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Study of Pear Fruit Fermentation and Pear Spirit Aroma Profile Analysis

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Approving signature of the supervisors and of the Doctoral School:

The PhD candidate fulfilled all requirements of the Szent István University, observations were considered, suggested modifications and corrections made during the defense workshop were applied in the corrected thesis. The thesis meets the standards of the Doctoral school and it can be subject of a public defense.

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Approval of the Doctoral School Approval of Supervisors

1. Background of the Work and its Objectives

Fruit spirits are very popular in Europe, but these spirits are consumed in other parts of the world as well. The first Hungarian data regarding the distilled beverages come from the 14th century royal court, when Queen Elisabeth used rosemary extract in a spirit to heal her husband's gout. Since then, the pálinka has become an important and popular drink in social occasions. Although consumer expectations are increasing, this brings new challenges to manufacturers. Generally, consumers like to buy the products they have got used to, this is due to fact that consumers are matching a memory to the flavor of a specific food or beverage. Because of the affore mentioned, the improvement efforts of the spirits' flavor and aroma composition are important and are increasingly coming into view in the product development. In the production of fruit based brandies, most of the volatile compounds get synthetized during the fermentation, going into the distillation process, ending up in the distillate, shaping the spirit's flavor profile and drinkability. The fruit-based distillates' primary flavor and aroma is defined by the fresh fruit's quality and ripeness, but there are other factors to the spirit quality that shape the taste and aroma quality of the spirit. Fermentation and the distillation methodologies have a major influence on the final taste and flavor quality of the spirits. The applied production techniques like washing method, grinding resolution, fermentation aids, acids and yeasts, as well as distillation and aging they all contribute to, or deteriorate the quality of the fruit based spirits. Yeasts selected for different purposes will synthesize specific metabolites at different rates, and this will be reflected in the quality of the product. Selecting the right yeast for getting the best potential out from a fruit during its fermentation, requires the information and understanding of the specific yeast's aroma producing abilities and other biological, physical properties (optimum, pH and temperature). The production of consistent aroma quality pálinka is nonexistent on the market. This variability is coming from the fruit's year-to-year difference as well as from the different fermentation and distillation methods and

protocols in the different vintages. Product consistency can be a key factor for a successful pálinka manufacturer.

The consumer experience is further enhanced by the origin designated (PDO) pálinka which has a higher market value due to its excellent quality and reputation. Despite of the high quality PDO spirits there are no tracking systems, no categorization mechanism (like for example for the whiskey and cognac), that can analyze the product compounds, and group the pálinka by their origin, variety or vintage of the fruit. Today if someone wants to demonstrate the higher quality of the PDO pálinkas through the region of origin of its fruit one has to rely on the pálinka manufacturers' honesty of filling out the documents of provenance. Because of the higher market value of the PDO pálinkas, counterfeit products appear on the market for easy extra profit. The issue of validation of PDO pálinkas could be solved through pálinka quality assurance and checking system based on an inexpensive analytical method combined with (big data based) powerful chemometric methods.

The topic of my PhD thesis is based on the above described two issues: development of the pálinka production, and the classification of the PDO pear spirits. In the first issue of the results the significance of different pH setting acid selection and the significance of different *Saccharomyces cerevisiae* strain selection for the pear fruit fermentation is presented. Mashing, fermentation, and distillation related issues are covered in the first part of my thesis results. Goal orientally selecting and applying of yeasts and additives at fermentation can minimize the variance coming from the pálinka production processes.

In the second part of my thesis results hyphenated analytical and chemometric methods for pálinka classification by their origin, vintage and fruit varietal are presented. These methods could be applied in the PDO-quality assurance and chekcing processes as a quick, inexpensive method.

To adress the above mentioned two major and different issues the following milestone targets were set:

1. Mapping the fermentation conditions that impact the flavor profile of pálinkas.
2. Setting a standard pálinka production protocol to minimize variability originating from different production protocols.
3. Adaptation of analytical methods for the qualification and quantification of the pear spirit volatiles
4. Adaptation of chemometric methods to analyze and evaluate results originating from the qualitative and quantitative analytical methods.
5. Finding a component link between pear spirits and their fruits
6. Finding marker molecules or molecule groups that are fruit varietal, fruit origin and vintage specific.

