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PRODUCTION AND QUALITY ANALYSIS OF SOME SPIRITS FROM FRUITS

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1. INTRODUCTION AND OBJECTIVES

Fruit spirits belong to the group known as “spirit-based” beverages, which are very popular worldwide, especially in Eastern and Central European countries. They are regarded as a traditional alcoholic beverage and a kind of gastronomic heritage. According to European Community Regulation EC 110/2008, 'Fruit spirit is a spirit drink produced exclusively by the alcoholic fermentation and distillation of fleshy fruit or must of such fruit, berries or vegetables, with or without stones'. The alcohol content of fruit spirits has more than 37.5 % v/v and less than 86 % v/v, and they should have an aroma and taste originated from the raw materials. In most cases, the maximum allowed methanol content of fruit spirit is 1000 g/hL absolute alcohol and cannot be allowed to be flavored artificially.

Pálinka is a traditional Hungarian spirit drink produced exclusively by the alcoholic fermentation and distillation of any native fruits cultured in Hungary. There are many kinds of pálinka-s with different characteristics based on specific types of fruit used in the fermentation. The most common fruits for production of pálinka are apricot, pear, plum, cherry, grape and apple as well as some exotic fruits such as blueberry, raspberry, black currant, cranberry etc. Fruit spirits as well as pálinka are widely consumed in European countries such as Hungary, France, Spain, Italy, Germany, Austria etc. and some on the world such as the USA, Canada, China, etc. It is protected as a geographical indication by the European Union. Therefore, only fruit spirits fermented, distilled and bottled in Hungary and four regions of Austria can be called “Pálinka”.

Spirits are often made from various sorts of fruits that have the common feature of high sugar content. They can be distributed into three groups, including pome fruits (apples, pears), stone fruits (sour cherries, peaches, plums, and apricots), and small fruits (blackberries and cranberries). Although the principal components of fruit spirits are ethanol and water, their flavor and taste are very varied, mostly coming from the natural aroma of fruits. 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.

The production process of spirits consists of the following stages: fermentation, distillation, and maturation. There are many factors influencing the quality of spirits such as fruit material (the type of fruit, the geographical origin, the method of cultivation, storage, and time of harvest), conditions of the alcoholic fermentation (temperature, pH, yeast strain, nutrient), distillation conditions (equipment type or parameters of distillation), and maturation conditions (time, temperature, the kind of wood). The correct separation of the three distillation fractions (heads, heart, and tails) will also be necessary. Much research focuses on investigating the distribution of volatile compounds during spirits distillation to find the appropriate cut-points for separating methanol and others having a negative sensory impact.

Main goal of my PhD research is the production and quality analysis of different pálinka-s made from different fruits. The main tasks are:

- Screening different commercial yeast strains for fruit spirits fermentation. Selection of best one for pálinka production from apple, apricot, cherry and pear
- Investigation of effects of different factors on the alcoholic fermentation process
    - temperature
    - pH
    - initial soluble solid contents
- Optimization of the fermentation process for production of pálinka
- Investigation of effects of the distillation process on aroma compounds distribution
- Classification of fruit spirits using different chemometric methods such as PCA and LDA.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Reagents, chemicals and standards

All chemicals and standards were analytical grades and purchased either from Sigma–Aldrich (USA), Lachner (Czech Republic), VWR Chemicals (USA), or Fluka (Hungary).

2.1.2 Yeast strains

Nine different yeast strains were provided by the Kokoferm Limited Company (Gyöngyös, Hungary), including Uvaferm SLO, Uvaferm PM, Uvaferm Danstil A, Fermiblanc Arom, Viniflora Melody, Vin-O-Ferm Roses, Fermicru AR2, Oenoferm X-treme F3, Oenoferm x-thiol F3. They are classical strains for red, white and sparkling winemaking.

These yeast strains were activated before fermentation by mixing 1 g dry yeast, 1 g yeast nutrients* (Uvavital^{TM}, Lallemand, Canada) and 100 mL warm water (28 °C), then the mixture was aerated by gentle agitation for 2 hours to grew. 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, yeast extract.

2.1.3 Fruit juice

Concentrate juices, including sour cherry of 68 °Brix, apple of 70 °Brix, apricot of 65 °Brix and pear of 70 °Brix, were provided by the INNIGHT Company (Budapest, Hungary).

2.2 Experiment design

2.2.1 Selection of yeast strain for fermentation of fruit spirits

Fruit juice fermentations with each yeast strain were carried out separately in 500 mL Erlenmeyer conical flasks. Each flask contained 300 mL juice of 18.0 °Brix and 2% v/v pre-culture of the activated yeast strain, then was mounted by twin bubble airlock to close the air and provide facultative anaerobic conditions. The fermentation was conducted at 20 ℃ statically. Sampling was carried out daily to determine pH, Brix, reducing sugar, alcohol and organic acid content, excepting volatile compounds analyzed on the last day of the fermentation. Three replicates of the fermentation were performed for each strain to select a suitable yeast strain among fruit juice based on evaluating alcohol production capacity and volatile profile.

