VOLATILE COMPOUNDS IN FRESHLY DISTILLED ŠAMPION APPLE WINES

Renata **Choińska***, Elżbieta **Bartosiak,** Antoni **Miecznikowski**
Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology
ul. Rakowiecka 36, 02-532 Warszawa, Poland
*E-mail: renata.choinska@ibprs.pl

Abstract
The production of the major volatile compounds of alcoholic fermentation (acetaldehyde, ethyl acetate, methanol and some fusel alcohols) as well as other flavor volatiles, is very complex process. Among many factors affecting the volatile profile of the apple wine, apple cultivar, fermentation microflora and distillation process employed, play a key role. The study of volatile compounds in fresh distillates will enable to verify the influence of the used fermentation microflora and overall process condition on the quality of final product.
In this manuscript, we present our preliminary study on the major volatile compounds of freshly distilled Šampion apple wines (fresh distillates). Distillates were prepared from Šampion apple wines fermented either by selected S. cerevisiae yeast strains (S. cerevisiae "32" and S. cerevisiae "Reneta") or by indigenous microflora present in juice (spontaneous fermentation). Distillation was conducted by using a distillation apparatus for small-scale laboratory distillation. The volatile compounds were determined using a gas chromatograph equipped with a flame ionization detector (FID).
Differences observed in the levels of the studied volatile compounds, are yeast strain specific. Distillates obtained through distillation of spontaneously-fermented apple mash were characterized by higher concentrations of acetaldehyde, ethyl acetate and methanol compared to distillates obtained from experimental fermentation. Distillates obtained from experimental fermentation showed higher concentrations of isoamyl alcohols. Differences in fusel alcohol concentrations were higher in wines fermented with S. cerevisiae "32" compared fermented with S. cerevisiae "Reneta".

Keywords: fermented apple distillates, aroma compounds, *Saccharomyces cerevisiae*

Submitted: 30.10.2015 Reviewed: 19.01.2016 Accepted: 25.02.2016

1. INTRODUCTION

Poland is one of the largest producers of apples in the EU and one of the largest exporters of apples following China and the United States of America. In spite of Poland's geographic location, it has ideal conditions for the cultivation of apples. Šampion is one of the most popular apple cultivars grown in Poland, and was deemed a good candidate for the production of fermented apple beverages. This cultivar is characterized by a high antioxidant activity, which is related to the relatively high concentration of chlorogenic acid and procyanidins. Apple wines produced from Šampion cv. possess positive and well-balanced taste and aroma (Satora et al., 2008).
The overall organoleptic characteristics of fermented apple wines depend on many factors including: apple cultivar, pretreatment of apple juice, type of the fermentation microflora, distillation technique, and wood maturation. The alcoholic fermentation carried out by yeast is the primary step in the production of apple wines. During alcoholic fermentation, aromatic compounds are formed, thereby establishing the aroma profile of the particular beverage. Yeasts metabolize apple juice constituents (primarily glucose) into alcohol and other minor metabolites such as esters, volatile fatty acids and carbonyls (Vidrih et al., 1999). These metabolites contribute to the sensory properties of the fermented apple juice. The concentrations and chemical properties of the synthesized flavor components depend on the yeast population during fermentation and fermentation conditions i.e.,. temperature, which can affect the growth and biochemical properties (Cabranes et al., 1997). Typically, the fermentation process is predominated by the yeast strains of *Saccharomyces* spp., mainly *S. cerevisiae.* The metabolic differences observed between strains that belong to this genus affect both the flavor and aroma of the

