $^{\circ}\text{C}$, it lead to 10.73% (v/v)

ethanol content with a desirability of 86.9% for the apple wine production. On the basis of desirability, these parameters were selected for the validation of apple wine fermentation. A validation experiment was conducted at 150 mL flask level for apple wine production. Results revealed that numerically optimized parameters provided by the RSM were better for further scale up studies because of its higher fermentation efficiency of 95.83% in *S. cerevisiae* KY069279.

Optimization for ethanol response

A model F-value of 207.17 implied that the model for ethanol response was significant. In this case linear factors A, C; interaction factors AB, AC, BC (Fig. 1a–c) and quadratic factors KY069279 , B^2 , C^2 were found to be significant model terms. The “Pred R-Squared” of 0.9829 was in reasonable agreement with the “Adj R-Squared” of

Table 2 Experimental response profile for optimization of fermentation process parameters in fresh apple juice using *Saccharomyces cerevisiae* KY069279

Run	Factor 1 Temperature ($^{\circ}\text{C}$)	Factor 2 Inoculum size (% v/v)	Factor 3 DAHP (mg/ 100 mL)	Response 1 Ethanol (% v/v)	Response 2 Total sugars (g/ 100 mL)	Response 3 Reducing sugars (g/ 100 mL)
1	20	7.5	200	11.12	0.145	0.012
2	25	10	300	9.03	0.156	0.072
3	11.591	7.5	200	5.98	0.741	0.295
4	20	7.5	200	11.49	0.29	0.025
5	20	7.5	200	10.96	0.27	0.019
6	25	5	100	8.91	0.267	0.041
7	28.409	7.5	200	8.92	0.16	0.021
8	15	5	100	6.71	0.506	0.258
9	20	7.5	318.207	6.07	0.66	0.129
10	15	5	300	6.16	0.725	0.199
11	20	7.5	200	11.07	0.295	0.019
12	20	3.29552	200	8.97	0.16	0.095
13	20	7.5	200	11.53	0.241	0.017
14	25	10	100	6.89	0.294	0.027
15	20	7.5	368.179	7.54	0.729	0.108
16	20	7.5	200	11.08	0.295	0.012
17	15	10	100	6.31	0.79	0.196
18	25	5	300	9.15	0.246	0.055
19	15	10	300	7.43	0.97	0.149
20	20	11.7045	200	8.95	0.165	0.067

Adjusted R^2 and predicted R^2 values of model responses

Yeast strain	Response	Model F-value	p value	Adjusted R^2 value	Predicted R^2 value
<i>Saccharomyces cerevisiae</i> KY069279	Ethanol	207.17	< 0.001	0.9899	0.9829
	Total sugars	24.19	< 0.001	0.9166	0.7454
	Reducing sugars	43.63	< 0.001	0.9952	0.9854

Table 3 Scale up of ethanol production by *S. cerevisiae* KY069279 under optimized fermentation conditions

Parameters	5 L		50 L	
	Initial	Final	Initial	Final
Total sugars %	18.01	0.781	18.76	0.012
Reducing sugars %	15.52	0.0	16.24	0.0
Ethanol % (v/v)	0.0	10.7	0.0	11.4
pH	3.7	3.6	3.0	3.5
Acidity % (w/v)	0.312	0.576	0.24	0.56
Ethanol yield (g/g)	0.475		0.484	
Fermentation efficiency %	92.8		95.0	
CD (5%) _{Ethanol}	1.26			

Values presented are mean values of triplicates for the years 2015 and 2016

Parameters: Temperature (20 °C); Inoculum size [7.08% (v/v)]; DAHP (154.41 mg/100 mL)

0.9899. By applying regression analysis, the equations (Eqs. 2 and 3) in terms of significant factors for the ethanol response are:

Final equation in terms of coded factors

$$\begin{aligned} \text{Ethanol} = & +11.21 + 0.90 \times A - 0.095 \times B + 0.40 \\ & \times C - 0.38 \times AB + 0.23 \times AC + 0.45 \times BC - 1.32 \\ & \times \text{KY069279} - 0.79 \times B^2 - 1.55 \times C^2 \end{aligned} \quad (2)$$

