

## INFLUENCE OF *OENOCOCCUS OENI* AND OAK CHIPS ON THE CHEMICAL COMPOSITION AND SENSORY PROPERTIES OF CIDER

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### Abstract

Cider quality is influenced by several factors, namely, apple variety, yeast strains, fermentation and maturation conditions. The aim of current research was to evaluate influence of lactic acid bacteria *Oenococcus oeni* and oak chips on the quality of apple cider. After main fermentation lactic acid bacteria *Oenococcus oeni* (LAB) and two types of oak chips: unroasted oak chips (U\_OC) and medium roasted oak chips (MR\_OC) were added to cider and samples were matured for four weeks. For matured ciders total phenolic compounds (TPC) were determined spectrophotometrically, individual phenolic compounds by HPLC, volatile compounds by SPME followed by GC/MS and sensory properties using line scale and ranking test. The TPC in ciders ranged from 1028 mg L<sup>-1</sup> in cider LA to 1526 mg L<sup>-1</sup> in cider MR\_OC. Among analysed phenolic compounds chlorogenic acid dominated in all samples. In cider MR\_OC comparing with control sample higher content of caffeic acid, epicatechin, ferulic acid and vanillin was determined. The highest total peak area was determined in cider U\_OC. Principal component analysis showed that profile of volatile compounds can be explained by three factors; the first two represent 81% of the total variances. The characteristic volatiles of cider LAB were acetic acid, ethyl-9-decanoate, etildecanoate, octanoic acid, and ethylhexanoate, in cider MR\_OC 3-methyl-1-butanol acetate, butyl acetate and ethyl acetate, whereas in cider U\_OC 3-methyl-1-butanol and phenylalcohol. Preference ranking test results showed that the assessors preferred cider LAB. Addition of *Oenococcus oeni*, unroasted and roasted chips during maturation significantly influence chemical composition and sensory properties intensity of cider.

**Keywords:** cider, maturation, *Oenococcus oeni*, oak chips

### Introduction

The quality of fermented drinks like cider depends on presence of aroma compounds in product, 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, Polo, 2003). Quality of ciders could be improved during maturation process by malolactic fermentation and maturation on oak chips. Malolactic fermentation can be considered as the part of maturation process (Buglass, 2011). *Oenococcus oeni* are the main bacteria responsible for malolactic fermentation (Fugelsang, 1997) converting L-malic acid to L-lactic acid and CO<sub>2</sub>. Bacteria's can adapt to rough environment of wines with high alcohol content, low pH and presence of sulphur dioxide (Lonvaud-Funel, 1999). In cider production malolactic fermentation is significant, and usually it starts after alcoholic fermentation. Exception is Asturian ciders because there both fermentation processes occurs simultaneously (Blanco Gomis et al., 2003) and efficiency depends on temperature and nutrients. In ciders from unpasteurized and unsulphured juices, can develop wild lactic acid bacteria (Lea, Drilleau, 2003). Winemakers assess the benefits of controlled malolactic fermentation (Krieger-Weber, 2009). Inoculation of starter, that mainly contains *O. oeni* bacteria, winemakers can reduce risks related to potential bacterial or bacteriophage spoiling, and accelerate beginning of malolactic fermentation and harmonize wine taste and fullness.

In traditional winemaking fermentation and/or maturation occurs in oak barrels and it influence wine quality positively. Wine is fortified with substances extracted from oak, forming more complex aroma and

taste of wines (Návojská et al., 2012). Oak barrels are expensive, takes up much area, and also it is more difficult to clean them. Based on these statements alternative methods for improvement of wine quality (Rodríguez-Rodríguez et al., 2011; Návojská et al., 2012) are developed, for example, use of wood chips. In England small and medium cider producers still use oak barrels and use of chips are not so popular comparing to wine industry (Buglass, 2011). Oak chips are obtained from wood processing byproducts, by using traditional methods of treatment – boiling and roasting (Bozalongo et al., 2007). Fan et al. (2011) investigations showed influence of different type oak chips (different raw materials, roasting degree) to the volatiles in cider. Oak typical aroma is influenced by wine and wood contact duration, temperatures, wood properties, for example, species, geographic origin, roasting degree (Garde-Cerdán, Ancin-Azpilicueta, 2006; Garde-Cerdán, 2010; Návojská et al., 2012). Several compounds are typical for unroasted oak (*cis* oak lactones) and others as vanillin, 4-methylguaiacol and furfural, mainly are formed from oak polymers that degrades and hydrolyses during storage (Hale et al., 1999). High roasting temperature influence degradation of wood polymers as lignins and cellulose, forming aldehydes, phenols, furfural derivatives, lactones (Nonier et al., 2006). In experiments about oak influence to the aroma of wine, positive correlation between concentration of vanillin and smoke and cinnamon aroma were determined (Spillman et al., 1998). Content of total phenolic compounds increases by increasing roasting intensity (Cabrita et al., 2011). Development and improvements of technology should be designed taking into account sensory evaluation. In order to match the instrumental analysis with the consumer requirements, a sensorial profile of the cider

is necessary. Sensory quality is related to consumer acceptance and confidence in the product, being defined by the interaction between the food and the consumer. Thus, sensory quality depends on both the sensory characteristics of the food and how consumers perceive them (Cardello, 1995; Costell, 2002; Ares et al., 2009). Sensory descriptive analysis is a primary tool of food scientists, which involves the evaluation of both the qualitative and quantitative sensory characteristics of products (Meilgaard et al., 1999). The aim of current research was to evaluate influence of lactic acid bacteria *Oenococcus oeni* and oak chips on the quality of apple cider.

