Optimization of Fermentation Conditions for Production of Hungarian Sour Cherry Spirit Using Response Surface Methodology

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Abstract: Pálinka is a traditional fruit spirit and a kind of gastronomic heritage in Hungary. In Pálinka production, fermentation is one of the most important processes affecting the quality and yield of spirits. 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 through three variables in a defined range of temperature (15–25 °C), pH (2.75–3.75), and total soluble solid (18–30 °Brix). After eight fermentation days, production yields of alcohol and volatile compounds were a maximum of 9.02% v/v and 337.37 mg/L at an optimized temperature of 24.71 °C, pH of 3.25, and total soluble solid of 22.49 °Brix. The GC-FID analysis results showed 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 spirits. These results provided important information in serving the basic to develop standard fruit spirits production from sour cherry.

Keywords: Pálinka; spirits; RSM; Plackett–Burman design; Saccharomyces cerevisiae; sour cherry; GC-FID

1. Introduction

Pálinka is a fruit spirit of Hungary that originated in the Middle Ages, produced exclusively by the alcoholic fermentation and distillation of fleshy fruit or must of such fruit, berries, or vegetables, with or without stones [1–3]. This spirit is regarded as a traditional alcoholic beverage and a kind of gastronomic heritage in this country. Pálinka is the commercial name for fruit spirit from Hungary and has been protected by the European Union law 2004 and local law LXVIII 2008 [4]. Accordingly, all producers outside of this country are not allowed to use the brand “Pálinka” for their products, but they freely make and sell under a different name. In these laws, a Pálinka must be fermented from domestic fruit, distilled, and bottled in Hungary with alcohol content no higher than 86% v/v and at least 37.5% v/v. Furthermore, it has a distinctive aroma and taste obtained from the distilled raw materials, not allowing the addition of flavorings or ethyl alcohol of agricultural origin. In addition, hydrocyanic acid is not permitted higher than 7 g/hL absolute alcohol, and most permitted methanol content in Pálinka cannot be higher than 1200 g/hL absolute alcohol [2]. Cherries are divided into sweet cherries (Prunus avium) and sour cherries...
Cherry has a high sugar content which is especially suitable to produce a good spirit [5]. The alcohol yield is extremely high, ranging from 5 to 7 L/100 kg of cherries [1]. Additionally, sour cherries contain high amounts of carboxyls, esters, acids, and terpenes comprising the flavor of the fruit [6]. Over 100 volatile compounds have been identified in both sweet and sour cherries [7]. Nikićević et al. [8] indicated that ethyl esters including ethyl decanoate, ethyl hexanoate, isoamyl acetate, etc. were the most abundant aroma compounds of five sour cherry varieties (Oblacinska, Celery’s 16, Rexle, Heiman’s Ruby, and Heiman’s Conserve) grown in Serbia.

Although volatile compounds account for a small amount compared to ethanol and water, they mainly contribute to the aroma and taste of beverages. The variety and characteristics in spirit flavor are caused by the differences in the composition and concentration of a complex matrix containing many volatile compounds. Januszek et al. [9] conducted a sensory analysis of apple spirits obtained from Saccharomyces cerevisiae fermentation of ten apple cultivars (Elise, Rubin, Topaz, Golden delicious, Szampion, Gloster, Pinova, Florina, Idared, and Jonagored) grown in Poland. The highest scores of brandies were observed in Topaz, Rubin, Elise, and Florina, which performed pleasant, sweet, fruity, citrus, and alcoholic aromas. According to these authors, the sweet and citrus aroma attribute could be associated with the variety and concentration of terpenes, e.g., α-phellandrene, o-cymene, α-terpineol, citric, and myrcene. In the production of spirits, fermentation is an important process that needs to be considered [10]. Some works reported that the changes of fermentation temperature can significantly impact the formation of aroma compounds and sensory profiles of beverages [11–13]. Gómez et al. [14] reported that fermentation pH greatly influenced the final quality of the spirits. In fermentation, changes in the initial sugar concentration of the grape juice impacted growth rates for yeasts and cell biomass [15].