Final equation in terms of actual factors

$$\begin{aligned} \text{Ethanol} = & -27.29598 + 2.42613 \times \text{temperature} \\ & + 2.09134 \times \text{inoculums size} + 0.043418 \\ & \times \text{DAHP} - 0.030100 \times \text{temperature} \times \text{inoculums size} \\ & + 4.52500\text{E} - 004 \times \text{temperature} \\ & \times \text{DAHP} + 1.78500\text{E} - 003 \times \text{inoculum size} \\ & \times \text{DAHP} - 0.052763 \times \text{temperature}^2 \\ & - 0.12563 \times \text{inoculums size}^2 - 1.54713\text{E} - 004 \\ & \times \text{DAHP}^2 \end{aligned} \quad (3)$$

3-D surface plots as shown in Fig. 1a–c represented the relation between the significant interaction factors for the ethanol response by keeping DAHP supplementation constant i.e. 200 mg/100 mL. Figure 1a demonstrated that temperature up to 20.71 °C and inoculum size of up to 7.02% resulted in the production of high ethanol levels. Further, increase in temperature and inoculum size lead to

reduction of ethanol levels. Figure 1b represented the interrelation between temperature and DAHP for the ethanol response at constant inoculum size (7.5% v/v). It indicated that maximum ethanol content was observed till temperature of 20.33 °C and further rise in temperature resulted in reduction of ethanol content. Also, the DAHP supplementation showed that maximum ethanol content was recorded up to DAHP concentration of 209.36 mg/100 mL. Figure 1c displayed the interconnection between inoculum size and DAHP for the response ethanol at constant temperature of 20 °C. It showed that an inoculum size of up to 7.6% (v/v) and DAHP concentration of up to 209.47 mg/100 mL provided an elevation to the ethanol content.

The regression equations of total sugars and reducing sugars were also generated (Sukhvir 2016) but have not been included in this manuscript since the major concern was to look factors responsible for maximum ethanol production. In literature, Cruz et al. (2002) reported that the accessible nitrogen content of the apple juice, ranging from 27 to 574 mg/L, is the main restraining factor for the yeast's growth. Alberti et al. (2011) evaluated the nitrogen content in musts of different apple cultivars and studied the effect of nitrogen concentration in apple wine fermentation. The apple must with 59.0, 122.0 and 163.0 mg/L of total nitrogen content displayed the maximum growth of 2.05×10^7 , 4.42×10^7 and 8.66×10^7 cell/mL, respectively. Hence, the fermentation rates were dependent on the initial nitrogen level corresponding to 1.4, 5.1 and 9.2 g/L day, respectively.

Herrero et al. (2006) reported that temperature had a more vital influence on the levels of certain volatile compounds in apple cider fermentation. It was recorded that when fermentation temperature increased to 22 °C, using the simultaneous method, the final concentrations of ethyl acetate and some of the higher alcohols decreased, while in the sequential inoculation model, after completion of the alcoholic fermentation at 15 °C, no statistically significant differences in the profiles of the volatile compounds tested. Musyimi et al. (2013) studied the effects of temperature and inoculum size on apple mango fruit wine. The rise in temperature (20–35 °C) and inoculum size (0.0065–0.1%) augmented the fermentation kinetics significantly. However, at high temperature (35 °C) and inoculum size (0.1%) the sugars were not completely consumed during fermentation. At low temperature of 25 °C, the alcohol yield was highest i.e. 9.44% in comparison to high temperature of 35 °C that gave the lowest ethanol yield of 6.93%. Peng et al. (2015) revealed optimum fermentation conditions for apple juice fermentation as 20 °C, 3.6 and 9% (v/v) of temperature, pH and inoculums size from RSM studies.

Fig. 1 3D surface plots representing interaction between significant fermentative parameters using *S.cerevisiae* KY069279.
a Temperature (A) and inoculum size (B) for the response ethanol.
b Temperature (A) and DAHP (C) for the response ethanol.
c Inoculum size (B) and DAHP (C) for the response ethanol

Malolactic fermentation (MLF)

Apple juice had 3.91 g/L of malic acid content which has a significant influence on the flavor and maturation of apple wine. However, desirable level of malic acid in apple wine is 0.4–0.6 g/L (Campo et al. 2007). Fresh wine (0 °B) was produced from *S. cerevisiae* KY069279 having malic acid content of 1.87 (g/L). It was subjected to malolactic fermentation using *Leuconostoc oenos* NCIM 2219 at already optimized conditions i.e. temperature 28 °C, inoculum size 7.1% (v/v) and yeast nitrogen base (YNB) supplementation 243.2 mg/L. Figure 2 showed that there was a gradual decrease in malic acid content till day 2 after which it declined rapidly up to day 8. After day 8, reduction in malic acid content was constant till day 16. Hence, apple wine revealed a declined range of 0.4 g/L of malic acid content in 6 days of MLF which is thus sufficient to prepare apple wine.

