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# THE ANALYSIS OF CORRELATIONS BETWEEN TERPENE TRANSFORMATIONS AND FERMENTATION TYPE OF APPLE MUSTS

– Research paper –

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**Abstract:** We measured the concentration of 13 terpenes in musts of 3 different apple cultivars fermented spontaneously, with Ethanol RED yeast (*Saccharomyces cerevisiae*) or Cider Yeast (*Saccharomyces bayanus*). Then we analysed obtained data with PCA with various packages in R to assess how different types of fermentation and apple cultivars affected terpene profile. The most informative results of PCA were obtained in 'psych' package and they indicated that there were groups of terpenes which concentrations increased simultaneously, while for some other groups the content of particular compounds was negatively correlated. This suggests that the first group of terpenes originated from different substrates while the latter group could be obtained from the same compound. Our findings indicated new possible pathways of terpene transformation, e.g. linalool, citral and geraniol could transform into one another. Moreover, the role of *S. bayanus* in those processes remains unclear and it requires further consideration.

**Keywords:** fermented apple must, terpenes, PCA, fermentation, GC-FID

## INTRODUCTION

Terpenes belong to the group of aliphatic long-chain hydrocarbons and are classified as natural compounds derived from active isoprene (isopenthyl diphosphate) or its isomer – dimethylallyl pyrophosphate. Among terpenoids, monoterpenes and sesquiterpenes are the main apple aroma components. The acyclic branched sesquiterpene (E,E)-α- -farnesene (fruity, herbaceous aroma) is synthesised mainly in epidermal and hypodermal cell layers of the apple and is most common in ripe apple fruit (Burdock, 2005; Espino-Díaz, Sepúlveda, González-Aguilar, & Olivas, 2016).

In addition to its aromatic characteristics, terpenes hold some biological properties. These compounds show strong cancer chemopreventive effects, antimicrobial, antiviral, anti-hyperglycemic, anti-inflammatory, and antiparasitic activities. Terpenes are also involved in the prevention and therapy of several inflammatory diseases (Paduch, Kandefer-Szerszeń, Trytek, & Fiedurek, 2007). At the same time, it must be highlighted that qualitative and quantitative profile of terpenes in apple musts has not been studied in detail so far.

Terpene compounds are formed by combining of two or more 5-carbon-atom molecules leading to the formation of C10 monoterpenes, C15 sesquiterpenes or C20 diterpenes. There are various derivatives of terpenes including alcohols (citronellol and linalool), aldehydes (citral and citronellal), ketones (tagetone, mircenon and o-cymenon), esters or oxides. Those might be formed during dehydration (by depleting those compounds of –OH groups) which leads to the formation of additional double bond, giving such substances as β-myrcene and o-cymene (Cal, 2006; Januszek, Satora & Tarko, 2020). There are many terpenes with characteristic aromas, e.g. β-damascenone has sweet, brown woody, tobacco, davana-like, fruity, with a spicy balsamic undernote (aroma threshold 0.0007 to 0.009 ppb); farnesol has floral, rose, sweet, fruity, green lavender and haylike nuances (aroma threshold 1 ppm); limonene has a pleasant, lemon-like odor free from camphoraceous and turpentine-like notes (4 to 229 ppb); linalool has a pleasant floral aroma, free from camphoraceous

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and terpenic notes (4 to 10 ppb); citronellol has characteristic rose-like odor: l-Citronellol - sweet, peach-like flavor d-citronellol – bitter taste (11 ppb to 2.2 ppm) (Burdock, 2005).

There are various statistical tools to optimise technological processes and principal component analysis (PCA) is one of them. It enables selecting variables which have significant impact on the outcomes (Smith, 2002) that are important from technological point of view. Therefore, in the current study we use it to select apple cultivar and fermentation type which enable obtaining the most desirable terpene profile.

## MATERIALS AND METHODS

### Fermentation

We obtained apple musts from three different cultivars (Elise, Rubin and Topaz) harvested in Garlica Murowana, Poland. Before collecting musts, fruits were washed, crushed and pressed using Zottel hydraulic press (35 L). Obtained liquid fraction was divided into 2 kg aliquots in 3-L sterile glass flasks, supplemented with $(NH_4)_2HPO_4$ (0.2 g/kg raw fruit) and inoculated (0.3 g/L) with Ethanol RED yeast strain (*Saccharomyces cerevisiae*, Starowar, Warsaw, Poland) or Cider Yeast (*Saccharomyces bayanus*, Young's brew, Bilston, England) or fermented spontaneously (Januszek et al., 2020; Januszek, Satora, Wajda, & Tarko, 2020). We obtained 9 experimental variants: musts from Elise cultivar (E), Rubin cultivar (R), Topaz cultivar (T); fermented spontaneously (S), with Ethanol RED (R) and Cider Yeast (C). The following abbreviations were assigned: ES, ER, EC, RS, RR, RC, TS, TR and TC. Fermented musts were distilled in one-stage process. Distillation was stopped when the ethanol concentration in the collected distillate was lower than 0.5% (w/v). In order to avoid the loss of aromas all fractions were kept at 4 °C until further analysis of terpene compounds.