According to Nwabueze [16], selecting process combinations for optimization, which has not analyzed relevant experimental designs, is scientifically unreliable and irreproducible. For successfully optimizing, Response Surface Methodology is one of the mathematical models, giving an accurate map. The RSM is a helpful method for mapping a response surface over a particular region of interest, optimizing the response, and selecting operating parameters to achieve the desired response [17]. However, the RSM is most frequently used to optimize the response surface. Three critical steps required in the process optimization by the RSM method include creating a statistical experimental design, estimating coefficients in the mathematical model and predicting the response, and checking the accuracy within predicted and observed variables. Recently, many works have applied RSM to evaluate the effects of individual variables and their interaction for optimizing alcohol fermentation. For instance, when optimizing the fermentation conditions for producing jabuticaba distillate by RSM, the temperature of 20 °C and °Brix of 22 were optimal to maximize ethanol, glycerol, volatile compounds, and volumetric productivity of ethanol and to minimize acetic acid and malic acid [18]. Tsengay et al. [19] reported that the temperature of 30 °C, pH of 3.9, and inoculum concentration of 16% were optimal values for the fermentation process of cactus pear fruit to produce wine. The sensorial acceptance and total antioxidant content were aimed to be maximized. In contrast, the alcohol content was targeted in a range of 8–15% v/v, and the total acidity was directed at 13.1 g/L of tartaric acid.

The objective of this study was to optimize the three basic variables of the fermentation process (including temperature, pH, and total soluble solid content) for producing Hungarian spirit from sour cherry by using response surface methodology coupled with central composite design, then evaluating the volatile compounds in fruit spirit obtained.

2. Materials and Methods

2.1. Fruit Juices

Concentrate juices of sour cherry were provided by the INNIGHT Company (Budapest, Hungary). Concentrate juices were diluted to the desired strength of initial soluble solids (18, 24, and 30 °Brix) with tap water, then adjusted to the desired pH (2.75, 3.25, and
3.75) by 3N phosphoric acid or 3N sodium hydroxide solution. Tap water was used to provide partially minerals such as calcium (132 mg/L CaO), lead (0.7 µL), iron (21 µg/L), manganese (1 µg/L), and arsenic (1.3 µg/L) [20], which enhanced the yeast enzymatic activity in the fermentation process [21].

2.2. Inoculum Preparation

*Saccharomyces cerevisiae* N0.342 with the commercial name of Uvaferm Danstil A obtained from Lallemand Inc. (Montréal, QC, Canada) was used throughout the study. The yeast was activated by adding 1.0 g of the dried yeast into a 250 mL conical flask containing 100 mL media 1% of yeast nutrient namely UvavitalTM (Lallemand Inc., Montréal, QC, Canada). The composition of yeast nutrients consisted of vitamins (thiamine, biotin, folic acid, etc.), amino acids, peptides, and polypeptides, proteins, ionic nitrogen, microelements, sterols, unsaturated fatty acids, oxygen-binding compounds, and inactive yeasts. Afterwards, the culture was incubated in a rotary shaker with a speed of 120 rpm at 28 °C for 2 h. The cell number reached approximately $2.5 \times 10^8$ cells/mL.

2.3. Alcoholic Fermentation

Each conical flask 500 mL contained 300 mL fruit juice with initial soluble solid contents and pH-s in Table 1. Then, 2% (v/v) pre-culture of the activated yeast was added and mounted by twin bubble airlocks to close the air and provide facultative anaerobic conditions. The fermentation was conducted at the desired temperature (Table 1). After 8 days, fermented fruit juice was sampled to analyze alcohol content and volatile compounds. Three replicates of the experiment were conducted.

Table 1. Independent variables in the experimental plan.

| Variables                         | Coded Levels |
|-----------------------------------|--------------|
| Temperature, $X_1$ (°C)          | −1 0 1       |
| pH, $X_2$                        | 2.75 3.25 3.75 |
| Soluble solids content, $X_3$ (°Brix) | 18 24 30    |

2.4. Distillation

With the optimum values obtained, 5.5 L sour cherry juice was fermented. After the alcoholic fermentation completed, the fermented mashes were distilled immediately twice. Cooling water of 15 °C had been circulated through the entire system before distillation began. The first distillation was stopped when the alcohol degree in the outlet was lower than 5%. The total volume of distillate in the first distillation reached approximately 1.8 L with an alcohol content of 22.8% v/v. In the second distillation, the head fraction was collected separately and standardized to a volume corresponding to about 1.5% of the distillate, while the heart fraction was collected until an alcohol content in the outflow dropped to 40% v/v. The final distillates were stored in glass bottles and kept at 4 °C until analysis in order to avoid the loss of aroma.

