INFLUENCE OF DIFFERENT YEAST STRAINS ON THE PRODUCTION OF VOLATILE COMPOUNDS IN FERMENTED APPLE JUICE

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Abstract

Aroma forming volatiles are important components of fermented beverages. The aim of current research is to evaluate the influence of different yeast strains on the volatile compounds of fermented apple juice of the variety 'Lietuvas Pepins'. Apples were harvested in the Latvia State Institute of Fruit Growing. Apple variety 'Lietuvas Pepins' juice was fermented with four different commercial yeast strains - *Saccharomyces bayanis* yeasts 'EC-1118', 'Cider yeast', *Saccharomyces cerevisiae* yeasts '71B-1122' and 'K1V-1116'. Fermentation was performed in laboratories of Latvia University of Agriculture, Faculty of Food Technology. Volatile aroma compounds of apple juice, yeasts and fermented juice were determined. Extraction of aroma compounds was performed using solid phase microextraction (DVB/Car/PDMS fibre). Analysis of volatile aroma compounds was made using a Perkin Elmer Clarus 500 GC/MS. The data obtained in the present study shows the influence of the yeast strain on the final chemical and volatile composition. The main group of volatiles in juice was esters, whereas in fermented juices – alcohols. The highest percentage of esters was determined in juice fermented with 'Cider yeast' whereas the highest percentages of alcohols – in juice fermented with yeast K1V-1116 and also free terpenes, associated with the floral note. The 71B-1122 strain produced the highest amount of identified volatile compounds. The strains potential ability to utilize and transform numerous apple must precursors.

Key words: apple juice, yeast strains, fermentation, volatile compounds.

Introduction

Fermented apple juice generally is regarded ascider. The quality of fermented drinks like cider is characteristic from presence of aroma compounds in product (Mangas et al., 1996), that are influenced by several factors, namely apple variety, yeast strains, fermentation conditions, the production process and fining treatments-(Hidalgo et al., 2004; Martinez-Rodriguez and Polo, 2003; Beech, 1993). Not all ciders are made from 'true' cider apples. Many modern ciders have a high proportion of dessert and culinary apple varieties (Lea, 1995). In this work, special attention is drawn to the use of culinary apples for cider production. The apple variety 'Lietuvas Pepins is one of the major commercially grown varieties in Latvia and is classified as autumn, early winter apple variety (stored until February), typically used for juice and wine production.

Cider flavour is composed by a wide range of compounds with different aromatic properties. Moreover, the main cider aroma holds a close relationship with the type and concentration of aromatic compounds derived from apples (varietal flavour), other compounds are produced by yeasts and bacteria during alcoholic and malolactic fermentation (fermentative flavour) and compounds that appear during the ageing process (post-fermentative flavour) (Rapp, 1987; Boulton et al., 1995) and it consists mainly of esters, higher alcohols, fatty acids, aldehydes, ketones, terpenes, lactones (Leguerinel et al., 1988; Leguerinel et al., 1989). The fermentation of apple must is a complex microbial reaction involving the sequential development of various strains of yeasts and bacteria (Duenas et al., 1994). Among these micro-organisms, yeasts are primarily

responsible for alcoholic fermentation. The different yeast species developed during fermentation and their dynamics and frequency of appearance determine the taste and flavour characteristics of products (Cabranes et al., 1997; Beech and Davenport, 1970). Ethanol and glycerol are quantitatively the dominating alcohols, followed by higher alcohols and esters. The main ester produced during alcoholic fermentation is ethyl acetate, but other esters of fusel alcohols and medium chain fatty acids also appear. Two main species, Saccharomyces cerevisiae and Saccharomyces bayanus, are currently recognized among wine yeasts (Masneuf-Pomarède et al., 2007; Naumov et al., 2000). Different strains of Saccharomyces cerevisiae can produce significantly different flavour profiles when fermenting the same must. This is a consequence of both, the differential ability of wine yeast strains in releasing varied volatile compounds from grape precursors, as well as the differential capacity to synthesise new yeast-derived volatile compounds (Swiegers et al., 2006; Ugliano et al., 2006; Vilanova and Sieiro, 2006; Wondra and Boveric, 2001). Saccharomyces cerevisiae is the main species of yeast in wine making although many other yeasts may be present at the beginning of fermentation (Heard and Fleet, 1986).