### Terpene compounds analysis (GC-FID - Gas Chromatography – Flame Ionization Detector)

Analysis of selected terpenes was carried out using gas chromatography as described by Satora et al. (2008) (Satora, Sroka, Duda-Chodak, Tarko, & Tuszyński, 2008) with modifications. GC-FID analysis was carried out on the Hewlett Packard 5890 Series II chromatograph system. Tested compounds were separated on the HP-INNOVAX capillary column (stationary phase – crosslinked polyethylene glycol; 30 m × 0.53 mm ID with 1.0 μm film thickness). The detector and injector temperature was 250 °C and column was heated using the following temperature program: 35 °C for one minute at an increment of 2 °C/min to 55 °C, then 15 °C/min to 230 °C and maintained a constant temperature for three minutes. The carrier gas was helium at a 20.0 mL/min flow. Hydrogen flow speed was 33.0 mL/min, and that of air was 400 mL/min. The following terpenes were analysed (qualitatively and quantitatively): linalool, linalool oxide, limonene, isoeugenol, methyl eugenol, citral, (+)-terpinen-4-ol, geraniol, myrcene, β-citronellol, β-ionone, eugenol, and guaiacol (Sigma Aldrich, Missouri, USA). We used anethole as an internal standard and then we compared retention times and peak surface area read from sample and standard chromatograms.

Concentration of terpenes in distillates was recalculated to express it as a concentration of those compounds in fermented musts [μg/L]. All tests were carried out in three replicates.

### Statistical analysis

We applied Shapiro-Wilk test to assess the normality of data distribution. Then we carried out multivariate analysis of variance (MANOVA) with post hoc Tukey test. PCA was carried out using functions demonstrated in Table 2. All statistical analyses were carried out using R: A language and environment for statistical computing, version 3.6.2 (Foundation for Statistical Computing, Vienna, Austria, 2019). MANOVA was carried out using "lm" function and Tukey test was done using "HSD.test" function in the "agricolae" package. The PCA was carried out in "psych" package (Beaumont, 2012). Cortest-Bartlett test was carried out using "cortest.bartlett" function in "psych" package as well. The data demonstrated normal distribution so its transformation was not necessary. Strong correlations between loads and scores were considered when values obtained in correlation matrix exceeded 0.3 or below -0.3.

## RESULTS

In our previous study (Januszek et al., 2020) we analysed chemical composition of musts before and after fermentation with Ethanol RED yeast and brandies of ten apple cultivars. Based on our results we selected three cultivars of apple (Topaz, Rubin, Elise) for the current study. Parameters of those were the most favourable for the production of alcoholic beverages. Moreover, brandies obtained from those cultivars demonstrated the most diverse profile of volatile compounds and some of terpenes were characteristic for particular cultivars e.g. limonene and mircene (Topaz and Elise) and β-citronellol (Rubin). In addition, those brandies reached highest overall scores in sensory analysis (Januszek et al., 2020).

In our current study limonene was present in all variants involving Elise and Topaz cultivar, with highest concentration in must fermented with *S. bayanus* and *S. cerevisiae*, respectively (Table 1). The concentration of linalool oxide was lowest in samples collected after spontaneous fermentation, while highest concentration was detected in Elise and Topaz musts fermented with *S. cerevisiae*. The highest content among all terpenes was noted for (+)-terpinen-4-ol in Rubin must fermented spontaneously (46.9 µg/l) and all musts obtained from that particular apple cultivar demonstrated significantly higher content of that compound when compared to other tested samples. Citral was detected in Rubin musts fermented with *S. bayanus* or *S. cerevisiae*. Concentrations of geraniol were lowest in all variants fermented spontaneously while it was absent in Elise fermented must. β-ionone was the most abundant in Elise and Rubin musts fermented with *S. cerevisiae*. Isoeugenol was not present in Elise and Topaz musts fermented with *S. bayanus*, while in the case of Rubin cultivar it was only present in musts fermented with that yeast. Significantly higher concentration of methyleugenol was detected in Topaz musts fermented spontaneously. β-citronellol concentration was slightly higher in variants fermented with *S. cerevisiae* and lowest in samples after fermentation with *S. bayanus*. Eugenol content was highest in Elise and Topaz musts fermented spontaneously, while in overall, it was the most abundant terpene in all tested samples. Guaiacol concentration was highest in all variants tested with *S. cerevisiae.* Mircene was detected in Topaz musts fermented spontaneously or with *S. bayanus*. Eugenol and linalool were the most common terpenes and they were detected in all or the majority of samples, min. 10 µg/L or min. 5 µg/L, respectively (Table 1). We carried out PCA to assess which fermentation type played major role in changes of terpene concentration. Moreover, we intended to examine how tested apple cultivar influenced that parameter. For that purpose, we used six different functions in R to verify which one could provide the most useful and informative results. Various terpenes were treated as loadings and apple musts obtained from three different cultivars fermented with various microorganisms were scores. 'Princomp' function did not provide any information about the most significant components, correlation matrix or eigenvalues for scores. Moreover, it did not demonstrate proportion variance (Table 2). Due to those facts data obtained in 'princomp' was not analysed further. 'Prcomp' function eliminated components which did not contribute significantly to the cumulative variance so only four first terpenes remained. It also provided eigenvalues for both loadings and scores. it did not provide correlation matrix or proportion variance for each selected component (Table 2) so it was rejected. For the same reasons we did not consider results obtained with 'ACP' function.