2.2.2 Optimization of fermentation process

2.2.2.1 Effect of temperature

Each conical flask 500 mL contained 300 mL fruit juice of 18.0 °Brix, pH 3.0 and 2 % v/v pre-culture of the activated Uvaferm Danstil A strain. The flasks were mounted by twin bubble airlocks to close the air and provide facultative anaerobic conditions. The fermentations were conducted at 10 ℃, 15 ℃, 20 ℃, 25 ℃, 30 ℃ and 35 ℃ statically. After eight days, fermented fruit juices were sampled to analyze Brix, reducing sugar, alcohol content, total higher alcohol and total ester.

2.2.2.2 Effect of pH

Each conical flask 500 mL contained 300 mL fruit juice of 18.0 °Brix, adjusted to the desired pH of 2.5, 2.75, 3.0, 3.5, 4.0 and 4.5 by 3n phosphoric acid or 3n sodium hydroxide. The alcoholic fermentation was initiated by adding pre-culture of the activated Uvaferm Danstil A strain in the ratio of 2 % v/v. The flasks were then mounted by twin bubble airlocks and kept at 20 ℃ statically. After eight days, fermented fruit juice was sampled and analyzed.

2.2.2.3 Effect of total soluble solids content

Different 500-mL conical flasks containing 300 mL fruit juices with pH 3.0 were prepared with initial total soluble solid contents of 12 °Brix, 18 °Brix, 24 °Brix, 30 °Brix and 36 °Brix. The fermentations were started by addition of pre-culture solution of the activated Uvaferm Danstil A strain in the ratio of 2 % v/v. Samples were taken and analyzed after eight fermentation days.

2.2.2.4 Optimization of fermentation conditions for alcohol production

The fermentation process from apricot, apple, sour cherry and pear 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 total volatile compounds (Y_2) were chosen as dependent variables.

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:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 \quad (1)$$

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.

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

2.2.3 Effects of distillation process on aromatic profile

2.2.3.1 Effects of distillation process on distribution of aroma compounds

The effects of the distillation process were investigated with the fermentation of 5.5L of each fruit juice at the optimum conditions. After the alcoholic fermentation was completed, the mashes were immediately transferred into the glass distillation system with a capacity of 3L (Figure 4.1). The cool water of around 15℃ – 18℃ was circulated through the entire system before distillation began. The cool water flow rate was adjusted for the alcohol product in the outflow not exceeding 9ml/min. The temperature of the heater was set at 102 ℃. Distillation was carried out slowly and continuously. It was stopped when the alcohol degree in the outflow was lower than 5 % v/v. The total volume of distillate in the first distillation reached around 1.8 L with an alcohol content ranging from 23 % v/v to 33 % v/v depending on the fruit mash applied. For describing the distribution of volatile compounds during the second distillation, the first cut volume was 1.5 % of the distillate. Other fractions were collected by volumes of each 100 mL cut until the outlet's alcohol content was below 5 % v/v. Besides, sensory evaluation was also performed adjunctively to find the appropriate cut-off point for the distillation process. Sensory samples for the cut-point of the head to heart fraction were 5 ml at 1%, 1.5% and 2% of the first distillate. Sensory samples for the cut-point of the heart to tail fraction were 20 mL of the distillate of around 50 % v/v, 45 % v/v, 40 % v/v and 35 % v/v. Three replicates of the distillation were performed with each fruit type. Samples of various kinds of fruit spirits were analyzed based on the alcohol content and the aroma compounds. In order to avoid the loss of aroma, all the fractions collected were kept at 4 °C until analysis.

2.2.3.2 Profile of spirit products from apple, cherry, pear and apricot

A similar experiment was conducted with the cut-points obtained. After the second distillation, fruit spirits (heart fractions) were stored at room temperature for two weeks for stabilizing their flavor and state. Then, they were diluted to 40% v/v and analyzed for volatile compounds to find the profile of these spirits.

2.2.4 Characterization and classification of pálinkas and fruits

A total of 48 pálinka samples (12 apple pálinkas, 12 apricot pálinkas, 12 pear pálinkas and 12 cherry pálinkas) were covered in this study. Chemometric statistics methods were applied to confirm the key aroma compounds and to classify fruit spirits and fruits used.

2.3 Analytical methods

2.3.1 Measurement of Brix and pH

The total soluble solids (°Brix) and pH were measured by use of refractometer (Atago, Japan) and pH meter (Mettler Toledo, Switzerland), respectively.