2.5. Chemical Analysis

2.5.1. Measurement of Total Soluble Solid and pH

The total soluble solid (°Brix) and pH were measured by using a refractometer (Atago, Tokyo, Japan) and a pH meter (Mettler Toledo, Greifensee, Switzerland), respectively.

2.5.2. Alcohol Content

Alcohol content of the fermented mash was determined by distilling and measuring the density of the distillate. In addition, 100 mL of fermented mash was taken out from each fermentation flask, then one drop of silicone oil was added to prevent the mashing.
foaming during the distillation process. The mash was distilled by using a steam injection distillation unit (Büchi K-350, Flawil, Switzerland) in 3 min. The distillate was collected in a 100 mL volumetric flask and diluted to the mark with distilled water. Alcohol content was measured by a digital density meter (Anton Paar DMA 35N, Graz, Austria).

2.5.3. Volatile Compound

Major volatile compounds were analyzed by a GC-FID system (Perichrom 2100, ALPHA MOS, Toulouse, France). The compounds were separated on a CHROMPACK CP-WAX 57CB Wcot (Agilent, Santa Clara, CA, USA) fused silica column (polyethylene glycol stationary phase, 50 m × 0.25 mm i.d. with 0.25 μm film thickness). The temperature program of the oven was as follows: initial 60 °C (isotherm for 6 min), ramp rate (6 °C/min to 83 °C and afterward to 220 °C at a rate of 10 °C/min), temperatures of injector and detector were 210 °C and 220 °C, respectively. Carrier gas was helium at 3 mL/min. Generally, 1–1 μL samples were injected twice with an automatic sampling system. The identification of volatile compounds was done by comparing the retention times of the samples with those of standard compounds injected at the same conditions. Calibration graphs used for the calculations and quantification were prepared by GC analysis of solutions containing known amounts (30 μL, 60 μL, 100 μL of each standard compound in 970 μL, 940 μL, and 900 μL of 40% v/v ethanol solution, respectively) of external standards including acetaldehyde, ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate, isooamyl acetate, methanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, n-heptanol, 2-phenylethanol, etc. Internal standards were also prepared by the addition of 10 μL of 2-methyl-1-propanol or n-heptanol into 990 μL of distilled samples, and 1 μL was injected in gas chromatography.

2.6. Experimental Design and Data Analysis

The fermentation process from sour cherry juice and *Saccharomyces cerevisiae* by RSM coupled with the central composite rotatable design were investigated to optimize fermentation conditions through three independent variables of temperature ($X_1$, °C), pH ($X_2$), and total soluble solid ($X_3$, °Brix). The production yields of alcohol ($Y_1$) and volatile compound ($Y_2$) were chosen as dependent variables. For $Y_1$ and $Y_2$ determination, the equations presented below were used:

$$Y_1 = P_1/S \times 100$$

$$Y_2 = P_2/S \times 100$$

where $S$ is total sugar (g/100 mL), $P_1$ is alcohol content (% v/v), and $P_2$ is total volatile compounds (mg/L). Major compounds in total volatile compounds include acetaldehyde, ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, isooamyl acetate, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, n-heptanol, 2-phenylethanol.

Following RSM proposed by Myers et al. [17], $2^3$ points in the corners of the cube representing the experimental domain, $2 \times 3$ axial points in the center of each face of the cube, and 3 replicated points in the center of the cube, leading to a total number of 17 experiments (a two-level factorial design), were employed (Table 2). The second-order polynomial function was used to obtain response surfaces on the chosen model for each response variable and for predicting the optimal value assessed as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3$$

where $Y$ is a predicted response, $X_1$, $X_2$, $X_3$ are predictor variables for temperature, pH, and total soluble solid content; $b_0$ is an offset term; $b_1$, $b_2$, and $b_3$ are linear effects; $b_{11}$, $b_{22}$, and $b_{33}$ are squared effects, and $b_{12}$, $b_{23}$ and $b_{13}$ are interaction terms.
Table 2. The central composite design matrix and experimental values.