'PCA' and 'dudi.pca' functions indicated that first five components explained the majority of cumulative variance. On the other hand, in 'PCA' five is a default value for the number of dimensions kept and there is no step in the analysis that allows selection and/or visualization of the most important components. On the other hand, the latter function does not allow elimination of nonsignificant variables. Moreover, none of those two functions provide data on the proportion variance (Table 2). Without that information we were not able to prepare figures demonstrating correlations of principal components. Because the analysis carried out in 'psych' package provided a detailed insight (Table 2) into analysed data, we presented graphical demonstration of the results (Figures 1-3). Five principal components explained the majority of variance. From the 8 obtained figures, we decided to show only those providing the most accurate demonstration of detected correlations: limonene vs. linalool oxide ($R^2$= 0.5647), isoeugenol vs. limonene ($R^2$=0.3207), ß-citronellol vs. limonene ($R^2$=0.4456), ß-citronellol vs. linalool oxide ($R^2$=0.3255), linalool oxide vs. guaiacol ($R^2$=0.3208), limonene vs. citral ($R^2$=-0.3732), limonene vs. mircene ($R^2$=-0.4252), linalool oxide vs. (+)-terpinen-4-ol ($R^2$=-0.4636), linalool vs. citral ($R^2$=-0.5519), linalool vs. geraniol ($R^2$=-0.5642),linalool vs. isoeugenol ($R^2$=-0.3907), linalool vs. eugenol ($R^2$=-0.5419), linalool vs. mircene ($R^2$=-0.4810), (+)-terpinen-4-ol vs. isoeugenol ($R^2$=-0.3815).