Apples are the most cultivated fruit in Latvia, and fermented beverage production from culinary apples could be perspective. The aim of current research is to evaluate the influence of different yeast strains on the volatile compounds of fermented apple juice of the variety 'Lietuvas Pepins'.

Materials and Methods

Raw materials

For analysis apples of the variety 'Lietuvas Pepins' ('LP') grown in the Latvia State Institute of Fruit Growing and harvested in October 2010 were used. Apple quality parameters were as follows: Streif index 0.18, starch index 8.6. Juice was obtained by the press Voran Basket Press 60K (voran Maschinen GmbH, Austria) and yield was $0.652 \pm 0.004 \text{ L kg}^{-1}$. For conservation of juice Tannisol (Enartis, Italy) was added (concentration 10 g L⁻¹).

Tannisol capsules consist of potassium metabisulphite (E 224) (9.5 g L⁻¹), ascorbic acid (0.3 g L⁻¹) and tannin (0.2 g L⁻¹). Sulphites have various permitted uses, their primary function is as a preservative or antioxidant to prevent or reduce spoilage (Fazio and Warner, 1990) and , they help to stabilize product colour and inhibit discolouration, thereby improving the appearance and flavour of many foods during preparation, storage and distribution (Adams, 1997). Quality parameters of the obtained apple juice are given in Table 1.

Table 1

Table 2

Parameters	Amount in juice		
pH	3.22 ± 0.01		
Titratable acidity, g L ⁻¹	8.31±0.12		
Soluble solids, Brix %	10.70±0.70		
Total sugars, g L ⁻¹	100.12±2.85		

Quality parameters of 'Lietuvas Pepins' apple juice

Fermentation conditions

Fermentation was performed using four commercial yeasts - *Saccharomyces bayanis* yeast EC-1118 (Lalvin, Canada), *Saccharomyces bayanis* 'Cider yeast' (Youngs Home Brew., UK), *Saccharomyces cerevisiae* yeast 71B-1122 (Lalvin, Canada) and *Saccharomyces cerevisiae* yeast K1V-1116 (Lalvin, Canada). The EC-1118 strain is recommended for all types of wines, including sparkling, and cider. 'Cider yeast' is especially selected for its ability to produce exceptional crisp and refreshing ciders. 71B-1122 abilities are to produce isoamil acetate, reinforcing the aromatic profile of wine. According to the manufacturer,

K1V-1116 under the right conditions of fermentations is one of the most floral ester (isoamyl acetate, hexyl acetate, phenyl ethyl acetate) producing yeasts. These esters bring fresh, floral aromas to neutral varieties or high yield grapes. Fermentation was carried out at 16 ± 1 °C for 28 days. The apple juice was fermented in a glass bottles (n=5) in the laboratories of Latvia University of Agriculture, Faculty of Food Technology. For analysis the average juice samples were combined from 5 bottles in equal proportions. All analysis of fermented drinks was performed immediately after 28 day fermentation. Chemical parameters of final fermented juices are presented in Table 2.

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Fermentatio	n conditions	and terme	entea juice	composition	

Yeast variety Abbreviation		Titratable acidity, g L-1	Soluble solids, g L ⁻¹	Alcohol, % vol.
EC-1118	SB1	9.40±0.04	7.74±0.21	5.42±0.05
Cider yeast	SB2	8.40±0.08	6.42±0.30	5.60±0.06
71B-1122 SC3		8.02±0.06	5.16±0.14	5.91±0.03
K1V-1116	SC4	8.32±0.08	15.43±0.27	4.30±0.08

Determination of volatile aroma compounds

Volatiles from apple juice, activated yeasts and fermented drinks were extracted using solid phase microextraction (SPME). 5 g of sample were weighed in a 20 mL headspace vial and capped with a septum.

Adivinylbenzene/carboxen/polydimethylsiloxane (DVB /Car/PDMS) fiber (Supelco Inc., Bellefonte, PA, USA) was used for headspace SPME sampling. SPME parameters were: incubation time 30 min, extraction temperature 22 ± 2 °C, extraction duration 30 min, desorption 15 min, 250 °C. For the analysis of the SPME extracts, a Perkin Elmer Clarus 500 GC/MS and an Elite-Wax ETR (60 m × 0.25 mm i.d.; DF 0.25 µm) were used. Working conditions were the following: an injector 250 °C; transfer line to MSD 260 °C; oven temperature start 50 °C, hold

2 min, programmed from 50 to 100 °C at 5 °C min⁻¹ hold 5 min, and from 100 to 210 °C at 5 °C min⁻¹, hold 15 min; carrier gas (He) 1 mL min⁻¹; split ratio 2:1; ionization EI+; acquisition parameters in full scan mode: scanned m/z 50-300. Compounds were identified by comparison of their mass spectra with mass spectral libraries (Nist98), and by calculation of linear retention indexes and comparison with literature data. All analyses were performed in triplicate. As a quantitative measure, the share in the total GC peak area for each compound is given.