| Run | Independent Variables | Dependent Variables |
|-----|-----------------------|---------------------|
|     | X₁ (°C) | X₂ | X₃ (°Brix) | Y₁ | Y₂ |
| 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      | 54.06 | 2008.55 |
| 14  | 20      | 3.25 | 30      | 54.06 | 1897.68 |
| 15  | 20      | 3.25 | 24      | 58.9  | 2149.30 |
| 16  | 20      | 3.25 | 24      | 58.48 | 2140.80 |
| 17  | 20      | 3.25 | 24      | 57.86 | 2174.91 |

X₁: temperature (°C), X₂: pH, X₃: total soluble solid content (°Brix), Y₁: production yield of alcohol (%) and Y₂: production yield of volatile compounds (%).

The experimental design matrix, data analysis, and optimization procedure were performed using Modde 5, Version 5.0 (Umetrics AB, Umeå, Sweden).

3. Results and Discussion

3.1. Optimization of Fermentation Conditions

Tables 1 and 2 showed the design matrix and the corresponding experimental data. The production yield of total volatile compounds reached a minimum of 1605.58 and a maximum of 2229.64, while the alcohol yield ranged from 44.14 to 59.79. Multiple regressions analyzing the experimental data were applied to find the second-order polynomial equations representing production yields of alcohol and volatile compounds. Full predictive equations were given below:

\[
Y₁ = 57.91 + 4.11X₁ + 0.97X₂ − 2.61X₃ − 2.87X₁² − 1.63X₂² − 1.98X₃² + 1.01X₁X₂ + 0.31X₁X₃ + 0.1X₂X₃
\]

\[
Y₂ = 2153.09 + 103.64X₁ − 76.26X₂ − 24.30X₃ − 20.44X₁² − 116.49X₂² − 198.54X₃² + 6.88X₁X₂ − 26.22X₁X₃ + 8.54X₂X₃
\]

ANOVA analysis results in Table 3 showed that these second-order regressions were statistically significant (all pANOVA < 0.05). An insignificant difference in p-values of the lack of fit (pLOF > 0.05) indicated that these models are sufficiently accurate for predicting the responses in the yields of alcohol and volatile compounds. In addition, all coefficients of determination values (R²) for all response variables were higher than 0.8, for which the value was considered sufficiently good [18]. Adjusted R² values of the three dependent variables in production yields of alcohol and volatile compound were 0.96 and 0.99, respectively. The adjusted determination coefficient values were high and close to the determination coefficient, which indicated the high significance of the models. In addition, the predictive powers Q² of all models from the dependent variables being higher than 0.8 (Q² of the production yields of alcohol and volatile compound were 0.889 and 0.942) revealed a perfect model with good predictive power.
The regression model’s coefficients, including three linear, three quadratic, three interaction terms, and one block term, were listed in Table 3. The effects of temperature, pH, and total soluble solid content of the fermentation process and their interactions on the production of ethanol and aroma compounds were found to be significant (p \leq 0.05). They were shown through the model terms with among their respective probability of 95%. The model terms of linear (X_1, X_2, X_3), quadratic (X_1^2, X_2^2, X_3^2), and interaction of X_1X_2 significantly affected the alcohol yield while other interactions (X_1X_3 and X_2X_3) were not significant. In the case of volatile compounds’ production yield, the model terms of linear (X_1, X_2, X_3), quadratic (X_2^2, X_3^2), and interaction of X_1X_3 were significant with a probability of 95%, except for model terms of X_1^2, X_1X_2, and X_2X_3. In addition, the quadratic response 3D surface plot in Figure 1 would illustrate clearly the optimization model of fermentation temperature, pH, and initial soluble solids content for the yields of alcohol and volatile compounds.

Figure 1A(a–c) showed that the alcohol yield was lowest at a high total soluble solid content (30 °Brix), a low temperature (15 °C), and a low pH (2.75). It went up as reducing total soluble solid content toward 18 °Brix, raising the temperature to 25 °C and combined with an increase of pH to 3.25; however, the yield will fall if pH increased continuously to 3.75. The increase of temperature in a range of 15–25 °C had a positive effect on the formation of volatile compounds (Figure 1B(a–c)). When rising total soluble solid to 24 °Brix and pH to 3.25, the total volatile compounds’ production yield rose. Still, their yield will reduce rapidly if continuing to increase total soluble solid content and pH.