Table 1. Terpene profiles of apple musts fermented by various microorganisms

| Apple Cultivar | Fermentation | Terpenes [µg/l of fermented must] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Limonene | Linalool oxide | Linalool | (+)-terpinen-4-ol | Citral | Geraniol | β-ionone | Isoeugenol | Methyleugenol | (-)-β-citronellol | Eugenol | Guajacol | Mircene |
| Elise | SF | 4.27c ± 0.64 | 1.56e ± 0.05 | 12.35ab ± 0.78 | 0.98c ± 0.13 | 2.15de ± 2.09 | 0.00d ± 0.00 | 9.82ab ± 1.31 | 7.31b ± 1.89 | 3.79cd ± 0.21 | 2.05b ± 0.64 | 17.42bcd ± 1.76 | 2.93a ± 1.03 | 0.00b ± 0.00 |
| | SB | 6.68b ± 0.54 | 7.43b ± 0.82 | 14.13a ± 1.32 | 2.30c ± 0.11 | 0.56e ± 0.08 | 0.05d ± 0.02 | 1.61c ± 1.67 | 0.00c ± 0.00 | 7.53bc ± 0.93 | 1.94bc ± 0.17 | 10.67e ± 2.27 | 3.42a ± 0.42 | 0.00b ± 0.00 |
| | SC | 4.58c ± 1.38 | 10.38a ± 1.38 | 12.32ab ± 2.32 | 1.34c ± 0.14 | 0.97e ± 0.70 | 0.49cd ± 0.03 | 14.49a ± 6.30 | 5.32b ± 0.53 | 9.16b ± 0.41 | 3.92a ± 0.59 | 13.59de ± 1.62 | 6.05a ± 0.65 | 0.00b ± 0.00 |
| Topaz | SF | 6.45b ± 0.26 | 2.78de ± 1.10 | 6.54c ± 2.78 | 3.33c ± 1.81 | 6.22bc ± 1.00 | 0.16cd ± 0.07 | 1.36c ± 0.98 | 10.16a ± 1.25 | 16.74a ± 2.97 | 4.02a ± 0.66 | 37.88a ± 3.34 | 4.22a ± 1.33 | 10.44a ± 1.47 |
| | SB | 9.23 a ± 0.63 | 4.28cd ± 1.01 | 7.27b ± 0.94 | 3.42c ± 0.82 | 1.51de ± 0.49 | 3.37a ± 0.49 | 3.15bc ± 1.21 | 0.00c ± 0.00 | 5.49bcd ± 3.23 | 2.19b ± 0.59 | 22.76b ± 2.47 | 5.11a ± 2.95 | 10.78a ± 0.33 |
| | SC | 4.94c ± 0.81 | 7.92ab ± 1.19 | 8.37bc ± 1.83 | 7.37bc ± 1.72 | 5.26cd ± 2.62 | 3.15a ± 1.26 | 2.03c ± 0.34 | 10.32a ± 1.06 | 3.28cd ± 0.72 | 3.98a ± 0.73 | 20.09bc ± 1.77 | 5.57a ± 1.40 | 0.00b ± 0.00 |
| Rubin | SF | 0.00d ± 0.00 | 0.90e ± 0.22 | 14.65a ± 2.21 | 46.91a ± 5.81 | 3.42cde ± 0.37 | 0.25cd ± 0.13 | 0.82c ± 0.11 | 0.00c ± 0.00 | 5.46bcd ± 1.49 | 1.54bc ± 0.07 | 15.34cde ± 0.51 | 3.88a ± 1.38 | 0.00b ± 0.00 |
| | SB | 0.00d ± 0.00 | 6.84bc ± 1.06 | 5.64c ± 1.90 | 6.37bc ± 2.52 | 12.84a ± 0.54 | 2.67ab ± 0.23 | 2.89bc ± 1.89 | 7.01b ± 0.20 | 3.94cd ± 2.29 | 0.52c ± 0.04 | 17.58bcd ± 1.91 | 2.94a ± 0.37 | 0.00b ± 0.00 |
| | SC | 0.00d ± 0.00 | 1.84de ± 0.43 | 8.71bc ± 0.55 | 12.11b ± 2.59 | 10.09ab ± 1.76 | 1.37bc ± 0.04 | 10.91a ± 2.03 | 0.00c ± 0.00 | 0.52d ± 0.05 | 1.65bc ± 0.37 | 17.76bcd ± 1.61 | 5.06a ± 0.75 | 0.00b ± 0.00 |
| Statistical significance | Apple cultivar | ** | * | ** | *** | *** | *** | ** | • | * | *** | *** | ns | *** |
| | Fermentation | * | *** | ns | * | ns | *** | ** | ns | • | *** | ** | * | * |

The same letters next to mean values within columns indicate the lack of statistically significant differences at p marked in the last two rows: *ns* not significant, 0 '***', 0.001 '**', 0.01 '*', 0.05 '•', n=3, Abbreviations: SF – spontaneous fermentation, SB - fermentation with *Saccharomyces bayanus* (Cider Yeast), SC – fermentation with *Saccharomyces cerevisiae* (Red Ethanol yeast)

Table 2. The summary of various functions for carrying out PCA in R

| Package properties | Package name | | | | | |
|---|---|---|---|---|---|---|
| | factorextra | stats | FactoMineR | FactoMineR | amap | psych |
| Function | prcomp | princomp | PCA | dudi.pca() | apc | principal |
| Correlation matrix | N/A | N/A | N/A | N/A | N/A | Available |
| Available rotation schemes | retx | No information | No information | No information | No information | viramax and rotamax |
| Information about proportion variance | N/A | N/A | Available | N/A | N/A | Available |
| Results for scores and loadings | Available for both | For scores only | For scores only | Available for both | Available for both | Available for both |