2.3.2 Alcohol content

Two different methods were used to determine the alcohol content of the samples. In case of metabolism analysis, the ethanol concentration was measured by HPLC with RI detector. In all other cases, alcohol content of the fermented mash was determined by distilling and measuring the density of the distillate.

2.3.3 Analysis of reducing sugars and organic acids

Samples were centrifuged at speed of 9,168 g and room temperature for 10 minutes before the analysis process. Sugars and organic acids were detected by HPLC system (Surveyor, Thermo Scientific, San Jose, USA) with Aligent Hi-Plex H column 7.7 x 300mm (Agilent, Santa Clara, USA).

2.3.4 Analysis of volatile compounds by GC-FID

The analyses of the volatile compounds were done with a GC-FID system (Perichrom, ALPHA MOS, France). The compounds were separated on a CHROMPACK CP-WAX 57CB Wcot fused silica column (polyethylene glycol stationary phase, 50 m*0.25 mm i.d. with 0.25µm film thickness).

2.4 Statistical analysis

The unpaired and paired Student's t-test were applied to compare the experimental results. In addition, the one-way analysis of variance (ANOVA), LSD test and Tukey-HSD test were applied to check the regression analysis. Before the statistical procedure, the data were checked for normality. All of the tests were done using R-studio and STATGRAPHICS Centurion XV with a significant level 5 % (α=0.05).

Response surface methodology was employed to optimization of fermentation conditions. Both the experimental design and data processing were carried on commercial software Modde 5.0.

3. RESULTS AND DISCUSSIONS

3.1 Selection of yeast strains for fruit spirit fermentation

During eight fermentation days, Brix value reduced rapidly from 17.20 °Brix to around 6.01 °Brix for apple, from 17.55 °Brix to around 9.36 °Brix for apricot, from 17.17 °Brix to around 9.30 °Brix for cherry, and from 17.87 °Brix to around 9.21 °Brix for pear (in where, 6.01 °Brix, 9.36 °Brix, 9.30 °Brix and 9.21 °Brix was the average of residual Brix values from nine yeast strain over apple, apricot, cherry and pear). Correspondingly, the total reducing sugar content decreased from 13.55 mg/100 mL to around 0.72 mg/100 mL, from 10.57 mg/100 mL to round 1.67 mg/100 mL, from 10.99 mg/100 mL to around 1.69 mg/100 mL, and from 10.54 mg/100 mL to around 2.10 mg/100 mL for apple, apricot, cherry and pear juices, respectively. At the end of fermentation, alcoholic content reached from 9.17 % v/v to 9.43 % v/v for apple, from 6.60 % v/v to 7.10 % v/v for apricot, from 5.93 % v/v to 6.20 % v/v for cherry, from 7.13 % v/v to 7.43 % v/v for pear. Alcohol yield ranged from 0.68 – 0.70, 0.59 – 0.68, 0.55 – 0.57 and 0.67 – 0.70 by apple, apricot, cherry and pear, respectively. However, for each fermented fruit juice, no significant difference in the alcohol production capacity among these yeast strains was found (p-value > 0.05). Although there was no significant difference among the alcohol production capacity of the yeast strains tested, the experimental results indicated that all these commercial yeast strains were strongly suitable for spirit production with a high yield of alcohol production, a short fermentation time and a stable pH during an alcohol fermentation process, especially strain Uvaferm Danstil A.

In fermented mashes from apple and pear, the content of total volatile compounds was the highest level in the case of strains Uvaferm Danstil A, Fermiblanc Arom, Vin-O-Ferm Roses and Fermicru AR2, whereas in fermented cherry mashes the total aroma compound peaked in the case of these strains and Viniflora Melody. In addition, in fermented mashes from apricot, it reached the maximum value in the case of strains Uvaferm Danstil A, Vin-O-Ferm Roses, Fermicru AR2 and Oenoferm x-treme F3. It suggests that Uvaferm Danstil A, Fermiblanc Arom, Vin-O-Ferm Roses and Fermicru AR2 are potential yeast strains in distilled alcohol production with high volatile compound content.

Based on these analysis results, it can be seen clearly that all these commercial yeast strains are suitable for use in the production of distilled alcohol in general and pálinkas in particular, especially Uvaferm Danstil A, Fermiblanc Arom, Vin-O-Ferm Roses and Fermicru AR2. Although there was no difference in alcohol production capacity among the commercial yeast strains applied in this study, strain Uvaferm Danstil A exhibited vigorous fermentation via the rate of sugar to alcohol conversion, short fermentation time, and pH being stable during alcohol fermentation. Besides, strain Uvaferm Danstil A is regarded as one of the strains with the high production capacity of volatile compounds. Therefore, the strain Uvaferm Danstil A was selected to conduct further studies.