High initial sugar content is more preferred in alcohol fermentation, which may raise the content of ethanol as well as other products in the fermented mash. Like optimizing fermentation conditions for ethanol production from palmyra jaggery, a substrate content of 398.5 g/L was optimal in a survey range of 316.9–484.1 g/L [22]. Nevertheless, yeast cells might be exposed to high osmotic stress if the media had sugar content that was too high, affecting the fermentation processes [23]. In the study, the lowest production yields of both alcohol and total volatile compounds were observed in the case of 30 °Brix. This suggested that the initial soluble solid of 30 °Brix influenced the fermentation performance of yeast negatively.

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

| Parameters | Y_1 | p-Values | Y_2 | p-Values |
|------------|-----|----------|-----|----------|
| Coefficient Values | 2153.09 | 0.0001 *** | 26.22 | 0.0132 * |
| Constant | 57.91 | <0.0001 *** | 8.54 | 0.3193 ns |
| X_1 | 4.11 | <0.0001 *** | −26.22 | 0.0132 * |
| X_2 | 0.97 | 0.028 * | 8.54 | 0.3193 ns |
| X_3 | −2.61 | <0.0001 *** | −198.54 | <0.0001 *** |
| X_1^2 | −2.87 | 0.0015 ** | 6.88 | 0.4163 ns |
| 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 | 0.4163 ns |
| X_1X_3 | 0.31 | 0.3753 ns | −26.22 | 0.0132 * |
| X_2X_3 | 0.10 | 0.7615 ns | 8.54 | 0.3193 ns |

Sig.: significance (*, **, ***)—display the significance at 0.05, 0.01 and 0.001 by least significant difference. ns: not significant.
A rising temperature from 15 °C to 25 °C led to an increase in total volatile compounds and alcohol yield. The results were consistent with the findings of Prusina and Herjavec [24] as well as Reddy and Reddy [25]. Beltran et al. [26] reported that, when the temperature rose from 13 °C to 25 °C, there were increases in yeast fermentation and growth rates, and yeast cells growing at a low temperature utilized less nitrogen than at 25 °C.

External pH can affect the cell wall structure and alter cell permeability to ions and other minor metabolites, which affects the growth and fermentation rate of yeast and influences the constitution of fermentation products [27]. A decrease in the production yield of the volatile compounds was recorded if pH raised from 3.25 to 3.75. García-Llobodanin et al. [28] reported that increasing pH from 3.2 to 4.25 significantly reduced the content of almost higher alcohols and increased the ethyl acetate content in the pear spirits. This led to the concentration of total volatile compounds dropping because total higher alcohol content predominated quantitatively over total ester content. A similar trend was found in fermenting durian wine [29].

3.2. Validation of the Optimized Conditions

Many studies have pointed out that higher alcohols and esters make an essential and positive contribution to the quality of alcohol fermentation products [18,30–32]. However, a high concentration of these compounds, especially higher alcohols, can cause the negative sensorial properties such as suppression of fruity and woody notes [31–33]. The total volatile yield of distillates ranged from 1605.58 mg/L to 2229.64 mg/L (Table 2), equivalent to total volatile compounds that went from 3167.79 mg/L of absolute alcohol to 3771.70 mg/L of absolute alcohol. All individual compounds used for optimizing were volatile components that had positive effects on the flavor and taste of the spirits, such as malty, fruity, sweetish, alcoholic, and pleasant odor. The characters of these volatile compounds were described in Table 4 as well as in this study, and the positive effects expressed in concentration of those compounds were aimed to optimize. Moreover, comparing with the minimum limit of volatile substances of 2000 mg/L absolute alcohol [2],
the concentration of total volatile compounds fluctuated in the safe range. Because the alcohol content of the distilled Pálinka is ranged from 37.5 % v/v to 86% v/v [2], during the optimization process, the production yields of alcohol and volatile compounds were aimed to be maximized in order to increase flavors for the final cherry spirit. After optimizing with the full regression models (Equations (4) and (5)), the optimal conditions including temperature, pH, and soluble solids content were 24.71 °C, 3.25, and 22.49 °Brix, respectively. Additionally, predicted values of alcohol and volatile compounds’ production yields were 59.68 and 2231.68, respectively, equivalent to 9.02% v/v and 337.37 mg/L (Table 5).