final product, thus determining its unique aroma profile. The selection of a suitable yeast strain is therefore an important aspect in this process (Peng et al. 2009). The next, relevant step in the apple brandy production is the distillation process. A number of reactions take place during distillation i.e., esterification, acetalization, and dehydration (Rodriguez Madrera et al., 2005).
The esterification of alcohols and acids results in increased esters formation during fermentation and the formation of carboxylic acid esters. Concentrations of low molecular-weight alcohols increase and high-molecular-weight alcohols decrease due both to the effects of differing volatility on recovery during distillation and of ester and acetal formation (Schreier et al., 1997).
The type of distillation apparatus, the material employed in the construction of the apparatus and the source of the heat are important factors affecting in the composition of distillates (Rodriguez Madrera et al., 2005). Distillation facilitates the incorporation of volatile compounds into the resulting ethanol-water mixture thereby affecting the volatile profile of fermented apple mash.
The study of volatile compounds in fresh distillates is important for several reasons. It can provide expedient information about raw materials and technological processes employed.
The composition of fresh distillates determines the aroma profile of maturated distillates. Several compounds generated during distillation have the capacity to react with oak wood constituents in the course of aging, which affects the organoleptic characteristics of the final product (Rodriguez Madrera et al., 2011).
The chemical composition of Šampion apple wines was recently reported on by Satora *et al*.(2008).
To the best of our knowledge, no information is available concerning the volatile compound profiles of freshly distilled Šampion apple wines.
In-depth knowledge of the volatile composition of Šampion apple wines and their distillates is important information which can contribute to the quality of the final product.
The aim of this study was to quantify the major volatile compounds in freshly distilled Šampion apple wines inoculated with two *S. cerevisiae* strains and natural occurring yeasts.

2. MATERIALS AND METHODS

Yeast, plant material and fermentation

S*accharomyces cerevisiae* strain, *"Reneta"* and *S. cerevisiae strain "32"* were selected from the Culture Collection of Prof. W. Dąbrowski Institute of Agricultural and Food Biotechnology in Warsaw. These strains were used for fermentation. *Saccharomyces cerevisiae "Reneta"* was isolated form apple mash, whereas *S. cerevisiae "32"* from grape mash.
All fermentations were conducted according to Dworska et al. (2011). Šampion apples were washed with water, ground, and pressed to obtain fresh juice (pH 3,4; titratable acidity 3,8 g/L). Fermentation was conducted using either indigenous microflora present in the juice (spontaneous fermentation) or by selected *S.cerevisiae* strains (inoculated; *S. cerevisiae "32"*, and S. *cerevisiae "Reneta",* respectively). In case of inoculated fermentation, 10 mL of each yeast strain were added to the Šampion apple juice.
The selected yeasts were cultured in a pasteurized apple juice at 25 °C for 48 h, following this, the cells were recovered by centrifugation, washed with sterile water and added to the mash at concentration 10^6cfu/ml. No yeasts were added to the spontaneous fermentation.
All fermentations were conducted for 28 days at 15 °C in 3-L glass flasks containing 2.2 L of apple mash. The fermentations were terminated after 28 days.
Inoculation and fermentations were performed in duplicate.
Table 1 summarize the total sugar and ethanol content of the mash before and after fermentation.

Table 1. Characteriztaion of the Šampion apple mash before and after fermentation (Dworska et al.2011)

	mash	*S. cerevisiae "32"*	S. *cerevisiae "Reneta"*	Spontaneous fermentation
Total sugar (g/L)	101,4	0,2	0,3	13,5
Ethanol (% v/v)	-	8,0	7,9	6,2

Distillation

The freshly distilled apple wines were prepared by distillation of the fermented mash, without decantation, using a distillation apparatus for small-scale laboratory distillation. The distillation apparatus consisted of a 2-L round-bottom flask, a rectifying column having 22 theoretical plates, three dephlegmators, and a condenser. The rectifying column used allows head-space collection, thus ensuring distillates with high ethanol content (approx. 96 % v/v). Apple wine distillates obtained through distillation contained approximately 65 % v/v ethanol. The distillates were further diluted with deionized water to obtain alcohol content of about 40 % v/v.

Chemicals

Acetaldehyde, ethyl acetate, methanol, isobutanol, 1-propanol and isoamyl alcohols were purchased from Sigma Aldrich, Poznań, Poland and were of GC purity.

