OPTIMIZATION OF FERMENTATION CONDITIONS FOR PRODUCTION OF HUNGARIAN SOUR CHERRY SPIRIT USING THE RESPONSE SURFACE METHODOLOGY

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INTRODUCTION

Pálinka is a traditional fruit spirit and a kind of gastronomic heritage in Hungary. In Pákinka production, one of the most important processes affecting the quality and yield of spirits is fermentation. Based on single-factor and three-factor influence level tests by following the Plackett-Burman design, the fermentation process from sour cherry juice concentrate and *Saccharomyces cerevisiae* by using Response surface methodology (RSM) coupled with the central composite rotatable design was investigated to optimize fermentation conditions.

MATERIALS

Yeast strain, Uvaferm Danstil A, were provided by the Kokoferm Limited Company (Gyöngyös, Hungary).

The yeast was activated before fermentation by mixing 1 g dry yeast with 1 g sucrose, 1 g yeast nutrient (Uvavital TM, Lallemand, France) and 100 mL warm water (28 °C), then the mixture was aerated by gentle agitation for 2 hours.

The fermentation medium was a cherry juice (concentrate) from the INNIGHT company (Hungary).

EXPERIMENTS

Fermentation temperature, pH and Brix were studied through a central composite experimental design (CCD). A quadratic model was applied to study the combined influence of three independent variables namely temperature (X_1,°C), pH (X_2) and soluble solids content (X_3, Brix) following table 1. The production yield of alcohol (Y_1) and volatile compound (Y_2) was chosen as dependent variables.

For Y_1 and Y_2 determination, the equations presented below were used.

$Y_1 = P_1/S*100$ (Eq.1)

$Y_2 = P_2/S*100$ (Eq.2)

where S is total sugar, P_1 is alcohol content, P_2 is total volatile compounds.

The majority of compounds in total volatile compounds include ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate, isoamyl acetate, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and 2-phenylethanol.

With the optimum values obtained, 4.8L sour cherry juice was fermented. After the alcoholic fermentation completed, the fermented mashes were distilled twice. The heart distillate was analysed volatile compounds.

RESULTS

Table 1. Independent variables in the experimental plan

Variables	Coded levels		
	- 1	0	+ 1
Temperature, X_1 (°C)	15	20	25
pH, X_2	2.75	3.25	3.75
Soluble solids content, X_3 (°Brix)	18	24	30

Table 2. The central composite design matrix and experimental values

Run	Independent variables			Dependent variables	
	X_1 (°C)	X_2	X_3 (°Brix)	Y_1	Y_2
1	15	2.75	18	50.7	1801.08
2	25	2.75	18	56.48	2035.88
3	15	3.75	18	50.7	1605.58
4	25	3.75	18	59.79	1895.42
5	15	2.75	30	44.47	1789.65
6	25	2.75	30	50.75	1947.09
7	15	3.75	30	44.14	1655.82
8	25	3.75	30	55.22	1813.25
9	15	3.25	24	50.22	2032.79
10	25	3.25	24	59.1	2229.64
11	20	2.75	24	54.76	2114.66
12	20	3.75	24	57.04	1955.67
13	20	3.25	18	57.04	2008.55
14	20	3.25	30	54.06	1897.68
15	20	3.25	24	58.48	2149.30
16	20	3.25	24	58.9	2140.80
17	20	3.25	24	57.86	2174.91

Table 3. Estimated regression coefficients and variance analysis results for response variables

Parameters	Y_1		Y_2	
	Coefficient values	p-values	Coefficient values	p-values
Constant	57.91	<0.0001***	2153.09	<0.0001***
X_1	4.11	<0.0001***	103.64	<0.0001***
X_2	0.97	0.0128*	-76.26	<0.0001***
X_3	-2.61	<0.0001***	-24.30	0.0113*
X_1^2	-2.87	0.0015**	-20.44	0.1809ns
X_2^2	-1.63	0.0239*	-116.49	0.0001***
X_3^2	-1.98	0.0101*	-198.54	<0.0001***
X_1X_2	1.01	0.0175*	6.88	0.4163ns
X_1X_3	0.31	0.3753ns	-26.22	0.0132*
X_2X_3	0.10	0.7615ns	8.54	0.3193ns
Q^2		0.889		0.942
R^2		0.984		0.993
R^2Adj.		0.964		0.985
RSD		0.926		22.52
p_{ANOVA}		<0.05		<0.05
p_{LOF}		>0.05		>0.05