Table 4. Volatile compounds of fruit spirits from sour cherry.

| Volatile Compounds | Descriptive                              | Threshold (mg/L) | Concentration (mg/L 40% v/v) |
|--------------------|------------------------------------------|------------------|------------------------------|
| methanol           | Alcoholic, solvent [34]                  | 10,000 [34]      | 650.12                       |
| higher alcohol     | 1-propanol                               | 720 [35]         | 207.29                       |
|                    | 2-propanol                               | 1500 [34]        | 14.73                        |
|                    | 1-butanol                                | 5 [35]           | 0.21                         |
|                    | 2-butanol                                | 10 [35]          | 0.29                         |
| 2-methyl-1-propanol| Malty, ethanol-odor [34]                 | 200 [34]         | 468.08                       |
| 2-methyl-1-butanol | Banana, malty, ethanol-odor [35,37]     | 32 [35]          | 268.20                       |
| 3-methyl-1-butanol | Sweetish, malty, banana [34,37]         | 70 [34]          | 847.01                       |
| 2-phenylethanol     | Roses, sweetish, perfumed [35,37]       | 7.5 [35]         | 18.00                        |
| total higher alcohol|                                         |                  | 1823.83                      |
| ester              | ethyl acetate                            | 17 [35]          | 116.17                       |
|                    | ethyl formate                            | 150 [34]         | 1.29                         |
|                    | ethyl lactate                            | 14 [35]          | 1.41                         |
|                    | ethyl hexanoate                          | 0.23 [34]        | 0.92                         |
|                    | butyl acetate                            | 1.83 [38]        | 0.12                         |
|                    | propyl acetate                           | 30 [34]          | 0.08                         |
|                    | isovalyl acetate                         | 1.6 [34]         | 6.72                         |
| total ester        |                                         |                  | 126.72                       |
| acetaldehyde       | Green leaves, fruity, sharp [34]         | 10 [34]          | 136.13                       |

Table 5. Estimated optimum values and predicted, experimental values of responses.

| Variables                               | Optimum Values | Predicted Values | Experimental Values |
|-----------------------------------------|----------------|------------------|---------------------|
| Temperature, X1, (°C)                   | 24.71          |                  | 60.86               |
| pH, X2                                  | 3.25           |                  | 9.20                |
| Total soluble solid content, X3, (°Brix)| 22.49          |                  | 2339.7              |
| Production yield of alcohol (%), Y1     | 59.68          | 60.86            |                     |
| Alcohol content, (% v/v)                | 9.02           | 9.20             |                     |
| Production yield of volatile compound (%), Y2 | 2231.68    | 2339.7           |                     |
| Volatile compounds, (mg/L)              | 337.37         | 353.71           |                     |

To evaluate the estimated optimum values obtained, a confirmatory experiment was carried out under these optimum values. Then, the experimental values would be compared to the optimum values. The obtained experimental values of the production yields of alcohol and volatile compounds were 60.86 and 2339.7, respectively, equivalent to 9.2% v/v and 353.71 mg/L. These experimental results were close to the predicted values. It suggests that the obtaining optimization values have been reliable and could be applied in the spirits fermentation from cherry juices.
3.3. Volatile Compound of Hungarian Spirits from Sour Cherry

The quality of spirits primarily depends on fruit type, climate, geographical origin, harvest method, and alcohol processing techniques [33]. The fruit spirits were constituted by a large variety of compounds, including methanol, higher alcohols, esters, fatty acids, carboxylic compounds (such as aldehydes, ketones and acetals), and others [39]. A total of 17 volatile compounds were identified and quantified in the Hungarian cherry spirit (Table 5). In addition, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and ethyl acetate were considered the major aroma compounds in the spirit. For the major compounds, isoamyl alcohols were the most abundant compounds recorded. These results correspond with the findings of Christoph and Bauer-Christoph [40] and Winterova et al. [41]. A high methanol concentration has been considered a characteristic of fruit spirits because methanol is formed by the enzymatic hydrolysis of fruit pectin, a natural component of fruit in high concentrations [40]. There were different views, but most of them confirmed that methanol had a mild, bland odor and un-impact on the spirits’ flavor due to its perceived threshold is very high. However, it was one of the most critical substances to observe and manage because its dangers might affect the health of consumers [30,37,40]. The methanol content in the obtaining spirit accounted for 650.12 mg/L, equivalent to 1625.30 mg/L absolute alcohol. Following EC Regulation, No. 110/2008 [2], the maximum limit approved for the methanol concentration is 10,000 mg/L absolute alcohol. It can be seen clearly that there was no limit to exceed.