GC-FID analysis

Major volatile compounds derived from alcoholic fermentation (acetaldehyde, ethyl acetate, methanol, isobutanol, 1-propanol and isoamyl alcohols) were were quantified using an Agilent Technologies model 7890A gas chromatograph equipped with a flame ionization detector (FID). The GC was equipped with an autosampler (model 7683B) and a split/splitless injector. Chemstation software (version B.03.01) was used for data acquisition. Separations were carried out on a DB-FFAP capillary column (30 m x 0.53 mm x 1µm). Temperature programming was as follows: 1.8 min isothermal at 50 °C, then a linear temperature rise of 70 °C/min to a maximum of 120 °C, and final isothermal of 120 °C (1.8 min). Other conditions were: injector temperature, 170 °C; detector temperature, 220 °C; injection volume, 1 µL in split mode (1/10). Quantitation was performed according to an external standard method. All analyses were done in triplicate.

Statistical analysis

STATISTICA (Statsoft) software was used to statistically analyse the results obtained. Statistical analysis of the distillate volatile compounds were evaluated by applying one-way analysis of variance (ANOVA) and the Tukey test to determine the statistically different values at a significance level of $\alpha \leq 0.05$.

3. RESULTS AND DISCUSSION

Several papers have reported on the impact of yeast strains on the chemical composition of wines (Lilly et al., 2000; Vilanova et al., 2006; Cortes et al., 2011).

The concentration of the major volatile compounds (acetaldehyde, ethyl acetate, methanol, isobutanol, 1-propanol and isoamyl alcohols) varied in distillates obtained from mashes fermented by selected *S. cerevisiae* strains and that of spontaneous fermentation. The concentration of acetaldehyde in the freshly distilled Šampion apple wine, produced by spontaneous fermentation, was higher, 0.66 g/L spirit 100% v/v, compared to the distillates produced with selected *S. cerevisiae* strains (0.20 g/L spirit 100% v/v, and 0.31 g/L spirit 100% v/v for *S. cerevisiae "32",* and *S. cerevisiae "Reneta*", respectively) (Table 2). The apple wines fermented with *S. cerevisiae "32"* had significantly less acetaldehyde compared fermented with *S. cerevisiae "Reneta"* (Table 2). The presence of acetaldehyde in young distillates is due to its formation during alcoholic fermentation. Differences in acetaldehyde concentration depend strongly on the yeast species or strain inoculated (Herrero et al., 2003).

Table 2. Concentrations of the major volatile compounds in the freshly distilled "Šampion" apple wines produced with selected *S. cerevisiae* strains and spontaneous fermentation.

Compound [g/L spirit 100 % v/v]	Strains of yeast		
	S. cerevisiae "32"	S. cerevisiae "Reneta"	Indigenous yeasts
Acetaldehyde	0.20 a ± 0.03	0.31 b ± 0.04	0.66 c ± 0.09
Ethyl acetate	1.69 a ± 0.13	1.76 a ± 0.14	2.93 b ± 0.23
Methanol	0.75 a ± 0.06	0.68 b ± 0.05	1.22 c ± 0.09
1-propanol	0.40 a ± 0.04	0.28 b ± 0.03	0.55 c ± 0.05
isobutanol	0.49 a ± 0.03	0.35 b ± 0.02	0.50 a ± 0.03
isoamyl alcohols	2.89 a ± 0.17	1.89 b ± 0.11	1.59 c ± 0.09

*Means followed by the different letters are significantly different at p < 0,05 (Tukey test)