Statistical analysis

The differences in the volatile profiles during fermentation were analyzed using the analysis of variance (ANOVA) procedure of SPSS, Version 17.0. HSD Tukey's test was applied to compare the mean values of the volatile

compounds of different fermentation conditions. P-value at 0.05 was used to determine the significant differences in content of volatiles in fermented juice samples. Mean values with standard deviations are reported.

Results and Discussions

Six volatile compounds with total peak area 4490.35×10^5 AU were detected in 'Lietuvas Pepins' apple

juice (Tables 3). Each group of volatile compounds gives a typical odour characteristic to the apple juice. The main identified compound classes were esters forming 85%(total peak AU 3613.62×10^5) of total volatile compounds and alcohols 19.5% (total peak AU 876.73×10^5) (Fig. 1). Butyl acetate, hexyl acetate and 2-methylbutyl acetate were identified as characteristic volatile compounds of 'Lietuvas Pepins' apple juice (Table 3).

Table 3

Volatile aroma compounds (AU ×10⁵± standard deviation) as measured by SPME-GC-MS in 'Lietuvas Pepins' apple juice and fermented juice

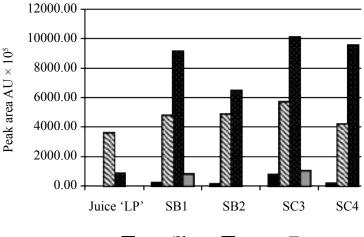
Compound	Juice 'Lietuvas Pepins'	SB1	SB2	SC3	SC4	
Acids						
acetic acid	n.d.	55.61 ±4.73ª	74.01±4.73 ^b	780.12±8.23 ^d	92.15±7.35°	
octanoic acid	n.d.	104.47±7.97	90.10±7.82	n.d.	49.64±2.91	
hexanoic acid	n.d.	64.26±4.23	n.d.	n.d.	70.34±3.62	
% from total volatile compounds	n.d	1.5	1.4	4.4	1.5	
Esters						
ethyl acetate	n.d.	161.55±1182 ^{ab}	112.24±7.08 ^{ab}	110.18±95.45ª	240.38±18.55b	
3-methylbutyl acetate	n.d.	314.32±744 ^a	312.02±20.80ª	659.24±13.44°	486.43±22.44 ^b	
2-methylbutyl acetate	701.46±61.02	739.33±3852 ^b	798.23±60.73 ^b	1573.93±5.56°	196.37±3.73ª	
butyl acetate	1586.78±55.75	n.d.	n.d.	n.d.	n.d.	
hexyl acetate	1086.51±94.12	1080.21±19.86ª	1136.93±77.48ª	1329.34±5.48 ^b	1495.67±107.77 ^b	
2,2-dimethylpropyl butyrate	238.87±23.16	n.d	n.d.	n.d.	n.d.	
octanoic acid ethyl ester	n.d.	1107.61±19.15 ^b	1079.94±79.76 ^b	679.68±12.53ª	713.46±56.44ª	
decanoic acid ethyl ester	n.d.	185.54±2.08°	189.36±5.54°	149.76±8.98 ^b	84.99±6.06ª	
hexanoic acid hexyl ester	n.d.	1201.95±31.61 ^b	1260.47±110.40 ^b	1197.10±11.24 ^b	972.32±32.62ª	
% from total volatile compounds	n.d	31.9	42.4	32.3	30.1	
Alcohols						
hex-2-en-1-ol	368.55±7.61	n.d.	n.d.	n.d.	n.d.	
2-hydroxy ethylhydrazine	n.d.	5694.87±145.19 ^b	3561.01±238.47ª	6512.58±9.08°	5107.14±396.29 ^b	
hexan-1-ol	508.18±29.14	546.39±9.87 ^b	453.02±36.95ª	613.81±14.55°	582.70±22.62 ^b	
3-methylbutan-1-ol	n.d.	1616.17±51.02ª	1262.10±90.17 ^a	1744.71±10.06b	2665.78±71.31°	
heptadecan-8-ol	n.d.	1057.92±13.29 ^b	990.62±96.55 ^{ab}	850.65±6.65ª	883.02±79.33ª	
phenylethyl alcohol	n.d.	233.85±11.04ª	221.67±11.46	385.08±14.55°	303.95±9.14 ^b	
% from total volatile compounds	n.d	61.0	56.2	57.3	68.4	
Terpenes						
limonene	n.d.	662.87±53.23	n.d.	1054.76±14.77	n.d.	
γ-terpinene	n.d.	180.46±14.59	n.d.	n.d.	n.d.	
% from total volatile compounds	n.d	5.6	n.d	6.0	n.d	