Acetaldehyde is a direct alcoholic fermentation by-product of the oxidation of ethanol by acetic acid bacteria in the presence of oxygen [30]. Acetaldehyde is the main compound of the aldehyde group presenting in the spirits and has a bit of a pungent odor [40]. Acetaldehyde content was recorded at 136.13 mg/L (Table 5).

Higher alcohols are alcohols having more than two carbons and are formed during alcoholic fermentation via the conversion of branched-chain amino acids presenting in mashes. For instance, S. cerevisiae can produce 3-methyl-1-butanol and 2-methyl-1-butanol during fermentation via the Ehrlich pathway from the catabolism of isoleucine and leucine, respectively [42,43]. Higher alcohols contribute importantly to the aroma profile of fruit spirit with a pleasant odor but a too high amount may give negative effects as being a strong, pungent flavor and taste [30]. 3-methyl-1-butanol and 2-methyl-1-butanol contribute to the alcoholic, aromatic, and fruity odors of fruit spirits [37]. 2-methyl-1-propanol contributes to alcohol, banana, and wine odors for alcoholic beverages [37,40,42,43]. In cherry spirit, 3-methyl-1-butanol had a higher alcohol content accounting for the highest concentration (847.01 mg/L) while 2-methyl-1-butanol and 2-methyl-1-propanol reached 268.20 and 468.08 mg/L, respectively. These results were in agreement with the previous finding [41]. However, 3-methyl-1-butanol content in this work was 1.8 times higher in comparison with the report of Schehl et al. [44]. In contrast to other higher alcohols, 1-propanol is known to be formed by the condensation of pyruvic acid and acetyl CoA [45]. 1-propanol provided pungent and alcoholic odor [37], and reached 207.29 mg/L. The result was similar to the results [41,44]. Christoph and Bauer-Christoph [40] and Spaho [30] reported that 1-propanol levels higher than 5000 mg/L absolute alcohol were indicators for the spoilage of fruit mashes. Obtaining 1-propanol content being lower than that level meant that the fruit juices we used were good quality. Other higher alcohols were in lower concentration including 2-propanol of 14.73 mg/L, 1-butanol of 0.21 mg/L, 2-butanol of 0.29 mg/L, and 2-phenyl-ethanol of 18.0 mg/L.

Esters are formed mostly through the esterification of alcohols with fatty acids during fermentation. Esters, especially ethyl esters, represented the largest group in the number and content of aroma components found [43], in which ethyl acetate was considered to be the dominant compound, accounting for 116.17 mg/L. A similar response was reported by Schehl et al. [44], whereas it was lower there than those reported by Winterova et al. [41]. In small quantities, ethyl acetate had fruity and sweetish odor, but in large amounts, it gave a sharp and glue smell for fruit spirits [30,37]. Other esters, though occupying a lower content, also contributed significantly to the fruity and floral flavor for the spirits,
such as ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate, and isoamyl acetate, accounting for 1.29, 1.41, 0.92, 0.12, 0.08, and 6.72 mg/L, respectively. Following EC Regulation, No. 110/2008 [2], the number of volatile substances should be at least 2000 mg/L absolute alcohol. The total volatile compounds in this work reached 1950.55 mg/L, equivalent to 4876.37 mg/L absolute alcohol, which showed that it was completely consistent with the regulation.

4. 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 solid) for alcohol and volatile compounds’ production yields. Maximum production yields of alcohol of 59.68 (equivalent to alcohol content of 9.02% v/v) 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 solid 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 suggested that the models obtained can be used to optimize the fermentation process in spirits production from sour cherry.

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