Ethyl acetate was, quantitatively, one of the compounds with the highest concentration in all freshly distilled Šampion apple wines (Table 2). The concentration varied in the analyzed distillates from 1.69 g/L (*S. cerevisiae "32")* to 2.93 g/L spirit 100% v/v (spontaneous fermentation). The highest concentration of ethyl acetate was determined in the samples produced by spontaneous fermentation. Ethyl acetate is the main ester of fresh distillates and is formed during fermentation or during distillation by esterification of acetic acid and ethanol (Rodriguez Madrera et al., 2006). Ethyl acetate can impart a glue/varnish flavor to the general aroma profile of the distillate. The presence of high levels of ethyl acetate is indicative of poor quality raw material used with probable acetic bacterial spoilage (Satora et al., 2008). It has been established that among wine yeasts, *S. cerevisiae* is one of the lowest ethyl acetate producers (Regodon Mateos et al., 2006). The second highest concentrations of compounds in the Šampion apple distillates were iso-amyl alcohols (Table 2). The level of these compounds was significantly higher in the distillates obtained from inoculated Šampion apple wines (2.89 g/L spirit 100% v/v, and 1.89 g/L spirit 100% v/v for *S. cerevisiae "32",* and *S. cerevisiae "Reneta*", respectively), compared to the distillates produced by spontaneous fermentation (1.59 g/L spirit 100% v/v). Iso-amyl alcohols, 1-propanol, and iso-butanol, are formed during the fermentation process, as a result of the degradation of an amino acid via the Ehrlich's pathway, or anabolically via biosynthesis from a carbon source (Satora et al., 2008; Molina et al., 2007). The synthesis of higher alcohols depends not only on the particular yeast species but also on the particular strain of species (Leguerinel et al., 1989; Giudici et al., 1990;Giudici et al., 1993).

Yeast kinetics during the formation of higher alcohols and ethyl acetate in wines fermented with selected *S. cerevisiae* strains and wild species have been reported by other authors (Cabranes et al., 1997; Regodon Mateos et al.; 2006; Patel et al., 2003). The production of ethyl acetate was higher in spontaneous mash fermentation compared to wines fermented with selected *S. cerevisiae* strains. The production of iso-amyl alcohols however, was higher in the wines produced with *S. cerevisiae*. The high content of ethyl acetate in the spontaneously-fermented wines results from the presence of wild yeasts other than *S. cerevisae*, while the high content of iso-amyl alcohols results from the greater fermentative metabolism of *S. cerevisiae*.

4. CONCLUSIONS

A comparative study was performed on major volatile compounds of freshly distilled "Šampion" apple wines fermented by selected *S. cerevisiae* strains (*S. cerevisiae "32"* and *S. cerevisiae "Reneta*") and by spontaneous microflora. The results obtained proved that the distillates differed in volatile compound concentrations depending on the *S. cerevisiae* strain used. The distillates produced from mashes fermented by selected yeast strains showed significantly higher contents of isoamyl alcohols compared to distillate obtained from spontaneous fermentation. In contrast, the distillate produced by spontaneous fermentation contained more acetaldehyde, ethyl acetate, and methanol. The yeast strain *S.*

cerevisiae "32" produces more iso-amyl alcohols and less acetaldehyde than yeast strain *S. cerevisiae "Reneta"*.

Differences observed in the levels of the major volatile compounds formed during alcoholic fermentation, are yeast strain specific. Spontaneous fermentation of apple mashes is a complex process performed by the sequential action of various *Saccharomyes* and non-*Saccharomyces* species (Suarez Valles et al., 2007; Morrissey et al., 2004; Styger et al., 2011). The addition of a yeast starter culture enables controlling the fermentation process and obtaining the end product with a desired volatile profile.

5. References

[1] Cabranes C., & Mangas J.J. Selection and biochemical characterization of *Saccharomyces cerevisiae* and *Kloeckera apiculata* strains isolated from Spanish cider. *Journal of the Institute of Brewing*, 103, 1997,165-169.

[2] Cortes S., & Blanco P. Yeast strain effect on the concentration of major volatile compounds and sensory profile of wines from *Vitis vinifera* var. Treixadura. *World J. Microbiol. Biotechnol.*, 27, 2011, 925 – 932.

[3] Dworska A., Zalewska T., Kupryś M., & Stecka K.M. Charakterystyka cech smakowo-zapachowych nowych napojów typu cydr i calvados. *Postępy Nauki i Technologii Przemysłu Rolno-Spożywczego*, 66(3), 2011, 45-61.

[4] Giudici P., Romano P., & Zambonelli C. A biometric study of higher alcohol production in *Saccharomyces cerevisiae*. *Canadian Journal of Microbiology*, 36, 1990, 61 – 64.