3.2 Optimizing alcohol fermentation for fruit spirit production

3.2.1 Effect of temperature

After 8 days of fermentation, the highest alcohol contents were observed as 7.23 ± 0.06 % v/v, 9.70 ± 0.00 % v/v, 6.67 ± 0.06 % v/v and 7.70 ± 0. 10 % v/v in mashes of apricot, apple, cherry and pear, respectively. In most cases, a reduction trend was found when the fermentation temperature was above 30 °C and below 20 °C, except in the cases of fermented cherry and apple mash. For the cherry case, a reduction trend was found if the temperature was above 25 °C and below 20 °C. For the apple case, the high alcohol content (9.5 ± 0.00 % v/v) was still detected at 36 °C.

The total higher alcohol reached the highest values at the temperature range of 25 °C – 30 °C, whereas in the case of apricot, the highest content was observed at the temperature range of 20 °C – 30 °C. The total higher alcohol content increased proportionally with the temperature from 10 °C to 25 °C, except for apricot from 10 °C to 20 °C, and then this trend turned to decrease. The amount of 3-methyl-1-butanol in fermented mashes from apricot, apple, cherry and pear increased from 13.50 mg/L to 103.05 mg/L, from 38.70 mg/L to 161.93 mg/L, from 22.08 mg/L to 117.87 mg/L and from 17.81 mg/L to 107.76 mg/L, respectively, as the temperature increased from 10 °C to 25 °C. In contrast, as the fermentation temperature at 35 °C, their 3-methyl-1-butanol content dropped to 83.81 mg/L, 145.78 mg/L, 100.28 mg/L and 85.80 mg/L, respectively. Likewise, in fermented mashes from apricot, apple, cherry and pear, 1-propanol rose from 4.67 mg/L, 4.51 mg/L, 5.35 mg/L and 7.56 mg/L to 25.18 mg/L, 30.71 mg/L, 32.08 mg/L and 35.56 mg/L, then reduced to 17.88 mg/L, 30.73 mg/L, 20.36 mg/L and 21.96 mg/L, respectively, corresponding to 10 °C, 25 °C and 35 °C.

The total ester content sharply grew with the increase of fermentation temperature from 10 ℃ to 15 ℃, except for apple case, it raised strongly as the temperature rose from 10 ℃ to 20 ℃ (p-value < 0.05). Total ester content was the highest value at the temperature level of the range 15 ℃ – 20 ℃ for cherry and pear, the range 20 ℃ – 25 ℃ for apple and the range 15 ℃ – 25 ℃ for apricot. The decrease in total ester was observed when the fermentation temperature was over 20 ℃ for cherry and pear and as it was over 25 ℃ for apricot and apple (p-value < 0.05). In the cases of temperatures of 10 ℃, 15 ℃ and 35 ℃, the ethyl acetate in fermented mashes from apricot, apple, cherry and pear raised from 6.11 mg/L, 9.37 mg/L, 14.55 mg/L and 10.18 mg/L to 21.08 mg/L, 22.93 mg/L, 32.40 mg/L and 31.17 mg/L then declined to 11.12 mg/L, 16.67 mg/L, 20.29 mg/L and 15.72 mg/L, respectively.

In general, the alcohol content at 20 ℃ and 25 ℃ was higher than that at 15 ℃. In addition, the total volatile compounds in fermented mashes from these fruits were highest in the range of 20 ℃ – 30 ℃, except for pear in the range of 25 ℃ – 30 ℃. Therefore, the fermentation temperature range of 15 ℃ – 25 ℃ was suitable for input on the RSM algorithm of the optimization process.

3.2.2 Effect of pH

The ability of ethanol production varied slightly with the pH change of fruit juices tested. As shown in Figure 5.4, in most cases, the ethanol amount of all fermented mashes at pH 2.75, 3.0, 3.5 and 4.0 was almost the same (p-value > 0.05), except for apricot at pH 2.75 and pear at pH 4.5, but vitally different from that at pH 2.5 level (p-value < 0.05). The alcohol amount of fermented juice from apricot, apple, cherry and pear at pH 3.0 level accounted for 7.60 % v/v, 9.50 % v/v, 6.70 % v/v and 7.50 % v/v.

In our results, the change of pH from 2.5 to 2.75 resulted in the increase in total higher alcohol and total ester contents (p-value < 0.05), except for the ester case of pear, pH from 2.5 to 3.0. Total higher alcohol tended to decrease at pH of over 3.5, except for apple and pear at pH of over 3.0 (p-value < 0.05). In contrast, in the case of apricot and cherry, total ester contents still raised as pH was over 3.5, and it rose at pH of over 3.0 in the case of pear. At this pH, the total esters content is relatively lower than the total higher alcohols. The content of total volatile compounds in fermented apricot, apple, cherry and pear mashes at pH 3.0 reached 251.44 mg/L, 277.46 mg/L, 259.25 mg/L and 200.31 mg/L, respectively.