n.d. - not detected

Different letters in the same row indicate statistically significant differences (Tukey's test, p<0.05).

The esters and alcohols which are the products of fatty acid metabolism were the major groups in the apple juice, respectively esters, associated with 'fruity' attributes of fruit flavour, accounted up to 80 - 98% (Lopez et al., 1998).

Hex-2-en-1-ol and hexan-1-ol found in 'Lietuvas Pepins' apple juices were also reported in apples 'Pink Lady' as one of the characteristic aroma compounds (Elss et al., 2006). In literature, butanol was found as the most abundant alcohol in the apple juice (Karlsen et al., 1999), but it was not identified in 'Lietuvas Pepins' juice. Also 3- methylbutan-1-ol was not identified in 'Lietuvas pepins' apple juice, but other authors (Nikfardjam and Maier, 2011) reported that it plays the major role in the apple juice quality. This alcohol is not genuine constituent of the apple fruit, but it is inevitably formed during apple juice production, probably through transamination and decarboxylation of the amino acids leucine and isoleucine (Hey et al., 2007).



Acids Esters Alcohols Terpenes

Figure 1. Changes in the peak area (AU \times 10⁵) of the main volatile compound chemical groups in apple 'Lietuvas Pepins' juice and fermented juice.

Yeast metabolism makes an important contribution to the flavour of the fermented juices. In addition to the reduction of sugars (glucose and fructose) to ethanol and carbon dioxide during alcoholic fermentation, the use of wine yeasts produces a number of intermediate products like acetaldehyde and several organic acids. Ten different volatile compounds were detected in yeasts representing two main volatile compound classes - alcohols 69.3 -97.3% and acids 2.5 - 29.4% (Table 4). Additionally, ketone 3-hydroxybuta -2-none were identified in the headspace of yeasts K1V-1116 and ester pentafluoropropionic acid hexyl ester in yeasts K1V-1116 and 71B-1122.

A total of 17 volatile compounds were detected in fermented juice of apples 'Lietuvas Pepins' (Table 3). Three sources of aroma compounds of fermented juices apple juice, yeast, and yeast metabolites - were analysed. It was not possible to identify such esters as butyl acetate, 2,2-dimethylpropyl butyrate and alcohol hex-2-en-1-ol found in apple juice and in fermented apple juices. In all fermented samples it is possible to identify 3 compounds previously detected in apples, namely, 2-methylbutyl acetate, hexyl acetate and hexan-1-ol. In the headspace of fermented drinks four yeast volatile compounds were identified: acetic acid, 2-hydroxyethylhydrazine, phenylethyl alcohol and 3-methylbutyl acetate. Literature data showed that different strains produce 3-methyl-1butanol and it is the major higher alcohol in wine (Romano et al., 2008; Garde-Cerdan and Anczn-Azpilicueta, 2007)