2. Methods and Materials

Three different yeast strains, selected for different purposes, were used in my research, Saccharomyces cerevisiae 342 (distillers yeast), Saccharomyces cerevisiae YS4 (baker's yeast), Saccharomyces cerevisiae 228 (wine yeast).

Pears used in my study were purchased by the Brewing and Distilling Department of the Corvinus University of Budapest (department now belonging to the Szent István University). For after-harvest ripening, pears were cold stored in a suitable storage room for 5 days prior to processing. Fruits procured for the three years long study were originating from the same orchard through the same supplier in each year (2010, 2011 and 2012).

Fruit processing in the lab scale studies started with the fruit washing, which step was followed by the grinding. Fruit juice and pulp coming from the grinding was measured for pH, sugar content and reducing sugar concentration. Pectolytic enzyme complex addition was followed by the yeast nutrition addition. The fruit mash resulting from the above described process was divided into 500 ml flasks and pH was set to pH 3 by using different acids (depending on the

experiment, phosphoric acid, sulfuric acid or lactic acid was used to set the desired pH of the mash)

To be able to mimic industrial condition for the pear spirits' aroma profile study it was necessary to run the experiments in pilot scale. Pilot scale conditions were also required to define and control the distillation protocol of the pear mash distillation. This later granted consistent distillation conditions between different distillation batches. Pilot scale distillation protocol was similar to the lab scale distillation steps, but distillation equipment and control of the parameters between lab scale and pilot scale varied a lot. Pilot scale equipment had better resolution and multiple points for temperature control than the lab scale equipment. 50-liter fermentation tanks were filled with 40 kg of pear mash, then yeast nutrient was added (11 g/100 kg) to each tank. pH, total sugar content (Brix %) and reducing sugar were measured, and registered. pH 3 of the mash was set, step to be followed by the pectolytic enzyme addition. Controlled alcoholic fermentation was started by adding the rehydrated yeast.

Effect of different commercial pectolytic enzyme mixtures on the pear mash was studied in lab scale. Three different, commonly applied enzyme mixtures were used in the lab experiment: Rapidase Clear, Lallzyme EXV and Lallzyme HC. 10 g of mash samples, each containing (manufacturer recommended dosage) one of the three different enzymes were centrifuged at 10000 g for three minutes. The supernatant resulting from this centrifugation process was measured in a 10-ml scaled cylinder. Results were compared to pectolytic enzyme blind samples regarding the quantity of the obtained supernatant (degree of liquifaction).

In the lab scale experiments where attributes (effect of pH, acids, yeast strain on the fermenting mash's sugar content, major flavor compounds, and rate of fermentation) of controlled fermentation were studied, fermentation flasks were weighed on analytical balance, then samples were closed with a bubble-cap (valve) and were put into a 16 °C thermostat. Fermentation was carried on for 7 days. Each day 40 cm^3 sample were taken from the flask. Samples collected each

day were put into a -50 °C freezer until the end of the 7-day experiment, when samples were analyzed.

During the lab-scale experiments pH, sugar concentration change and weight loss (through CO_2 formation) of the fermenting mashes were monitored. At the end of fermentation, a Gibertni lab distillation unit was used to distill the fermented mashes.

Fermentations at pilot scale, like lab scale fermentations, were started with the addition of the rehydrated yeast. In the rehydration process dry yeast was put into a sterile flask, NaCL at 0.1 M concentration, glucose at 2 g/l concentration and 7 g/l sterilized yeast nutrient were added. Duration of pilot scale fermentations were 10 days, by the end of these 10 days fermentation was complete. Controlled temperature was set to 16 °C, while pH 3.0 was set. In the pilot scale spirit production experiments samples were taken at three stages of the production process: 1.) at the beginning of the fermentation from the fresh fruit grind, 2.) from the fully fermented mash and 3.) from the final spirit (heads, hearts and tail fractions were analyzed separately).