Meanwhile, the changes of total higher alcohol content are mainly due to changes in the content of 3-methyl-1-butanol, 2-methyl-1-butanol, 1-propanol, 2-methyl-1-propanol, whereas the total ester variation was from changes in ethyl acetate content. Generally, ethyl acetate peaked at pH 3.5 – 4.5, except for apricot, the highest value at only pH 4.5. The ethyl acetate content in fermented apricot, apple, cherry and pear mash at pH 4.5 was 38.80 mg/L, 33.61 mg/L, 38.67

mg/L and 37.71 mg/L, respectively. Additionally, in most cases, the highest 3-methyl-1-butanol concentration was observed in pH of 2.75 – 3.5, except for pear in the range of pH 2.75 – 3.0 and apple in only pH 3.0. The 3-methyl-1-butanol value in fermented apricot, apple, cherry and pear mash at pH 3.0 was 110.16 mg/L, 141.43 mg/L, 106.00 mg/L and 89.76 mg/L, respectively.

Finally, the pH range of 2.75 – 3.75 was suitable for input on the RSM algorithm of the optimization process.

3.2.3 Effect of initial soluble solid contents

An increase in initial soluble solids content from 12 °Brix to 30°Brix had a positive effect on alcohol and volatile compound production. However, they reduced considerably if total soluble solids content in the medium increasing up to 36 °Brix. In most cases, the maximum ethanol was found at 30 °Brix levels; inhere, the ethanol content from fermented apple mashes peaked at both 24 °Brix and 30 °Brix levels. The highest alcohol contents in fermented fruit from apricot, apple, cherry and pear were recorded 10.47 % v/v, 14.17 % v/v, 11.0 % v/v and 12.10 % v/v, respectively. Total volatile compounds reached the highest amount when fermentation of apple and cherry mashes at 30 °Brix level, while it was found in the cases of apricot and pear mashes at both 24 °Brix and 30 °Brix levels. The concentration of the total volatile compounds in apricot, apple, cherry and pear at 30 °Brix were 295.86 mg/L, 379.34 mg/L, 375.48 mg/L and 275.43 mg/L, respectively.

Generally, the changes of concentration of individual aroma compounds tended similarly to the case of alcohol in different Brix, including ethyl acetate, 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol. Ethyl acetate, 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol peaked 25.72 mg/L, 29.18 mg/L, 37.64 mg/L, 132.50 mg/L and 35.28 mg/L in fermented apricot juice, respectively; 36.81 mg/L, 25.06 mg/L, 39.43 mg/L, 198.43 mg/L and 70.96 mg/L in fermented apple juice, respectively, 24.52 mg/L, 32.08 mg/L, 77.97 mg/L, 118.64 mg/L and 36.17 mg/L in fermented cherry juice, respectively, and 37.66 mg/L, 41.30 mg/L, 21.81 mg/L, 116.47 mg/L and 34.32 mg/L, in fermented pear juice, respectively.

In most cases, the residual sugar content did not significantly different as the soluble solids content ranges from 12 °Brix and 24 °Brix, except for apricot of 12 °Brix – 18 °Brix (p-value > 0.05), but initial solid content of over 24 °Brix caused higher the residual sugar concentration after fermentation (p-value < 0.05). Accordingly, the initial soluble solid content of lower than 30 °Brix was appropriate for alcohol fermentation, thus the range of 18 °Brix – 30 °Brix was selected for the RSM algorithm of the optimization process.

3.2.4 *Optimization of some fermentation factors*

Full predictive equations for optimization of fruit alcoholic fermentation were given (Eq. 1-8).

Apricot juice:

$Y_{1\ P/S} = 69.09 + 4.47*X_1 + 2.53*X_2 – 5.40*X_3 – 4.91*X_1^2 – 2.37*X_2^2 – 3.44*X_3^2 + 1.46*X_1*X_2 - 0.77*X_1*X_3 - 0.72*X_2*X_3$ (Eq.1)

$Y_{2\ CV/S} = 1997.1 + 169.38*X_1 + 34.66*X_2 – 42.57*X_3 – 127.24*X_1^2 – 82.01*X_2^2 – 190.51*X_3^2 – 21.39*X_1*X_2 – 49.65*X_1*X_3 – 2.01*X_2*X_3$ (Eq.2)

Apple juice:

$Y_{3\ P/S} = 69.68 + 5.34*X_1 + 1.96*X_2 – 3.10*X_3 – 2.69*X_1^2 – 3.12*X_2^2 – 4.83*X_3^2 + 0.48*X_1*X_2 + 1.42*X_1*X_3 + 1.03*X_2*X_3$ (Eq.3)