Elsewhere, Volschenk et al. (2006) reported that malolactic fermentation induced by the addition of malolactic starter cultures, regarded as the preferred method for naturally reducing wine acidity, efficiently decreases the acidic taste of wine, improves the microbial stability and modifies to some extent the organoleptic character of wine.

Scale up of apple wine production

Apple wine was scaled up to 5 L and 50 L under the optimized fermentation parameters obtained by RSM using *S. cerevisiae* KY069279 at sugar concentration of 20 °B. Brix of raw apple juice was 11 °B. Cane sugar was ameliorated 90 g/L into apple juice to raise the brix to 20 °B. Table 3 shows that 10.7 and 11.4% (v/v) ethanol was produced at 20 °B for 5 L and 50 L, respectively.

Fig. 2 Malolactic fermentation in apple wine produced by *S. cerevisiae* KY069279 strain

Sensory analysis

Apple wine samples stored over a period of 90 and 180 days were subjected to sensory evaluation by a panel of 10 judges on a scale of 80 points (Modified Davis Score Card) to find out the acceptability among the tasting panelists. Results showed that apple wine prepared using *S. cerevisiae* KY069279 was good in terms of appearance, aroma, taste and overall feel. Apple wine showed comparative scores in each of sensory evaluation parameter. Sensory parameters improved during the storage period of non-MLF treated apple wine and this lead to increase in sensory scores to 74.5 from 68.25 classifying it as ‘superior’ category. MLF treated apple wine revealed an increase in mean score from 59.75 to 69.5 over a storage period of 180 days.

In literature, Liu (2002) and Davis et al. (1985) reported that though impact of MLF on the taste of wine due to deacidification was well known, the effect of MLF on wine aroma and mouth feel was imprecised. According to Henick-Kling (1993) and Henick-Kling et al. (1994), MLF improved the fruity aroma and buttery note but reduced the vegetative, green/grassy aroma. Besides aroma, MLF was believed to augment the body and mouthfeel of wine and gave a longer after-taste (Henick-Kling et al. 1994). Other flavour attributes associated with MLF were described as nutty, yeasty, ropy, roasted, toasty, oaky, sweaty, spicy, smoky, earthy, bitter, honey and floral (Henick-Kling 1993; Henick-Kling et al. 1994; Laurent et al. 1994; Sauvageot and Vivier 1997).

Gas chromatography mass spectrometry analysis

The primary characterization of apple wine is a ‘spicy, aromatic, apple-like note’ (Jarvis et al. 1995). Quantitatively most important volatiles in apple wine are the higher alcohols, esters and lower fatty acids formed during fermentation (Jarvis et al. 1995; Williams 1974). Aroma components in apple wine are formed mainly during fermentation and monitoring aroma components in apple wine during fermentation can provide greater insight into yeast metabolism and flavor formation. A total of 38 volatile compounds were detected from the wine and are listed in Table 4, including 11 esters, 6 alcohols, 6 ethers, 9 amines, 2 ketones, other aldehydes, amides, phenols and fatty acids. Glycerin was detected as the main volatile compound showing high relative abundance at a retention time of 11.55 min. Esters impart fruity flavor, acids sourness and tart, alcohols and glycerin flavor, smoothness and texture, aldehydes and ketones originate from yeast metabolism and impart bitterness to wine. Hexanal, a typical of apple was also observed in the GCMS analysis (Table 4). Elsewhere, 51 volatile substances were detected by Zhao et al.