Assimilation test was carried out to compare the three yeast strains regarding their carbon source preference. To prepare clean yeast for the test 3 test tubes per yeast strain were filled with 5 ml YPG nutrient broth, then rehydrated yeast was added to each tube. After an overnight incubation the tubes were centrifuged, supernatant was discarded and pellet was washed with NaCl solution. Finally, pellets were suspended in 1.5 ml NaCl (0,85%) solution and incubated at 25 °C in a shaker incubator for 2 hours. After incubation the centrifugation and pellet suspension was repeated. After the preparation phase of the test different carbon sources, glucose, fructose, ribose, melibiose, lactose, raffinose, inulin, celibiose, methanol and ethanol were put in sterile test tubes. Each test tube contained one type, 0.5 g (crystal form) of carbon source dissolved in 4.5 ml of sterile water. Nine sets of tubes were prepared to allow triplicate tests for the three yeast strains. 0.5 ml of sterile YNB broth was pipeted into the tubes. One

inoculation loop of yeast was added to each test tube. Duration of the assimilation test was 14 days. Samples were evaluated on the 6^{th}, 10^{th} and 14^{th} day.

Fermentation test of the three different yeasts were carried out with the following carbon sources: glucose, fructose, ribose, melibiose, lactose, raffinose, inulin, cellibiose, ethanol and methanol. At the beginning of the test yeasts were propagated in YEPD broth. Vidal test tubes were filled with alcalic broth and incubated at 25±2°C for 10 days. Turbidity of the broth was the positive test of the yeast propagation. The raising of the VazPar (Vaseline and paraffin mixture) plug with the broth's color change to yellow hues meant that the yeast fermented the tested carbon source.

Lab scale distillation of the fermented pear mashes was carried out either by using a bench top conductive mash heated distillation unit or by a bench top steam injected distillation unit, depending on the goal of the trial. To define volatile acidity and total ester content of the pear mash steam injected distillation unit was used. In the study of the aroma compounds of the fermented pear mash conductive heated distillation unit was used, due to the higher low boilers concentration in the product (compared to the steam heated unit).

Fermented mashes and their spirits were analyzed with hyphenated methods. Sugars, ethanol and methanol content were analyzed by using HPLC method. The HPLC used in this research was equipped with RI and PDA detectors. Separation of the compounds were carried out on a Bio-Rad Aminex HPX-87H column by using 0.005 N sulfuric acid as eluent. Sample run time was 25 minutes with a flow rate of 0.5 ml/second. pH, alcohol content (based on density), total sugar content, reducing sugar content, volatile and total acid content were determined with classical analytics methods

Effect of acids used as fermentation aids for pH setting, were tested in lab scale trials, using Bosc Cobak pear mash. pH set at the beginning of the fermentation was pH 3 (+/- 0.08), by using phosphoric acid, lactic acid and sulfuric acid. Resulted mashes were analyzed for sugar content and alcohol

concentration. prior to distillation. Distillates were analyzed with GC/FID method.

All distillate samples, coming from lab scale or pilot scale trials, were analyzed with GC/FID or GC/MS methods. GC/FID unit was equipped with a CP-WAX-57 CP capillary column, manufactured by Varian. GC/MS unit consisted of an Agilent 6890 GC and an Agilent 5973 hyperbolic monolythic quadrupole MS-detector. The Agilent GC was equipped with a ZB-WAX column. MS was operated in SIM mode at 70 eV, ion source temperature was 230 °C.

Results obtained from the hyphenated methods were processed and analyzed by using variance analysis and linear discriminant analysis statistical methods.