N/A – not available

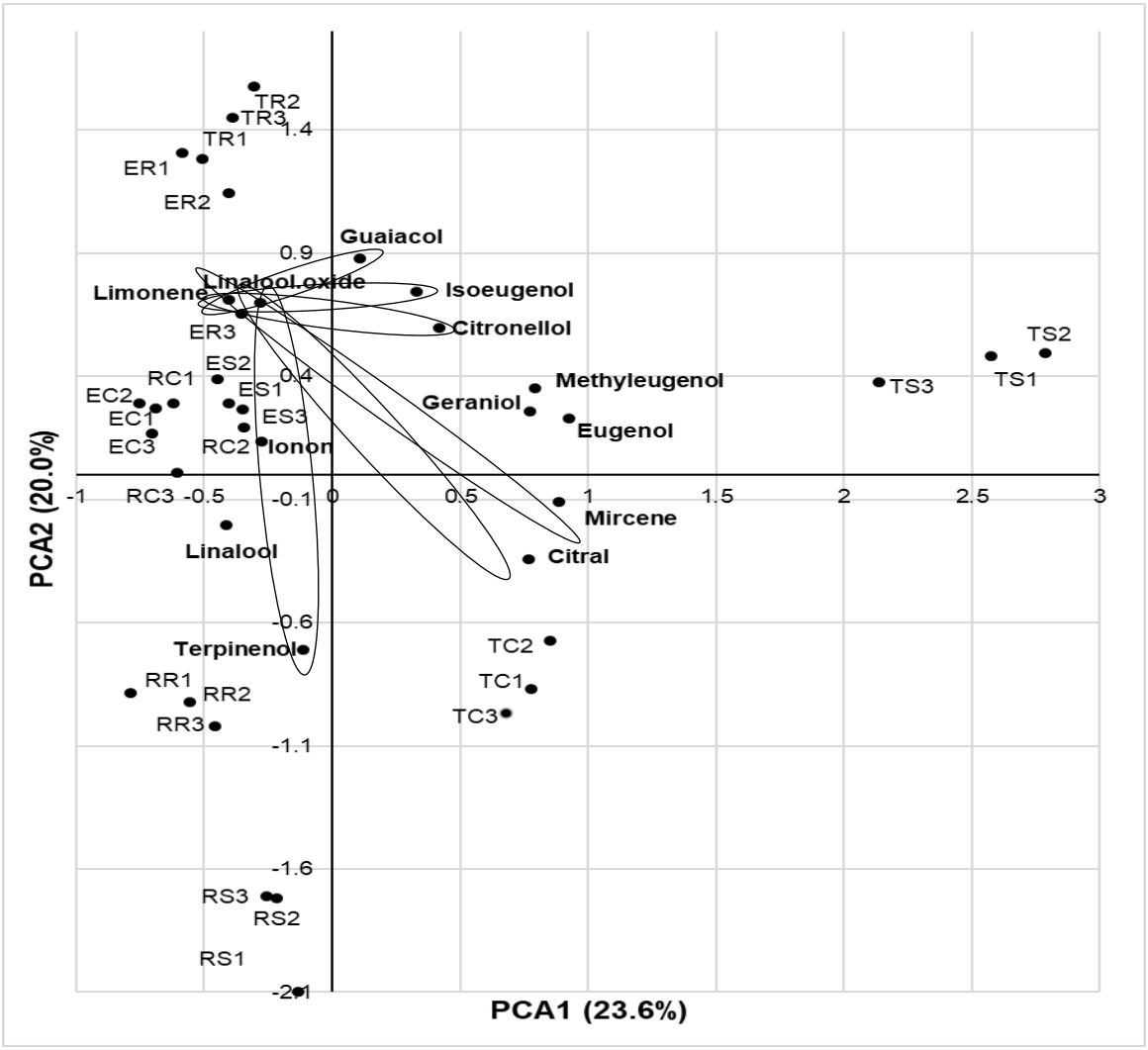



Figure 1. Principal component analysis describing correlations between quantitative/qualitative terpene profiles of head fractions obtained after various fermentation variants (PCA1 vs. PCA2): S – spontaneous fermentation; C – cider yeast (*S. bayanus*); R –Ethanol RED yeast; from various apple cultivars: E – Elise; T – Topaz; R – Rubin; 1, 2 and 3 refer to replicates of particular experimental variant; n = 3, p>0.05

The first pair of terpenes was located in the same quarter (Figure 1). In the case of other pairs of terpenes where positive correlations were noted, analysed compounds were located in the same half of the figure but in opposite quarters (Figures 1-3).
In the case of negative correlations, compounds were located in opposite quarters located diagonally. This means that citral and limonene concentrations increase simultaneously, while (+)-terpinen-4-ol was increasing when linalool oxide concentration was decreasing.
There are certain groups of terpenes which occur together in fermented musts:
i) geraniol, methyleugenol and eugenol (Figure 1);
ii) limonene and linalool oxide (Figure 1);
iii) limonen and β-citronellol (Figure 3);
iv) linalool oxide and β-ionon (Figure 2).
There are also some negative correlations between groups of terpenes:
i) β-ionon, limonene and linalool oxide vs. mircene and citral (Figure 1);
ii) linalool and (+)-terpinen-4-ol vs other terpenes (Figure 1);
iii) linalool oxide and β-ionon vs. β-citronellol and methyleugenol (Figure 2);
iv) (+)-terpinen-4-ol and linalool vs. linalool oxide, isoeugenol and β-ionon (Figure 3).