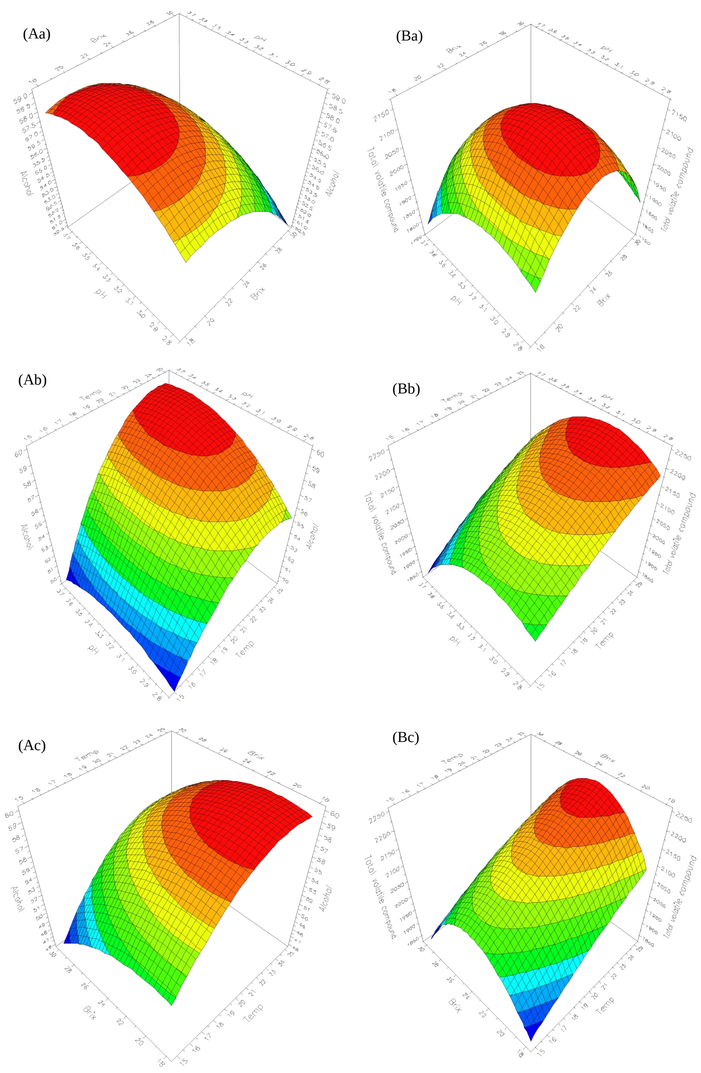

Figure 1. Response surface (a) pH vs. Brix, (b) pH vs. temperature, and (c) Brix vs. temperature on production yield of alcohol (A) and volatile compounds (B) from the fermentation of cherry juice

Full predictive equations were given below:

$Y_1 = 57.91 + 4.11*X_1 + 0.97*X_2 - 2.61*X_3 - 2.87*X_1^2 - 1.63*X_2^2 - 1.98*X_3^2 + 1.01*X_1X_2 + 0.31*X_1X_3 + 0.1*X_2X_3$ (Eq.3)

$Y_2 = 2153.09 + 103.64*X_1 - 76.26*X_2 - 24.30*X_3 - 20.44*X_1^2 - 116.49*X_2^2 - 198.54*X_3^2 + 6.88*X_1X_2 - 26.22*X_1X_3 + 8.54*X_2X_3$ (Eq.4)

Table 4. Estimated optimum values and predicted, experimental values of responses

Variables	Optimum values	Predicted values	Experimental values
Temperature, X_1, (°C)	24.71		
pH, X_2	3.25		
Total soluble solids, X_3, (°Brix)	22.49		
Alcohol's production yield, Y_1		59.68	60.86
Alcohol content, (vol.%)		9.02	9.20
Volatile compounds' production yield, Y_2		2231.68	2339.7
Volatile compounds, (mg/L)		337.37	353.71

Table 5. Volatile compounds of fruit spirits from sour cherry

Volatile compounds	Descriptive	Threshold (mg/L)	Concentration (mg/L 40% vol.)
Methanol	Acohol, solvent	10000	650.12
Higher alcohol			
1-Propanol	Alcoholic, pleasant odor	720	207.29
2-Propanol	Sweet odor, ethanol-odor	1500	14.73
1-Butanol	Alcoholic, pleasant odor	5	0.21
2-Butanol	Alcoholic, pleasant odor	10	0.29
2-methyl-1-propanol	Banana, ethanol-odor	200	468.08
2-methyl-1-butanol	Floral, fruity, almond	32	268.20
3-methyl-1-butanol	Sweet, malty, rancid	70	847.01
2-Phenylethanol	Roses, sweetish, perfumed	7.5	18.00
Total higher alcohol			*1823.83*
Ester			
Ethyl acetate	Ethereal, fruity, sweet	17	116.17
Ethyl formate	Rum-like, peach, apple	150	1.29
Ethyl lactate	Penetrating odor	5.8	1.41
Ethyl hexanoate	Apple, fruity, sweetish	1	0.92
Butyl acetate	Banana, fruity	1.83	0.12
Propyl acetate	Ethereal, fruity, perfumed	30	0.08
Isoamyl acetate	Bitter, green leaves	15	6.72
Total ester			*126.72*
Acetaldehyde	Pungent, fresh, green	10	136.13

DISCUSSION

ANOVA analysis results showed these second-order regressions were statistically significant. Insignificant difference in p-values of the lack of fit (pLOF> 0.05) indicated these models are sufficiently accurate for predicting the responses in production yield of alcohol and volatile compound. Besides, all R^2, adjusted R^2, and Q^2 for all response variables were higher than 0.8, which revealed a perfect model with good predictive power.

The obtaining experimental values were close to the predicted values, which suggests that the obtaining optimization values have been reliable and could be applied in the spirits fermentation from cherry juices.

A total of 17 volatile compounds were identified and quantified in the Hungarian cherry spirit. In which, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and ethyl acetate were considered the major aroma compound in the spirit.

CONCLUSIONS

The results showed that response surface methodology coupled with the central composite rotatable design are powerful tools for modelling, optimizing, and studying the interactive effects of fermentation conditions (including temperature, pH, and total soluble solids) for alcohol and volatile compounds' production yield. Maximum production yields of alcohol of 59.68 (equivalent to alcohol content of 9.02% vol.) and volatile compounds of 2231.68 (equivalent to volatile compounds of 337.37 mg/L) were obtained at an optimized temperature of 24.71°C, pH of 3.25, and total soluble solids of 22.49 Brix. 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and ethyl acetate were considered the major aroma compound in the cherry spirit. The results revealed that the production yields of alcohol and volatile compounds could be enhanced by optimizing fermentation conditions. It is suggested that the models obtained can be used to optimize the fermentation process in spirits production from sour cherry.

References

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