Table 4 GCMS analysis of apple wine

S. no.	Compound	Molecular formula	RT	PEAK AREA
1	Morphinan-4,5-epoxy-3,6-di-ol,6-[7-nitrobenzofurazan-4-yl]amino	C ₂₆ H ₂₇ N ₅ O ₆	4.63	16,019,963.45
2	2,7-Diphenyl-1,6-dioxypyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	C ₂₀ H ₁₃ N ₅ O ₂	5.15	12,342,869.69
3	á-D-Glucopyranose,4-O-á-D-galactopyranosyl	C ₁₂ H ₂₂ O ₁₁	11.02	688,881.97
4	Glycerin	C ₃ H ₈ O ₃	11.55	23,182,152.15
5	Methanamine, N-methoxy	C ₂ H ₇ NO	12.38	3,055,265.38
6	N,N-Dimethyl-O-(1-methyl-butyl)-hydroxylamine	C ₇ H ₁₇ NO	12.46	3,416,627.15
7	Erythritol	C ₄ H ₁₀ O ₄	13.31	816,617.28
8	3-Pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-oxo-4a,7a-epoxy-5H-cyclopenta[a]cyclopropa[f]cycloundecen-11-yl ester	C ₃₂ H ₃₉ NO ₁₀	13.61	1,733,644.67
9	N-Methoxy-N-methylacetamide	C ₄ H ₉ NO ₂	13.64	715,568.08
10	2,7-Diphenyl-1,6-dioxypyridazino[4,5:2',3']pyrrolo[4',5'-d]Pyridazine	C ₂₀ H ₁₃ N ₅ O ₂	16.31	897,443.47
11	Ergosta-5,22-dien-3-ol, acetate	C ₃₀ H ₄₈ O ₂	16.36	816,755.53
12	Acetic acid,17-acetoxy-3-hydroxyimino-4,4,13-trimethyl-hexadecahydrocyclopenta[a]phenanthren-10-ylmethyl ester	C ₂₅ H ₃₉ NO ₅	24.33	571,720.78
13	Dodecanoic acid,1a,2,5,5a,6,9,10,10a-octahydro-5a-hydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-5-yl ester	C ₃₂ H ₄₈ O ₆	24.50	556,852.33
14	(5á)Pregnane-3,20á-diol,14á,18á-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	C ₂₈ H ₄₃ NO ₆	24.65	643,658.84
15	3,6,9,12-Tetraoxatetradecan-1-ol,14-[4-(1,1,3,3-tetramethylbutyl)phenoxy]	C ₂₄ H ₄₂ O ₆	25.02	575,895.04
16	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol	C ₂₇ H ₄₄ O ₃	25.49	499,928.66
17	2,2-Bis[4-[[4-chloro-6(3-ethynylphenoxy)-1,3,5-triazin-2-yl]oxy]phenyl]propane	C ₃₇ H ₂₄ Cl ₂ N ₆ O ₄	26.00	544,932.18
18	4-Piperidineacetic acid,1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1H-indol-2-yl]-á-methyl-, methyl ester	C ₂₃ H ₃₂ N ₂ O ₄	26.06	821,784.48
19	Pregna-1,4-dien-3-one,11á,17,20á,21-tetrahydroxy-,Cyclic 20,21-acetal with acetone	C ₂₄ H ₃₄ O ₅	27.08	2,524,511.53
20	Olean-12-ene-3,16,21,22,28-pentol,21-(2-methyl-2-butenolate)	C ₃₅ H ₅₆ O ₆	28.33	580,903.92
21	Ergosta-5,22-dien-3-ol,acetate	C ₃₀ H ₄₈ O ₂	28.38	567,865.99
22	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl] Cyclohex-1-en-1-carboxaldehyde	C ₂₃ H ₃₂ O	29.06	532,413.32
23	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	29.88	946,584.40
24	1-Heptatriacotanol	C ₃₇ H ₇₆ O	30.08	1,451,829.90
25	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester	C ₂₈ H ₄₄ O ₄	31.11	493,074.81
26	Morphinan-4,5-epoxy-3,6-diol-6-[7-nitrobenzofurazan-4-yl] Amino	C ₂₆ H ₂₇ N ₅ O ₆	31.35	712,734.36
27	2,7-Diphenyl-1,6-dioxypyridazino[4,5:2',3']pyrrolo[4',5'-d] Pyridazine	C ₂₀ H ₁₃ N ₅ O ₂	31.43	814,215.29
28	Butanoic acid,1a,2,5,5a,6,9,10,10a-octahydro-5a-hydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-5-yl ester	C ₂₄ H ₃₂ O ₆	31.96	498,510.26
29	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	C ₂₈ H ₄₀ O ₁₀	32.79	798,242.92
30	8,14-Seco-3,19-epoxyandrostane-8,14-dione,17-acetoxy-3á-methoxy-4,4-dimethyl	C ₂₄ H ₃₆ O ₆	33.63	480,059.54
31	Dasycarpidan-1-methanol, acetate (ester)	C ₂₀ H ₂₆ N ₂ O ₂	33.76	809,575.30
32	Milbemycin b,13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)	C ₃₃ H ₄₆ ClNO ₇	34.09	603,023.67
33	7,8-Epoxylostan-11-ol, 3-acetoxy	C ₃₂ H ₅₄ O ₄	34.27	600,200.14
34	(22S)-21-Acetoxy-6á,11á-dihydroxy-16á,17á-propylmethylenedioxypregna-1,4-diene-3,20-dione	C ₂₇ H ₃₆ O ₈	34.33	522,062.92
35	Acetate,10,13-dimethyl-17-(1-methyl-4-oxo-4-[1,2,4]-triazol-1-yl-butyl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3-yl	C ₂₈ H ₄₁ N ₃ O ₃	34.58	549,219.47
36	Chol-8-en-24-al,3-hydroxy-4,4,14-trimethyl	C ₂₇ H ₄₄ O ₂	35.29	476,313.80
37	Ergosta-5,22-dien-3-ol,acetate	C ₃₀ H ₄₈ O ₂	35.33	569,297.14
38	Pregnan-20-one,3-(acetyloxy)-5,6:16,17-diepoxy	C ₂₃ H ₃₂ O ₅	35.41	660,101.51