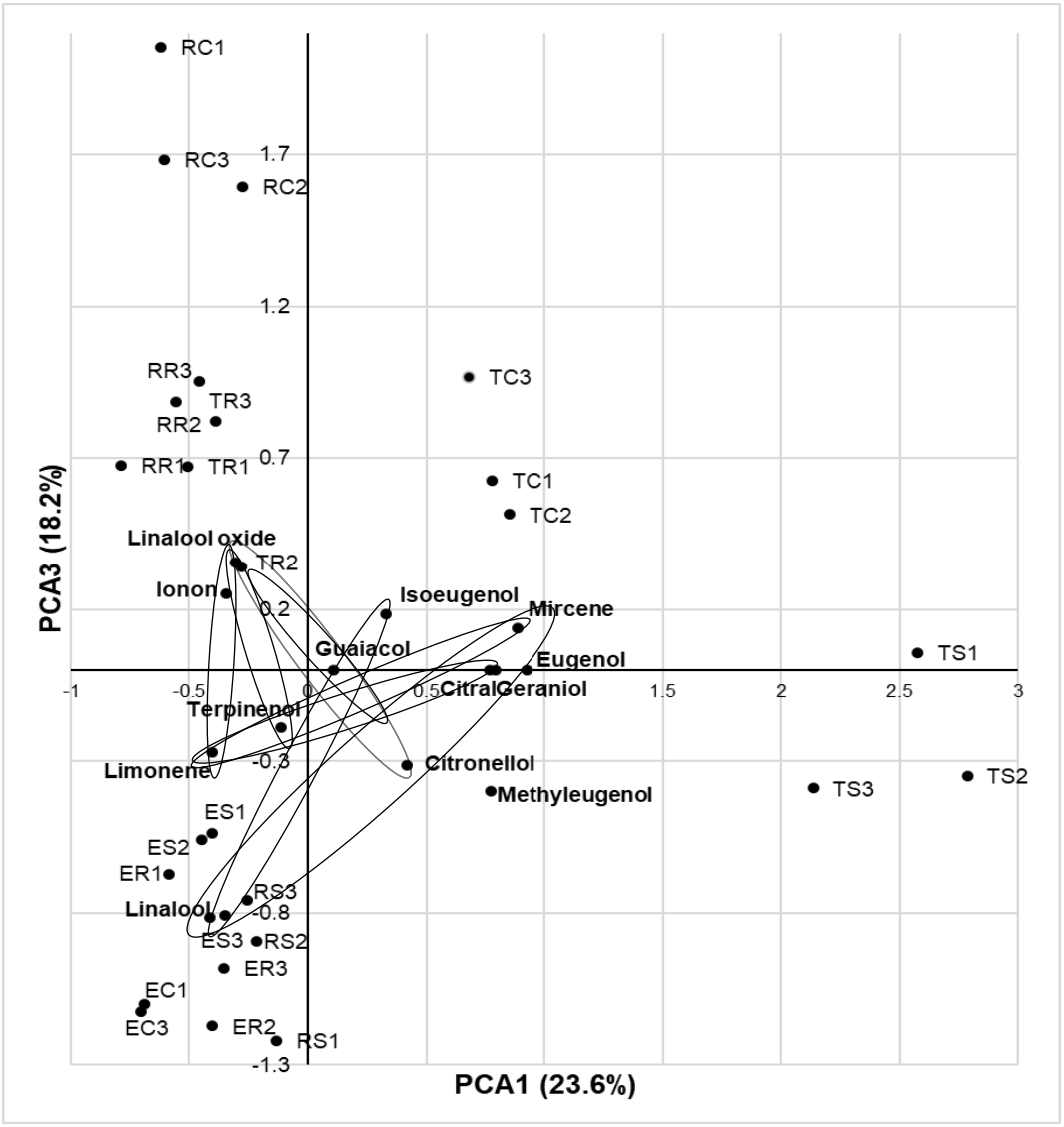



Figure 2. Principal component analysis describing correlations between quantitative/qualitative terpene profiles of head fractions obtained after various fermentation variants (PCA1 vs. PCA3): S – spontaneous fermentation; C – cider yeast (*S. bayanus*); R –Ethanol RED yeast; from various apple cultivars: E – Elise; T – Topaz; R – Rubin; 1, 2 and 3 refer to replicates of particular experimental variant; n = 3, p>0.05

## DISCUSSION

Based on the results obtained in PCA it might be stated that apple cultivar and fermentation type significantly influenced terpene profile in obtained fermented musts. It has been previously reported that both qualitative and quantitative composition of terpenes might be affected by plant cultivar (Jin, Jin, Yu, Lee, & Chen, 2017) but this phenomenon has not been investigated in fermented apple musts so we could not compare our results to findings of other authors. We demonstrated that the presence and concentration of the majority of tested terpenes was related to the presence of certain microorganisms which carried out fermentation (Table 1, Figures 1-3). Moreover, mircene was present only in the case of certain fruit cultivar (Topaz), while guaiacol could be rather assigned to the certain microorganism carrying out fermentation (*Saccharomyces cerevisiae*).