3. Summary of The Results

3.1. Adaptation of GC/FID and GC/MS methods

By adapting a general GC-MS method, we have ended up with a comprehensive analytical method that is suitable for both quantitative and qualitative determination of the pálinka compounds. In the beginning of the method adaptation, the separation of the sample components were performed with two columns (CP-Wax 57 CB, 50m x 0,33mm x 0,2um and ZB-WAX 60m x 0,25 mm x 0,25 0,25 µm). The use of CP-WAX 57 CB column has been discarded for two reasons a.) the separation on the acetone/methyl acetate peak pair was lower efficiency, b) peak tailings appeared at the early-eluting compounds (especially acetaldehyde and methanol peaks). The 2870/2000/EK ENACTMENT allows the amyl alcohols to determine, not selectively, but jointly (2-methyl-butanol and 3-methyl-butanol), which can also be easily performed with a flame ionization detector.

The GC-FID analytical system equipped with a CP-WAX column (50 m x 0,33 mm x 0,2 µm, injector temperature was set to 220 °C, whereas the

temperature of the detector was set up 250 °C) appeared to be a powerful asset in the pálinka production methodologies related studies. The method allows excellent stability and robustness in the analysis of alcohol/water-based samples.

3.2. Comparing different S. cerevisiae strains

Several trials and experiments were set and conducted to identify differences between yeast strains selected for baking, wine making and spirit mash fermentation. Results show that various acid treatments during mashing, has different effect on the fermenting mash effecting its reducing sugar content, and thus the alcohol yield. Samples treated with phosphoric acid showed a more intense sugar concentration drop in the fermenting mash than samples treated with other acids. Fermentation showed, even if little, to have a more elongated dynamic under the influence of phosphoric acid treatment compared to the samples treated with lactic and sulfuric acid.

The results of assimilation tests showed that the applied *S. cerevisiae* strains showed little differences between the tested carbon source capabilities. According to the findings *S. cerevisiae* 342 (distiller's yeast) strain was the most capable of assimilating carbon sources. Fermentation test was carried out on the assimilated carbohydrates and was found that the different *S. cerevisiae* strains utilized all tested carbohydrates but lactose and xylose, these can be considered as negative control samples. According to the fermentation test, the YS4 baker's yeast could not ferment the galactose, however it surpassed the 228 and 342 strains in the lab-scale fermentation and assimilation. These differences were not significant under pilot scale conditions. According to the findings, assimilation capacities of the different yeasts selected for the three different industries are distinct, but all three yeast strains are suitable for the fermentation of the sugars (mainly glucose, fructose, the DP2 -mostly sucrose) of the pear mash. The baker's yeast appeared to be similarly efficient at fermentation capacity as the wine or distiller's yeast.

I have experienced that yeasts well propagated in pear mashes, even without any additional alimentary compounds, and the available carbohydrates were

fermented with similar efficiency in laboratory conditions, producing similar amount of ethanol. At lab scale conditions the tested yeast strains achieved similar alcohol yields and cell count growths without the addition of any yeast nutrients, no significant difference was found in the fermentation rate of the different *S. cerevisiae* strains

3.3. Effect of different fermentation additives and treatments on the quality of the spirits

Different commercial pectolytic enzyme (Lallzyme EXV, Lallzyme HC és a Rapidase Clear) products' effect on the fermenting pear mash were tested. It was observed that the mashes treated with the Lallzyme products were less susceptible to foaming, compared to Rapidase Clear treated pear mashes. Since the pear mashes were identical in all trials I concluded that the foam formation difference between the mashes is due to the differences between the enzyme products' composition. The fastest liquefication and the highest clear juice output was observed at the samples containing Lallzyme EXV enzyme. During pear pálinka production pear skin and zest wasn't separated from the fermenting broth, thus during fermentation maceration occurred as well. Based on my findings I suggest the usage of the Lallzyme EXV enzyme in the pear pálinka production.

Depending on the acid (sulfuric-, phosphoric- or lactic acid) used to regulate initial fermenting mash pH, fermenting mashes could be grouped into three classes by their registered pH curves. Samples with phosphoric acid treatment have very similar pH chart for the 7 days of fermentation. These samples show the most pH drop at day 2 and day 3 of the fermentation. These samples, regardless of the yeast strain fall into one group. Another sample group forms with the sulfuric acid treated samples, where pH of the fermenting mashes stayed the same as the initial pH set at the beginning of the fermentation. Lactic acid treated samples fall into the third group, where pH increase of the fermenting mashes started at day 1 and continued until day 7, when fermentation was stopped.