The main groups of compounds formed during the fermentation are alcohols and esters. Literature data showed that the main groups of compounds that form the fermentation bouquet are the acids, alcohols and esters and, to a lesser extent, aldehydes and ketones (Lambrechts and Pretorius, 2000). In analysed fermented juice samples ketones and aldehydes were not identified. Alcohols were the most abundant group in the tested fermented juice and represented more than 56 - 68% of total volatile compounds. Main alcohols were 2-hydroxyethylhydrazine, hexan-1-ol and phenylethyl alcohol. Juices inoculated with S.cerevisiae show higher content of alcohols, and the same tendency was observed for wines (Mateo et al., 2001). In fermented products, esters can be classified into two groups, those formed enzymatically and those formed during ageing, by chemical esterification between alcohol and acids at low pH (Margalit, 1997). Esters represented more 30 - 42% of aroma compounds. The main esters found in fermented samples are hexyl acetate, ethyl octanoate, hexyl hexanoate, 2-methylbutyl acetate. The C_4 - C_{10} ethyl esters of organic acids, ethyl esters of straight chain fatty acids and acetates of higher alcohols are largely responsible for the fruity aroma of wine (Ebeler, 2001). The fatty acids ethyl esters (ethyl hexanoate, octanoate and decanoate) and fusel alcohols are also important aroma compounds in white wines (Mateo et al., 2001). Ethyl acetate that intensifies the

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acetified cider perciption (Campo et al., 2008) was identified in all fermented juice samples, with the highest peak area in the sample SC4. Three acids - acetic acid, octanoic acid and hexanoic acid- were detected in fermented samples. Production of acetic acid is frequently accompanied by a greater production of ethyl acetate (Campo et al., 2008), but in our experiment such a tendency was not observed. Hexanoic acid was found in two samples (SB1 and SC4) in small concentrations. Synthesis of this compound mainly is associated with spontaneous fermentation, and its concentration decreases when concentration of inoculated yeast increases (Mateo et al., 2001).

Table 4

Volatile aroma compounds (AU ×10⁵± standard deviation) as measured by SPME-GC-MS in yeasts

Compound	EC-1118	Cider yeast	71B-1122	K1V-1116
Acids			•	
acetic acid	151.71±1.78	61.94±4.03	10.86±0.86	80.04±5.27
2-methylpropanoic acid	51.63±1.39	38.77±2.82	5.63±0.23	90.29±6.03
4-methylpentanoic acid	49.46±0.78	42.60±2.54	3.61±0.01	116.75±6.09
butanoic acid	n.d.	5.35 ± 0.40	n.d.	8.79±0.40
% from total volatile compounds	27.4	17.9	2.5	29.4
Esters				
pentafluoropropionic acid hexyl ester	n.d.	n.d.	1.33±0.13	6.29±0.60
% from total volatile compounds	n.d.	n.d.	0.2	0.6
Ketones				
3-hydroxybuta-2-none	n.d.	n.d.	n.d.	6.37±0.25
% from total volatile compounds	n.d	n.d	n.d	0.6
Alcohols				
2-hydroxyethylhydrazine/ 4-penten-2-ol	138.75±2.32	229.49±0.73	259.05±10.92	285.96±8.96
2-methylpropan-1-ol	41.12±0.56	22.77±0.07	46.80±0.45	27.33±0.21
2-ethylhexan-1-ol	50.70±1.28	n.d.	n.d.	n.d.
3-methylbutan-1-ol	381.51±11.35	411.26±4.81	445.03±3.76	335.58±16.61
phenylethyl alcohol	58.73±0.72	19.03±1.78	32.70±3.08	47.82±4.28
% from total volatile compounds	72.6	82.1	97.3	69.3

n.d. - not detected

In general, peak area of total and individual compounds in fermented juices were influenced by used yeast strain, and it is not possible to distinguish differences between *S. cerevisiae* and *S.bayanus* yeasts. In wine *S. cerevisiae* strain represents one of the primary parameters affecting wine fermentative volatile composition, the strain-specific aromatic potentiality becomes a selective tool in the choice of the starter that will drive the alcoholic fermentation (Mauriello et al., 2009).

Conclusions

The data obtained in the present study showed the effect of yeast strain on the final chemical and volatile composition. Yeast strains represent one of the primary parameters affecting wine fermentative volatile composition; the strain-specific aromatic potentiality becomes a selective tool in the choice of the starter that will drive the alcoholic fermentation. The main group of volatiles in juice was esters, whereas in fermented juice – alcohols. The highest percentage of esters was determined in juice fermented with 'Cider yeast' whereas the highest percentages of alcohols in juice fermented with yeast K1V-1116. The 71B-1122 strain produced the highest amount of identified volatile compounds and also free terpenes, associated with the floral note. The 71B-1122 strain also produced the highest amounts of alcohols, acetates and esters. The strains potentially producing a higher number of volatile compounds could contribute to a more complex aroma of the final product, due to their potential capability to utilize and transform numerous apple must precursors.

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