There are few likely explanations to the latter phenomenon. The first possible pathway of that processes is that microorganisms that carried out fermentation produced enzymes which released terpenoids from their glycosidic forms. Some yeast are responsible for the transformation of certain aroma precursors into aromatic compounds, e.g. linalool, geraniol, nerol, citronelol, α-terpineol and

linalool oxide, which are linked to diglycosides: 6-O-α-L-rhamnopyranosyl-, 6-O-α-L-rhamnofuranosyl- and 6-O-β-D-apiofuranosyl-β-D-glucosides (Bisotto, Julien, Rigou, Schneider, & Salmon, 2015; Gunata, Bayonove, Baumes, & Cordonnier, 1985; Rivas, Parra, Martinez, & Garcia-Granados, 2013; Schneider, Razungles, Augier, & Baumes, 2001; Williams, Sefton, & Wilson, 1989). Reactions are as follows: α-L-rhamnosidase or β-D-apiofuranosidase cleaves the (1→6)- glycosidic linkage, and then, flavour compounds are liberated from the monoglucosides by the activity of a β-glucosidase (Bisotto et al., 2015; Gunata et al., 1985; Günata, Dugelay, Sapis, Baumes, & Bayonove, 1993).

Linalool was detected in all samples. Some strains of *S. cerevisiae* have got natural biotechnological ability to produce that compound (Jongedijk, et al., 2016) and that yeast species is one of the most dominant in spontaneous fermentation of apple beverages (Cousin et al., 2017). Since the role of *S. bayanus* in the synthesis of that compound was not described in the literature we can speculate that it can also produce it. *S. bayanus* is the major fungal species which occurs during fermentation of apples (Cousin et al., 2017). Moreover, it is possible that since the majority of analysed terpenes (linalool oxide, linalool, (+)-terpinen-4-ol, citral, geraniol, β-ionone, methyleugenol, (-)-β-citronellol, eugenol and guaiacol) were found in all analysed samples, it is possible that those compounds can transform into one another.

Citral can be synthesised from geranic acid and converted promptly to geraniol (Esmaeili & Tavassoli, 2010) and linalool (Pardo, Rico, Gil, & Orejas, 2015) or be further transformed by some fungi to citronellol (Esmaeili, Rohany, Safaiyan, & Zarei, 2011). Furthermore, geranic acid tends to be easily transformed to citronellic acid which is then converted into citronellal and citronellol.