[5] Giuidici P., Altieri C., & Gambini G. Effects of yeast strain on minor products of alcoholic fermentation: studies on Apulia wines. *Industrie delle Bevande*, 22 (126), 1993, 303 – 306.

[6] Herrero M., Garcia L. A., & Diaz M. The effect of SO_2 on the production of ethanol, acetaldehyde, organic acids, and flavour volatiles during industrial cider fermentation. *Journal of Agriculture and Food Chemistry*, 51, 2003, 3455 – 3459.

[7] Leguerinel I., Mafart P., Cleret J. J., & Burgeois C. Yeast strain and kinetic aspects of the formation of flavor components in cider. *Journal of the Institute of Brewing*, 95 (6), 1989, 405 – 409.

[8] Lilly M., Lambrechts M.G., Pretorius I. S. Effect of increased yeast alcohol acetyltransferase activity on flavor profiles of wine and distillates. *Applied and Environmental Microbiology*, 66 (2), 2000, 744 – 753.

[9] Molina A. M., Swiegers J. H., Varela C., Pretorius I. S., & Agosin E. Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. *Appl. Microbiol. Biotechnol.*, 77, 2007, 675 – 687.

[10] Morrissey W. F., Davenport B., Querol A., & Dobson A. D. W. The role of indigenous yeasts in traditional Irish cider fermentations. *Journal of Applied Microbiology*, 97, 2004, 647 – 655.

[11] Patel S., Shibamoto T. Effect of 20 different yeast strains on the production of volatile components in Symphony wine. *Journal of Food Composition and Analysis*, 16, 2003, 469 – 476.

[12] Peng B., Yue T., & Yuan Y. Analysis of key aroma components in cider from Shaanxi (China) *Fuji* apple. *International Journal of Food Science and Technology*, 44, 2009, 610-615.

[13] Regodon Mateos J. A., Perez-Nevado F., & Ramirez Fernandez M. Influence of *Saccharomyces cerevisiae* yeast strain on the major volatile compounds of wine. *Enzyme and Microbial Technology*, 40, 2006, 151 – 157.

[14] Rodriguez Madrera, R., & Magnas Alonso, J.J. Typification of cider brandy on the basis of cider used in its manufacture. *Journal of Agricultural and Food Chemistry*, 53, 2005, 3071-3075.

[15] Rodriguez Madrera R., Valles B.S., Garcia Hevia A., Garcia Fernandez O., Tascon N., & Mangas Alonso J.J. Production and composition of cider spirits distilled in "Alquitara". *Journal of Agricultural and Food Chemistry*, 54, 2006, 9992-9997.

[16] Rodriguez Madrera R., Suarez Valles B., & Picinelli Lobo A. Chemical and sensory changes in fresh cider during maturation in inert containers. *Journal of the Science of Food and Agriculture*, 91, 2011, 797-804.

[17] Satora P., Sroka P., Duda-Chodak A., Tarko T., & Tuszyński T. The profile of volatile compounds and polyphenols in wines produced from dessert varieties of apple. *Food Chemistry*, 111, 2008, 513 – 519.

[18] Schreier P., Drawert F., & Schmid M. Changes in the composition of neutral volatile components during the production of apple brandy. *Lebensmittel Unters. u. Forsch*., 1997, 728 -736.

[19] Suarez Valles B., Pando Bedrinana R., Fernandez Tascon N., Querol Simon A., Rodriguez Madera R. Yeast species associated with the spontaneous fermentation of cider. *Food Microbiology*, 24, 25 – 31 (2007).

[20] Styger G., Prior B., & Bauer F.F. Wine flavor and aroma. *Journal of Industrial Microbiology and Biotechnology*, 38, 2011, 1145-1159.

[21] Vidrih R., Hribar J. Synthesis of higher alcohols during cider processing. *Food Chemistry*, 67, 1999, 287-294.

[22] Vilanova M., & Sieiro C. Contribution by *Saccharomyces cerevisiae* yeast to fermentative flavour compounds in wines from cv. Albarino. *J. Ind. Microbiol Biotechnol.*, 33, 2006, 929 – 933.