(2014) including 10 esters, 15 alcohols, 7 acids, 8 ketones, other aldehydes, phenols, fatty acids, terpene. Glycerin was also reported as main volatile component and there was a significant increase in the concentration of glycerine after MLF (Santos et al. 2004). Glycerol may improve the fruity character of the wine, but has no direct effect on its aroma. Wang et al. (2004) reported the presence of 43 aroma compounds in ‘Fuji’ apple wine and 47 aroma compounds in ‘Fuji’ apple juice by SPME analysis. Lorrenzini and Zapparoli (2019) screened 18 yeasts for apple juice fermentation and used *S. uvarum* for cider production and studied its chief volatiles as alcohol esters and fatty acids.

Conclusion

The present study optimized fermentation parameters of apple wine from “Golden delicious” using an indigenous yeast *S. cerevisiae* KY069279 employing RSM optimized conditions of 20 °C, 7.08% (v/v) and 154.4 mg/100 mL of temperature, inoculums size and DAHP for production of 10.73% (v/v) ethanol. Apple wine with and without MLF fermentation were prepared and evaluated for high sensory attributes of 69.5 and 74.5, respectively thus presenting development of an efficient apple processing technology to produce a good quality apple wine.

References

- Alberti A, Vieira RG, Drilleau JF, Wosiacki G, Nogueira A (2011) Apple wine processing with different nitrogen contents. *Braz Arch Biol Technol* 54(3):551–558
- Amerine MA, Roessler EB (1976) *Wines: their sensory evaluation*. Freeman, San Francisco
- Cabranes C, Mangas JJ, Blanco D (1997) Selection and biochemical characterisation of *Saccharomyces cerevisiae* and *Kloeckera Apiculata* strains isolated from Spanish cider. *J Inst Brew* 103:165–169
- Campo GD, Berregi IA, Santos JI, Duen M, Irastorza A (2007) Development of alcoholic and malolactic fermentations in highly acidic and phenolic apple musts. *Biores Technol* 99:2857–2863
- Cheema HS, Singh B (1991) Software Statistical Package CPCS-1. Developed at Department of Statistics, Punjab Agricultural University, Ludhiana
- Cruz SH, Cilli EM, Ernandes JR (2002) Structural complexity of the nitrogen source and influence on the yeast growth and fermentation. *J Inst Brew* 108:54–61
- Davis CR, Wibowo DJ, Eschenbruch R, Lee TH, Fleet GH (1985) Practical implications of malolactic fermentation: a review. *Am J Enol Vitic* 36:290–301
- Downing DL (1989) Apple cider. In: Downing DL (ed) *Processed apple products*, 1st edn. Van Nostrand Reinhold, New York, pp 169–188
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Anal Chem* 28:350–356
- GenBank (2016) GenBank Submissions Handbook www.ncbi.nlm.nih.gov/books/NBK51157. Accessed 2 Nov 2016
- Goodban AE, Stark JB (1957) Rapid method for determination of malic acid. *Anal Chem* 29:283–287
- Henick-Kling T (1993) Malolactic fermentation. In: Fleet GH (ed) *Wine microbiology and biotechnology*, 1st edn. Harwood Academic Publishers, Switzerland, pp 289–326
- Henick-Kling T, Acree TE, Krieger SA, Laurent MH, Edinger WD (1994) Modification of wine flavour by malolactic fermentation. *Wine East* 4:8–15
- Herrero M, Garcial LA, Diaz M (2006) Volatile compounds in cider: inoculation time and fermentation temperature effects. *J Inst Brew* 112(3):210–214
- Jarvis B, Forster MJ, Kinsella WP (1995) Factors affecting the development of cider flavour. *J Appl Bacter Symp Sup* 79:5S–18S
- Jolicoeur C (2013) *The new cider maker’s handbook: a comprehensive guide for craft producers*. Chelsea Green Publishing, Hartford
- Joshi VK, Attri D (2005) Panorama of research and development of wines in India. *J Sci Nat Res* 64:9–18