Correlation between fermentation pH and volatile composition of the spirits (volatile synthesis) was confirmed. Depending on the correlation's direction three types of spirit compound groups could be distinguished. Iso-butanol, 1-propanol, 2-phenilethanol, 3-methyl-1-butanol, and the methyl butanols fall into one class, concentration of these compounds significantly increases with the rise of the pH. Acetaldehyde and acetal form another group of chemicals. These compounds were found to be more abundant in lower pH mashes. pH had no impacted, but no direction can be determined of the 1-butanol, methyl-acetates and methanol, these compounds form the third group of chemicals.

Types of acids applied impacted the glucose and fructose uptake in the fermenting mash. Fast monosaccharide concentration decrease was observed in the samples, except in the case of the phosphoric acid treated distiller's yeast samples. Generally, fructose concentration dropped to 2g/100ml, while glucose concentration dropped into the unquantifiable ranges, below 1 g/100 ml concentrations. Samples of baker's yeast and wine yeast showed a slight fructose increase in the late section of the fermentation, which could be due to the invertase activity of the yeasts. Generally, yeast prefers glucose over fructose, this leads to quicker and fuller glucose uptake, while fructose is still present at late fermentation stages, when alcohol is also abundant in the mash, limiting the yeast activity. Disaccharide (DP2) concentration of the fermenting mashes changed dynamically, significant differences were detected between the different yeast/acid combinations. At early stages of the fermentation disaccharide concentration rapidly drops at the lactic acid treated mash samples of the *S. cerevisiae* 228 yeast, then, after the first 2 days of fermentation disaccharide concentration starts to increase, which trend then was again followed by a concentration drop. This phenomenon could be explained through the enzymatic system of the pear fruit and yeast, where oligo- and polysaccharides are degraded into smaller molecules, and finally fermentable sugars. Samples treated with

phosphoric acid also showed a disaccharide concentration increase which phenomenon could also be explained by the hydrolytic effect of the acids.

During the alcoholic fermentation weigh reduction of the mashes, due to CO_2 formation was monitored. *S. cerevisiae* 342 combined with lactic acid generated the most CO_2, especially at the first three days of the fermentation. The combination of *S. cerevisiae* 228 and phosphoric acid showed intensive gas formation after the first three hours, which intensive gas formation lasted for hours only. The sulfuric acid treated *S. cerevisiae* YS4 showed little to no gas formation at the first hours, gas formation was observed 7 hours after inoculation YS4 yeast with the other two acids generated the most CO_2 in the first two hours. *S. cerevisiae* 342 yeast samples treated with sulfuric and phosphoric acids gave similar results to the *S. cerevisiae* YS4 samples, treated with similar acids. Measured weight loss due to CO_2 formation was slower but continuous at the lactic acid treated samples, the largest weigh loss was registered at these samples.

The general observation was that the early 3-8 hours are most intense in terms of gas formation, but at specific yeast and acid pairings extremely intense or very moderate gas formation can occur. It was also found that lactic acid increases CO_2 formation, while yeasts with phosphoric acid appeared to have a more moderate CO_2 generation rate.

Alcohol content of the fermenting pear mashes was monitored daily. Alcohol accumulation in the samples treated with sulfuric acid was slower. At other samples no alcohol formation was detected at the first days of fermentation, then a rapid alcohol formation was registered. By day 7 of the fermentation all pear mash samples had similar alcohol concentrations

3.4. Distillation protocol development for the distillation of the fermented pear mashes

Parallel to the bench top fermentation and distillation trials pilot scale experiments were performed as well to mimic real industrial conditions for

scalability and reproducibility. A distillation protocol was made for the pear mash distillation processes. The protocol was programmed and run through a computer which computer was connected to the distillation unit to control the distillation conditions. The protocol starts with a pre-heating process, this process heats the still and the mash to the operating temperatures. As operating temperatures are reached the computer switches to the distillation protocol, which consists of the following steps:

- At the beginning of the distillation (t0) 20% heating intensity in the mantle which rises to 22.5% heating intensity. Calculated slope of increase is 0.041 °C/min.
- At t0 cooling water temperature of the dephlegmator is set to 28 °C, which value is rising to 37 °C with a slope of 2 °C/min.
- After reaching the 37 °C in the dephlegmator, temperature of the cooling water starts to decrease back to 34 °C with a slope of 0.057 °C /min (for 56 minutes)
- Fluid level of the 1st stage (the lowest stage /tray in the column) was set to 45% (approx. 2.25 cm liquid layer) for 4 minutes, for the rest of the distillation process a 55% fluid layer was held on the lower stage.
- The second stage of the three (or middle stage) holds 60% of fluid layer for 4 minutes, then it drops to 50 % for the remaining time of the distillation.
- 70% of liquid layer was set on the 3rd stage for 4 minutes, then this value dropped to 60% and it was held for the rest of the distillation process

During the protocol setup it was found that the critical setting, that had the most impact on the spirit quality was the dephlegmator's temperature profile, with its maximum and minimum values. The rising and dropping temperature between 28°C and 37 °C gave me a very good separation of the low and high boiling compounds.

3.5. Distillate analysis

Volatile acidity of the distillates is mostly due to acetic acid. Non-volatile acids used in the mashing processes had an indirect impact on the volatile acidity of the mashes. The lactic acid pH regulated mash samples contained higher concentrations of volatile acids (baker's yeast 68,2 mg/100 ml, wine yeast 71,8 mg/100 ml, distiller's yeast 66,1 mg/100 ml) than the samples of the sulfuric and phosphoric acids.

Volatile acidity by yeast shows that baker's yeast produced 161,1 mg/100ml, wine yeast fermented samples ended up with 177,5 mg/100 ml, and in the distiller's yeast containing samples 173,9 mg/100 ml volatile acid accumulation was found by the end of the fermentation trial.

Total ester content of the mashes was measured with gravimetric method. It was found that wine yeast fermented mashes had a higher total ester content than samples of the other two yeasts. Type of the acid used also impacted the ester concentrations of the spirits, it was found that spirits coming from lactic acid containing mashes had a higher total ester content compared to the spirits made with the other two acids.

3.6. Effect of the different acids on spirit aroma quality

The influence of the different yeasts and pH setting acids on the aroma profile of the pear distillates was studied by using the adopted GC/FID method. Principal component analysis (PCA) was applied on the GC/FID results to discover differences between yeast and acid combinations, from the resulted spirits. It was found that the quality differences of the samples are due to the following compounds: 1,1-diethoxy-etane, 2-butanol, 2-methyl-1-butanol, 2-methyl-2 butanol, 2-pentanol, 2-phenylethyl-etanol, 3-hydroxi-2-butanone (acetoin), 4-decanol, benzyl alcohol, diethyl succinate, ethyl decanoate, ethyl formiate, ethyl hexamate, hexil acetate, i-butil-D-lactate, i-amyl-acetate, methyl-carbamate, n-butil-alcohol, n-propil alcohol, n-heptanol, Phenethyl alcohol, trans-3-hexen-1-ol, urethane.

Pear spirits made with the different yeasts and acids form clear groups, or classes on the PCA charts (by D1 and D2 functions). This means that these spirit groups show clear dissimilarities, despite of the fact that they were made of the same fruit with the same production protocol, at the same time, the only two variables being the acid and the yeast.

3.7. Classification of pear distillates by their fruit's variety

Four pear variety (Bosc Kobak, Fétel, Packham's Triumph and William's pear) was used to make pilot scale mashes and distillation with a precise distillation and fermentation protocol. The different pear distillates resulting from this trial were analyzed with an adopted GC/MS method. GC/MS result data was fed into linear discriminant analysis method to further evaluate data. It was found that there are detectable and significant differences between the spirits of the different variety pears. Eight marker compounds were identified, as responsible for the variety classification (acetaldehyde, 1-butanol, ethyl-propionate, 2-methyl-butil-acetate, 3-methyl-butil-acetate, ethyl-hexanoate, ethyl-dodecanoate and 2-phenilethanol). The magnitude of the factors of the discriminant analysis show how much influence one compound has on the classification. According to the result it was determined that the most impact on the variety classification was due to the ethyl-propionate, 2-methyl-butil-acetate and to the ethyl-hexanoate.