$Y_{4\ VC/S} = 1797.83 + 171.98*X_1 – 83.58*X_2 – 127.91*X_3 – 86.94*X_1^2 – 140.33*X_2^2 – 22.82*X_3^2 + 9.56*X_1*X_2 – 28.22*X_1*X_3 + 3.2*X_2*X_3$ (Eq.4)

Cherry juice:

$Y_{5\ P/S} = 57.91 + 4.11*X_1 + 0.97*X_2 – 2.61*X_3 – 2.87*X_1^2 – 1.63*X_2^2 – 1.98*X_3^2 + 1.01*X_1*X_2 + 0.31*X_1*X_3 + 0.1*X_2*X_3$ (Eq.5)

$Y_{6\ VC/S} = 2153.09 + 103.64*X_1 – 76.26*X_2 – 24.30*X_3 – 20.44*X_1^2 – 116.49*X_2^2 – 198.54*X_3^2 + 6.88*X_1*X_2 – 26.22*X_1*X_2 + 8.54*X_2*X_3$ (Eq.6)

Pear juice:

$Y_{7\ P/S} = 78.37 + 5.3*X_1 + 2.67*X_2 – 3.95*X_3 – 6.39*X_1^2 – 4.97*X_2^2 – 5.93*X_3^2 + 0.68*X_1*X_2 + 1.89*X_1*X_3 + 1.34*X_2*X_3$ (Eq.7)

$Y_{8\ VC/S} = 1881.02 + 156.70*X_1 + 58.39*X_2 – 53.19*X_3 – 0.38*X_1^2 – 106.56*X_2^2 – 126.56*X_3^2 – 4.74*X_1*X_2 – 45.37*X_1*X_3 – 9.09*X_2*X_3$ (Eq.8)

The optimal conditions of temperature, pH and soluble solids content were determined to be 23.02 °C, pH 3.50 and 20.94 °Brix; 24.66 °C, pH 3.25, and 21.28 °Brix; 24.71 °C, pH 3.25, and 22.49 °Brix; 24.33 °C, pH 3.42, and 21.95 °Brix, respectively. Additionally, predicted values of the responses were calculated that of alcohol and volatile compounds' production yield were 73.38 (8.98 % v/v) and 2031.64 (248.66 mg/L) for apricot, 72.20 (12.10 % v/v) and 1947.76 (326.39 mg/L) in the case of apple, 59.68 (9.02 % v/v) and 2231.68 (337.37 mg/L) in the case of cherry, 78.63 (10.12 % v/v) and 2039.77 (262.60 mg/L) in the case of pear.

Confirmatory experiments were carried out, and the experimental results were closed to the predicted values. It suggests that the optimization process was success.

3.3 Effects of distillation process on aromatic profile

3.3.1 Effects of distillation process on distribution of aroma compounds

The presence of acetaldehyde concentration from apricot, apple, cherry and pear changed significantly in the distillation process and in the first fraction, its' content was up to 2072.13 mg/L, 2980.19 mg/L, 3580.34 mg/L and 1797.30 mg/L, respectively. Then, in the second fraction, it rapidly decreased to 218.48 mg/L, 464.54 mg/L, 500.84 mg/L and 234.98 mg/L, respectively (p-value < 0.05). Finally, in the sixth fraction, it went steadily down 0.87 mg/L, 5.4 mg/L, 2.93 mg/L and 3.41 mg/L, respectively. From the seventh fraction, it was not detected in distillates. In comparison with the classification of volatile compounds mentioned by Douady et al. (2019), the distribution of acetaldehyde in all these cases similarly belonged to type 1.

During second distillation in the case of apricot, apple, cherry and pear distillates, ethyl acetate concentration reached the maximum value in the first fraction with 2549.61 mg/L, 3789.38 mg/L, 2799.48 mg/L and 2760.97 mg/L, respectively. Then, in the second fraction of distillate, it fell significantly to 314.52 mg/L, 316.67 mg/L, 380.03 mg/L and 339.95 mg/L, respectively (p-value < 0.05). Eventually, from the 8th fraction, except for in the case of apricot and apple from the 9th

During the distillation process of the mashes of apricot, apple, cherry and pear, methanol content ranged from 17591.82 mg/L to 489.31 mg/L, from 2781.57 mg/L to 47.26 mg/L, from 3948.22 mg/L to 76.69 mg/L and from 6651.74 mg/L to 141.60 mg/L, respectively. In the cases of apple and cherry mashes, the methanol distribution was compatible with type 3 of the classification of volatile compounds. There was a difference between the methanol distribution in apricot and pear distillates compared to in apple and cherry distillates. In the cases of apricot and pear, methanol content relatively remained high from the 2nd to the 7th fraction, resulting in high concentration in heart fractions from 3238.37 mg/L of alcohol 40 % v/v and 1320.53 mg/L of alcohol 40 % v/v, respectively.