- Joshi VK, Kumar V (2017) Influence of different sugar sources, nitrogen sources and inocula on the quality characteristics of apple tea wine. *J Inst Brew* 123(2):268–276
- Kahle K, Kraus M, Richling E (2005) Polyphenol profiles of apple juices. *Mol Nut Food Res* 49:797–806
- Laurent MH, Henick-Kling T, Acree T (1994) Changes in the aroma and odor of Chardonnay wine due to malolactic fermentation. *Wein Wissenschaft* 49:3–10
- Liu SQ (2002) Malolactic fermentation in wine- beyond deacidification: a review. *J Appl Microbiol* 92:589–601
- Lorrenzini M, Zapparoli G (2019) Assessment of yeasts for apple juice fermentation and production of cider volatile compounds. *LWT Food Sci Tech* 99:224–230
- Malik CB, Singh MB (1991) *Plant enzymology and histoenzymology*. Kalyani Publishers, New Delhi, pp 274–279
- Miller GL (1959) Use of Dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31:426–428
- Musyimi SM, Sila DN, Okoth EM, Onyango CA, Mathooko FM (2013) The influence of process optimization on the fermentation profile of mango wine prepared from the Apple mango variety. *J Animal Plant Sci* 17(3):2600–2607
- NHB (2015) World Wide Web source for National Horticulture Board data. http://nhb.gov.in/area-pro/NHB_Database_2015.pdf. Published 11 Feb 2015. Accessed 5 Apr 2016
- Nikhanj P, Kocher GS, Boora RS (2017) Fermentative production of guava wine from pectinase treated and untreated juice of ‘punjab pink’ cultivar of *Psidium guajava* L. *Agric Res J* 54(2):244–247
- Novak A, Wilk MK, Pogorzelski E, Czyowska A (2013) Effect of nitrogen sources on fermentation process and formation of H₂S and ethyl carbamate by wine yeast. *Biotech Food Sci* 77(1):11–23
- Oyeleke O (2007) Extraction of juice from some tropical fruits using a small scale fruit-juice extractor. *Afr Crop Sci* 8:1803–1808
- Peng B, Lei Y, Zhao H, Cui L (2015) Response surface methodology for optimization of fermentation process parameters for improving apple wine quality. *J Food Sci Technol* 52(11):7513–7518
- Polshettiwar SA, Ganjiwale RO (2007) Spectrophotometric estimation of total tannins in some ayurvedic eye drops. *Ind J Pharm Sci* 69(4):574–576
- Pooja (2016) Optimization of enological practices for production of quality wines from grapes. Ph.D., Dissertation. Punjab Agricultural University, Ludhiana, India
- Santos JP, Arroyo T, Aleixandre M, Lozano J, Sayago I, García M, Fernández MJ, Arésa L, Gutiérrez J, Cabellos JM, Gilb M, Horrillo MC (2004) A comparative study of sensor array and

- GC–MS: application to Madrid wines characterization. *Sens Actuators B Chem* 102:299–307
- Sauvageot F, Vivier P (1997) Effects of malolactic fermentation on sensory properties of four Burgundy wines. *Am J Enol Vitic* 48(2):187–192
- Shankar S, Babu JD, Reddy YN (2006) Fermentation of guava pulp with grape grown yeast (*Saccharomyces cerevisiae* var. *ellipsoideus*) for wine production. *Ind J Hort* 63(2):171–173
- Sukhvir (2016) Evaluation of apple cultivars for fermentative production of cider. M.Sc. thesis, Punjab Agricultural University, Ludhiana, India
- Volschenk H, Van Vuuren H, Viljoen-Bloom M (2006) Malic acid in wine: origin, function and metabolism during vinification. *S Afr J Enol Vitic* 27(2):123–136
- Wang D, Xu Y, Hu J, Zhao G (2004) Fermentation kinetics of different sugars by Apple wine Yeast *Saccharomyces cerevisiae*. *J Inst Brew* 110:340–346
- Williams AA (1974) Flavour research and the cider industry. *J Inst Brew* 80:455–470
- Zhao H, Zhou F, Dziugan P, Yao Y, Zhang J, Lv Z, Zhang B (2014) Development of organic acids and volatile compounds in cider during malolactic fermentation. *Czech J Food Sci* 32:69–76

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.