3.8. Classification of pear distillates by their fruits' vintage

Temperature and humidity are the two most important factors of a climate, microclimate, but plant growth and development is defined by many other factors as well, such as sunny days in the vegetative period, air composition, etc. Pear coming from different vintages should carry the differences of the climate as well, which differences are passed on and are detectable in the spirits as well. Pear spirits were classified by their vintage by using LDA method on the GC/MS results. Five volatile compounds were found to be responsible for the successful classification, these compounds are the following: acetaldehyde, 3-metgyl-1-

butanol, 2-methyl-butil-acetate, allyl-alcohol, 2-phenylethanol. GC/MS data showed that acetal concentration of the 2012 spirits was higher than what was measured at other vintages. Spirits made in 2010, a rainy year, had one magnitude lower acetal concentrations compared to 2012, which was a dry, hot year. Confidential ellipses of the spirit classes show no overlapping

3.9. Classification of pear spirits by their fruits' origin of cultivation

Spirits were classified by the origin of the pear that they were made of. Similar to the previous classification methods, LDA (stepwise forward) was applied on GC/MS data. Pear spirits made of pear cultivated in Nagykanizsa were distinguished from the spirits that were made of Göcsej originated pears. The confidential ellipses of the two classes of spirits didn't show any overlapping. It was found that methyl-butil acetates, and acetate concentration differences are the differentiating feature between the two classes. Spirits made of Göcsej originating pear contained significantly higher levels of methyl-butil acetates. Due to the low sample variability this later classification should be re-tested with a sample set, that contains higher variability, spirits should have at least three different origins.

4. New scientific results

1. Correlation between fermenting mash pH and volatile compounds was found, at low pH values (pH 3) low acetaldehyde and acetal formation was observed, while at higher pH values (pH 3.8) an increased synthesis of methyl acetate, ethyl acetate, 2-phenylethanol, 1-propanol, i-butanol, metil-1-butanol and 3-metil-1-butanol was found

2. A distillation protocol was developed that reduces the batch to batch variations and improves the efficiency of the fractionation. The optimized dephlegmator temperature profile allows a steady flow of distillate while improves the separation of the high boiling compounds.

3. Pear spirits were analyzed by hyphenated methods and classical analytic methods to find pear specific characteristics. Identified compounds were quantified, analytic data was used to build a data base which data base can be used to classify unknown pear spirits, or to identify specific unknown characteristics of a pear distillates. The database contains every relevant information that is required to classify a pear spirit by its vintage or fruit variety.

4. Strong correlation was confirmed between the pear spirits and their fruits. LDA data shows that pear varieties contain specific, and inherent patterns of relative concentration of the following compounds: acetaldehyde, 1-butanol, ethyl propionate, 2-methylbutil acetate, 3-methylbutil acetate, ethyl-hexanoate, ethyl dodecanoate and 2-phenyethanol.

5. It was found and validated that the measurement of five volatile compounds is sufficient to classify the pear spirits by their vintages. These 5 compounds are the following: acetaldehyde, 3-methyl-1-butanol, 2-methylbutil-acetate, allyl-alcohol, 2-peniletanol.
   Furthermore, I found that GC/MS data evaluated with LDA method could be also used to classify the pears by their cultivation site (Nagykanizsa or Göcsej). The results of cultivation site classifications looked promising at the early stages of the research, but it was found that a higher number of class variability is required to fully test and validate the applicability of this methodology

5. Conclusions and proposals

It was confirmed that good quality pear distillate can be made with either of the tested yeasts (*S. cerevisiae* 228, YS4 and 342). Acids used for the pH setting of the mashes had more significant effect on the spirit quality than the yeasts had. Strong correlation was found between the spirit quality and acid type, the alcohol

yield was lower, but ester content was higher in the experiments made with lactic acid compared to the other two acids. The usage of lactic acid could become relevant at the end of fermentation, a few hours or days prior to distillation, this way not limiting the yeast. Administration of the phosphoric acid should be carried out at the early stages of fermentations.