The concentrations of these higher alcohols, including 1-propanol, 2-metyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol were relatively low in the head fraction, gradually increased at 2nd fractions. In addition, most cases from 4th fraction or 5th fraction, they gradually decreased during the distillation. At the end of the distillation process, all their contents were close to zero. The 2-methyl-1-butanol and 3-methyl-1-butanol concentration reached the highest at 4th fraction in the case of pear, in a range of 2nd fraction – 4th fraction in the case of cherry and apricot, in a range 4th fraction – 5th fraction in the case of apple. Besides, 1-propanol reached the highest in range of 2nd fraction – 4th fraction in case of pear and cherry, 2nd fraction – 3rd fraction in the case of apricot and 2nd fraction – 5th fraction in the case of apple.

3.3.2 Profile of spirit products from apple, cherry, pear and apricot

In my work, 17 aroma components mainly including methanol, higher alcohols (1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and 2-phenylethanol), esters (ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate and isoamyl acetate) and acetaldehyde in the apple, apricot, cherry and pear spirits were identified by GC–FID techniques.

Methanol contents in the spirits samples varied from apricot, apple, cherry and pear accounted for 3238.37 mg/L alcohol 40 % v/v, 386.61 mg/L alcohol 40 % v/v, 650.12 mg/L alcohol 40 % v/v and 1320.53 mg/L alcohol 40 % v/v, respectively. These values are lower than the limit.

High acetaldehyde content was recorded in cherry spirits (136.13 mg/L alcohol 40 % v/v compared to pear, apple and apricot spirits with 74.44 mg/L alcohol 40 % v/v, 59.30 mg/L alcohol 40 % v/v and 55.73 mg/L alcohol 40 % v/v, respectively).

The 1-propanol, 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol and 2-methyl-1-butanol were considered as higher alcohols accounting for high levels in these fruit spirits. Total higher alcohol content in apricot, apple, cherry and pear spirits reached 1529.93 mg/L alcohol 40 % v/v, 1281.56 mg/L alcohol 40 % v/v, 1823.83 mg/L alcohol 40 % v/v and 1330.72 mg/L alcohol 40 % v/v, respectively. The total esters in apricot, apple, cherry and pear spirits accounted for 109.56 mg/L alcohol 40 % v/v, 107.46 mg/L alcohol 40 % v/v, 126.72 mg/L alcohol 40 % v/v and 155.83 mg/L alcohol 40 % v/v, respectively.

The principal component analysis (PCA) explained 65.9 % of the variability of volatile compounds in two components: PC1 (42.5 %) and PC2 (23.4 %). Cherry, apricot, pear and apple spirits were clearly located at the quarters of 1st to 4th in turn. Aroma compounds extremely contributed to PC1-2 including propyl acetate, 2-methyl-1-propanol, 2-butanol, 1-propanol, 2-phenylethanol, 2-propanol, 2-methyl-1-butanol, acetaldehyde.

3.4 Classification of fruit spirits by PCA and LDA

The linear discriminant analysis explained 97 % of the total variance, with 55 % from LD1 and 42 % from LD2. The results illustrated that there were significant differences between these four groups of tested samples. Although these fruit spirits groups were unequally distributed and concentrated. Depending on the fruit type, the distance between groups was still close. The distribution of the apricot spirit was central and surrounded by apples, pears and cherry spirits. Apricot spirits were located near pear spirits, so there was a relatively high confusion in classifying them with others. The cherry spirits were easily distinguished by LD1, while LD2 supported recognizing the apple spirits well.

Conducting Fisher's classification function coefficients for multiple classes via the LDA, pálinka discrimination among the different fruits related to 2-propanol, 2-butanol, butyl acetate, isoamyl acetate, ethyl hexanoate, 2-phenylethanol. The correct classified capacity of the LDA model was 100 %. To validate the predictive ability of the model, the leave-one-out cross-validation method was utilized to generate the appropriate model. As a result, this model's predictive ability was 91.66 %, which revealed that the LDA model showed relatively satisfactory results for the classification of pálinkas from different fruit types.