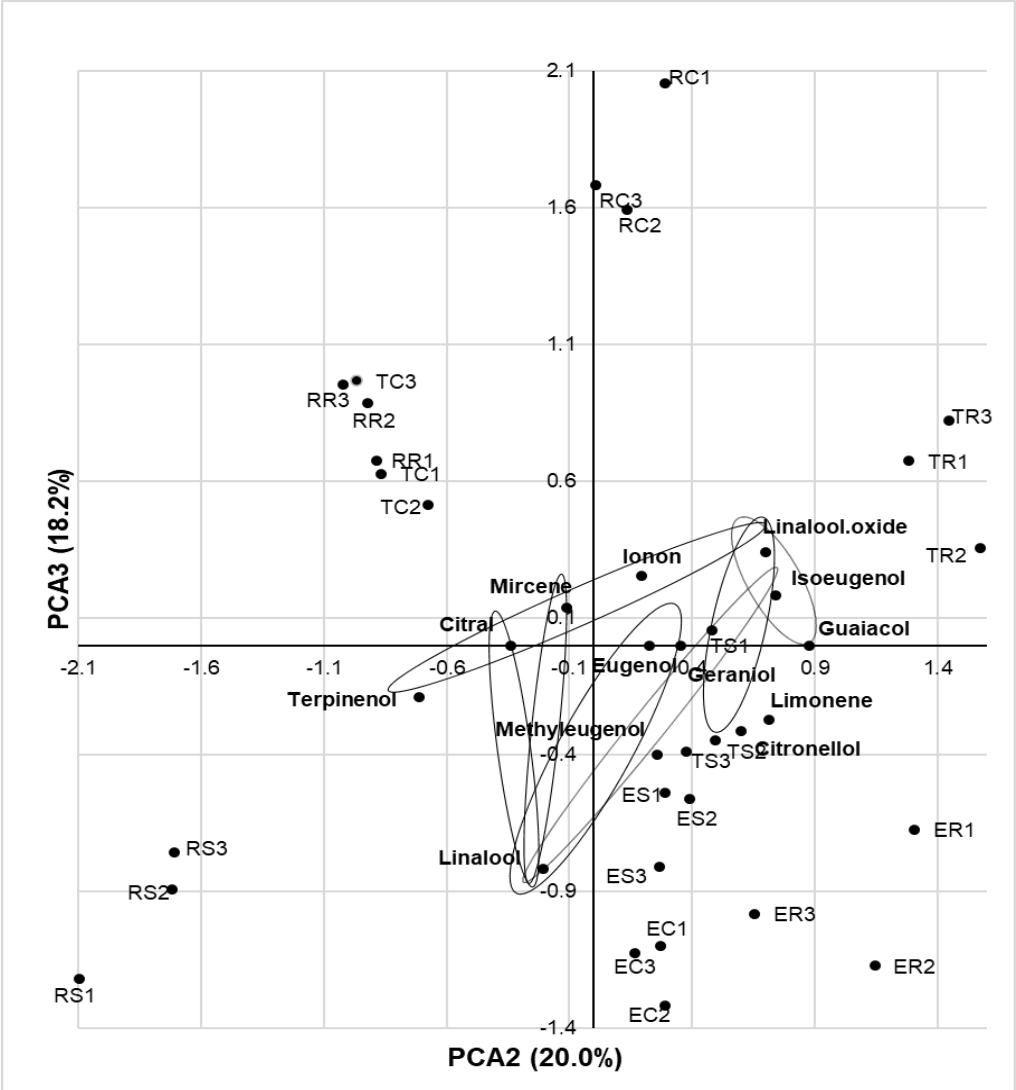



Figure 3. Principal component analysis describing correlations between quantitative/qualitative terpene profiles of head fractions obtained after various fermentation variants (PCA2 vs. PCA3): S – spontaneous fermentation; C – cider yeast (*S. bayanus*); R –Ethanol RED yeast; from various apple cultivars: E – Elise; T – Topaz; R – Rubin; 1, 2 and 3 refer to replicates of particular experimental variant; n = 3, p>0.05

Moreover, in the presence of water, linalool converts (isomerises) into geraniol. This would explain the negative correlation between those two compounds (Figure 2). This process can also be reverted. Geraniol is a fine base to prompt the production of numerous volatile compounds e.g. citronellol, linalool, nerol, citronellyl acetate and geranyl acetale. Geraniol synthase (GES) gene in *Saccharomyces cerevisiae* substantially changes the profile of terpenes in alcoholic beverages (Pardo et al., 2015).

Citral, which was present in each analysed samples easily underwent aldol condensation with acetone to obtain ψ-ionone, which can be transformed during cyclisation (under acidic conditions) into a mixture of α- and β-ionones (Isac-García, Dobado, Calvo-Flores, & Martínez-García, 2016).

The fact that linalool might transform into ionones, could explain the presence of β-ionon in all samples (Table 1). The appearance of methyleugenol in fermented musts can prove that this compound is a part of eugenol methylation. The methylation of eugenol to methyleugenol **(**methyl eugenol**)** it catalysed by an S-adenosylmethionine dependent O-methyltransferase (IEMT). The two compounds might appear codependently in alcoholic beverages (Yuwono Siswandono et al., 2002).

Negative correlations detected in PCA could be explained by the fact that microbial enzymes compete for the same substrates for the synthesis of particular compounds, e.g. limonene and citral (Figures 1-3) or (+)-terpinen-4-ol and linalool oxide (Figure 3). Geraniol and linalool are both aliphatic monoterpenes and they can be further transformed into other compounds that belong to the same group (Paramasivan & Mutturi, 2017) which could explain negative correlation in their concentration (Figure 1).

The role of *S. bayanus* in terpene biotransformation is unknown. It is clear that the strain which was used in current study demonstrated different enzymatic activity to *S. cerevisiae*. In most of cases musts obtained from the same apple cultivar fermented with different microorganisms contained unique terpenes, e.g. isoeugenol was characteristic for Rubin must fermented with *S. bayanus*. Some terpenes were correlated only with spontaneous fermentation because there are other microorganisms that play a significant role in terpene transformation. It has been previously demonstrated that *Yarrowia lypolitica*, *Rhodosporidium toruloides, Escherichia coli* or *Streptomyces* species could be involved in those processes (Wang et al., 2018) but only *R. toruloides* could naturally occur on apples and survive fermentation conditions. Since in the majority of cases, terpene profiles between variants fermented by apple microflora were similar to those obtained by *S. bayanus* or *S. cerevisiae*, it is possible that yeast belonging to those species were present in tested apples. On the other hand, in the case of mircene, it only occurred in the case of Topaz musts subjected to fermentation carried out spontaneously or with *S. bayanus*.

## CONCLUSIONS

PCA with various packages in R contributed to understanding of terpene transformations and to assess how different types of fermentation and apple cultivars affected this profile. Various microbial enzymes compete for the same substrates in terpene transformations. Some of terpenoids probably are formed during fermentation with specific yeasts, e.g. guaiacol is present when *S. cerevisiae* is used for fermentation of apple must. PCA analysis indicated that there were groups of terpenes which concentrations increased simultaneously, while for some other groups the content of particular compounds was negatively correlated. Example is linalool which converts (isomerises) into geraniol - negative correlation between those compounds. Some of terpenoids can transform into one another e.g. linalool might transform into ionones, methyleugenol might formed from eugenol during methylation process. In the current paper, based on our previous research, we decided to investigate how different microorganisms used for fermentation may influence profiles of terpenoids. The role of *S. bayanus* in those processes remains unclear and it requires further investigation.

**Declarations of interest**: none.

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## LITERATURE


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