Constant distillate quality was obtained through a well-defined and detailed distillation protocol. Dephlegmator temperature profile was proved to have a major effect on the accumulation and concentration of the volatiles in end product.

The classification of the distillates by their fruit's origin, vintage and cultivar can be achieved through chemometric methods. Since the low number of origin variety of the pears, further analysis is required with larger class variety. Pear cultivar and vintage of spirits could be analyzed and checked by measuring their marker volatiles and classifying them with known sample data from a database containing the GC/MS of commercial and craft PDO pear pálinkas.

6. Publications related to the thesis

Impact Factor Publications

Attila G. Kovács, Attila Szöllősi, Dániel Szöllősi, Ilona A. Panyik, László Nagygyörgy, Ágoston Hoschke, Quang D. Nguyen. Classification and Identification of Three Vintage Designated Hungarian Spirits by Their Volatile Compounds. Periodica Polytechnica - Chemical Engineering, Paper: 11078 (2017) DOI: 10.3311/PPch.11078

Attila Szöllősi, László Narr, **Attila G. Kovács**, Gabriella Styevkó**.** Relationship between kinetics of growth and production of exo-electrons: case study with *Geobacter toluenoxydans.* Acta Microbiologica et Immunologica Hungarica, 62: 307-316 (2015) DOI: 10.1556/030.62.2015.3.8

Attila Szolosi, Quang Duc Nguyen, **Attila Gabor Kovacs**, Attila Levente Fogarasi, Szilard Kun, Beata Hegyesne-Vecseri. Production of low or

nonalcoholic beer in microbial fuel cell. Food and Bioproducts Processing, 98: 196-200 (2016) DOI: 10.1016/j.fbp.2016.01.012

Non- Impact Factor Publications

Attila G. Kovács, Marat Mamedov. The Art and Science of Fruit Distillate Fermentation
(part 1), Artisan Spirit, Pages 81 – 83, 2015, October

Attila G. Kovács, Marat Mamedov. The Art and Science of Fruit Distillate Fermentation
(part 2), Artisan Spirit, Pages 60-62, 2015, December

Attila G. Kovács, Marat Mamedov. The Art and Science of Fruit Distillate Fermentation
(part 3), Artisan Spirit, Pages 84 – 87, 2016, April

Symposium publications

International symposium (Full paper)

Attila Gábor Kovács, Ilona Panyik-Lánszki, Ágoston Hoschke, Quang D. Nguyen. Effects of Acids and Yeast Strains On Alcoholic Fermentation of Bosc Kobak Pear Mash. In: Dalmadi I, Engelhardt T, Bogó-Tóth Zs, Baranyai L, Bús-Pap J, Mohácsi-Farkas Cs (Editor): Food Science Conference 2013 - With research for the success of Darányi Program: Book of proceedings. Location and time of symposium: Budapest, Hungary, 2013.11.07-2013.11.08. Budapest: Corvinus University of Budapest, Faculty of Food Science, 177-180 (2013) ISBN:978-963-503-550-2

International Symposium Publication (Summary)

Attila Gábor Kovács, Ilona Panyik-Lánszki, Ágoston Hoschke, Quang D. Nguyen. Effects of Yeast Strains and Acids On Alcoholic

Fermentation of Pear. Acta Microbiologica et Immunologica Hungarica 60:(suppl. 1)161-162. (2013).

Hungarian Symposium Publications (Summary)

Kovács Attila Gábor, Nguyen Duc Quang, Hoschke Ágoston. Development of pálinka production technologies at the Faculty of Food Science of the Corvinus University of Budapest, MÉTE Beverage industry section, 2014. 05. 15., Budapest.