4. NOVEL CONTRIBUTIONS

1. Nine commercial yeast strains were screened for alcoholic fermentation of fruit juices. The production capacity of volatile compounds reached the highest level in the cases of strains Uvaferm Danstil A, Fermiblanc Arom, Vin-O-Ferm Roses, Fermicru AR2, and the lowest level in the cases of strain Oenoferm x-thiol F3. Strain Uvaferm Danstil A exhibited strong fermentation ability through the conversion rate of sugar to alcohol in the cases of apple, apricot, cherry and pear and short fermentation time. Thus, it was selected for production of pálinkas
2. The optimal conditions for alcoholic fermentation of fruit juices for production of pálinkas were determined and optimised. The temperature, pH and soluble solids content for fermentation of apricot, apple, cherry and pear juices were 23.02 °C, pH 3.50 and 20.94 °Brix; 24.66 °C, pH 3.25 and 21.28 °Brix; 24.71 °C, pH 3.25 and 22.49 °Brix; 24.33 °C, pH 3.42 and 21.95 °Brix, respectively.
3. The effects of the distillation process over apple, apricot, cherry and pear spirits on aroma compounds distribution were described. And the suitable cut-point for the distillation process scientifically was determined experimentally. In the distillation of spirits from apricot, apple, cherry and pear juice, the suitable cut-point of the head faction was at around 1.5% of the wine volume, while the cut-point of the heart fraction was appropriate when the alcohol content in the outflow dropped to 40 % v/v.
4. Chemometric statistics were conducted to classify both obtained and commercial fruit spirits. The linear discriminant analysis method was suitable to verify the origins of spirits from apricot, apple, cherry and pear with a model's predictive ability of 91.66 %. In addition, the spirits discrimination among these different fruits related to 2-propanol, 2-butanol, butyl acetate, isoamyl acetate, ethyl hexanoate, and 2-phenyethanol.

5. PUBLICATIONS

❖ Journal articles

1. **Tuan M. Pham**, Weizhe Sun, Erika Bujna, Ágoston Hoschke, László Friedrich, Quang D. Nguyen. *Optimization of fermentation conditions for production of Hungarian sour cherry spirit using response surface methodology*. Fermentation 7, 209, 2021
2. Csilla Farkas, Judit M. Rezessy-Szabó, Vijai Kumar Gupta, Erika Bujna, **Tuan M. Pham**, Klára Pásztor-Huszár, László Friedrich, Rajeev Bhat, Vijay Kumar Thakur, Quang D. Nguyen. *Batch and fed-batch ethanol fermentation of cheese-whey powder with mixed cultures of different yeasts*. Energies 12, 4495, 2019.
3. **Pham, M. T**, Varjú, R., Bujna, E., Hoschke, Á., Csernus, O., **Nguyen, B. T.**, Gupta, K. V., Nguyen, D. Q. (2021): Chemical and volatile composition of fermented apple juice with different commercial yeast strains of Saccharomyces cerevisiae. *International Journal of Food Microbiology* (**under review**)

❖ Poster presentations

1. **Tuan M. Pham**, Réka Varjú, Erika Bujna, Ágoston Hoschke, Quang D. Nguyen. *Chemical and volatile composition of fresh spirit from apple fermented with different Saccharomyces Serevisiae yeast strains*. EuroFoodChem XIX Conference. 2017, Budapest – Hungary.
2. **Tuan M. Pham**, Réka Varjú, Agócs Gergely, Erika Bujna, Ágoston Hoschke, Quang D. Nguyen. *Effect of different commercial yeast strains on physic-chemical characterizations and volatiles production in fermented apricot juice*. 3rd FoodConf. 2018, Budapest-Hungary.
3. **Tuan M. Pham**, Weizhe Sun, Erika Bujna, Ágoston Hoschke, Quang D. Nguyen. *Study on response surface methodology (RSM) of alcohol fermentation from apple juice by Saccharomyces cerevisiae*. 18th International congress of the Hungarian society for microbiology. 2019, Budapest-Hungary.
4. **Tuan M. Pham**, Weizhe Sun, Erika Bujna, Ágoston Hoschke, László Friedrich, Quang D. Nguyen. *Application of response surface methodology for fermentation optimization of cherry by Saccharomyces cerevisiae*. 4th International Conference on Biosystems and Food Engineering BIOSYSFOODENG 2021, Budapest-Hungary.
5. **Tuan M. Pham**, Mohan Malkani Anbalagan, Fanni Hegedűs, Réka Varjú, Quang D. Nguyen. *Fermentation profile of different yeast strains on sour cherry. Chemical Engineering Conference*. 2017, Veszprem – Hungary.
6. **Tuan M. Pham**, Réka Varjú, Agócs Gergely, Erika Bujna, Ágoston Hoschke, Quang D. Nguyen. *Effects of commercial Saccharomyces Cerevisiae strains on fermentation of pear juices and production of volatile compounds*. FIBOK2018 Conference. 2018, Budapest-Hungary.
7. **Tuan M. Pham**, Weizhe Sun, Erika Bujna, Ágoston Hoschke, László Friedrich, Quang D. Nguyen. *Application of response surface methodology for fermentation optimization of pear by Saccharomyces cerevisiae*. Műszaki Kémiai Nap '21. 2021, Budapest-Hungary.