## Materials and Methods

Experiments were carried out at the Faculty of Food Technology, Latvia University of Agriculture in 2012.

### Materials

Apples grown at the Latvian State Institute of Fruit Growing were used in the experiments. Apples were harvested and stored for 1–2 weeks at  $+3\pm 1$  °C with relative ambient humidity of 90–95%. In the present study juices of three varieties ‘Auksis’, ‘Lietuvas Pepiņš’, ‘Kerr’ in proportion 2 : 1 : 2 (by volume) were used. Fermentation was performed using the commercial *Saccharomyces cerevisiae* yeast strain ‘71B-1122’ (Lalvin, Lallemand Inc., Canada). Fermentation was carried out at  $16\pm 1$  °C for 28 days.

In order to reduce the sharp acidity of cider (malic acid) lactic acid bacteria (*Bacillus Malolactic Bacteria Culture Oenococcus oeni*) (Lalvin, France) was added to the cider at a concentration of  $0.05\text{ g L}^{-1}$  ( $7.3\times 10^5\text{ cells L}^{-1}$ ).

In order to improve the taste and aroma of cider, two types of oak chips were added:

- unroasted oak chips ‘French Oak Chips’ (Young’s Brew, UK);
- medium roasted oak chips ‘American oak chips’ (Browland, Belgium).

Oak chips were added in concentration  $1.5\text{ g L}^{-1}$  of cider.

All cider samples were matured at  $+16\pm 1$  °C for four weeks.

In experiment the following samples were analysed:

- control – control sample stored without additives;
- LAB – sample matured with addition of *Oenococcus oeni*;
- U\_OC – sample matured with unroasted oak chips;
- MR\_OC – sample matured with medium roasted chips.

### Determination of total acids, solids and alcohol content

Determination of titratable acidity (expressed as total acids) was performed according to method LVS EN 12147:2001 and expressed in  $\text{g L}^{-1}$ . Alcohol content was determined by volume % (ГОСТ 12787–81) and solids were determined after removal of alcohol gravimetrically.

### Determination of total phenolic content

The total phenolic concentration was determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Singleton, 1999). Cider was diluted with ethanol/acetic acid solution (1:20 v/v). The ethanol/acetic acid solution was prepared using an acetic acid water solution (2.5%) and ethanol (98% vol.) in ratio the of 10:90 (v/v). To 0.5 mL of aliquot 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times with water) was added and, after 3 minutes 2 mL of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) ( $75\text{ g L}^{-1}$ ) was added. After 30 minutes of incubation at room temperature, the absorbance was measured at 765 nm using a JENWAY 6300 spectrophotometer (Baroworld Scientific Ltd., UK). Results were expressed as chlorogenic acid equivalents.

### Determination of individual polyphenols

The concentration of all individual polyphenols was determined by High-performance liquid chromatography (HPLC) Shimadzu LC-20AD Prominence with diode array detector (SPD-M20A). Separation was performed in a PerkinElmer C18 4.6 mm×250 mm column (thermostated at 27 °C). Eluting solvents are methanol (A, 20%), water (B, 78.4%) and acetic acid (1.6%) used in a gradient mode and at 17.50 minutes solvents ratio are as follows – A concentration 40.3%, B concentration 58.5%, C concentration of 1.2%. 10  $\mu\text{L}$  of the sample was injected in the chromatograph using automatic sample injection system SIL-20 AC. The total duration of the analysis was 35 minutes. For the detection and quantification of compounds, several wavelengths were used: 253 nm for 4-hydroxybenzoic acid and rutine, 263 nm for gallic acid, 278 nm for catechin, caffeic acid, syringic acid, 298 nm for chlorogenic acid, epicatechin, coumaric acid, sinapic acid and ferulic acid.

### Determination of volatile compounds

Volatiles from ciders were extracted using solid phase microextraction (SPME). 5 g of cider were weighed in a 20 mL headspace vial and capped with a septum. A divinylbenzene/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  $22\pm 2$  °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 a Elite-Wax ETR (60 m×0.25 mm i.d.; DF 0.25  $\mu\text{m}$ ) was used. Working conditions were: injector 250 °C; transfer line to MSD 260 °C; oven temperature start 50 °C, hold 2 min, programmed from 50 to 100 °C at  $5\text{ }^\circ\text{C min}^{-1}$  hold 5 min, and from 100 to 210 °C at  $5\text{ }^\circ\text{C min}^{-1}$ , hold 15 min; carrier gas (He)  $1\text{ mL min}^{-1}$ ; 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.

#### Sensory analysis

Sensory evaluation of the ciders was carried out with trained panellists (33 women and 3 men, aged 21–71). The panellists had studied the basics of sensory evaluation methods and were experienced in sensory panels. This group included students and staff of the Latvia University of Agriculture Faculty of Food Technology. Line scale (ISO 4121:2003) for determination of the intensity of sensory properties (clarity, apple, fruit and yeast aroma, apple, yeast, sour, astringent and bitter taste) was used. Ranking test (ISO 8587:2006) was used to rank samples according to their degree of liking.

#### Statistical analysis

The analysis of variance was performed by the ANOVA procedure and  $p < 0.05$  was considered as statistically significant. Principal component analysis was performed with the software Multibase 2014 for Windows.

### Results and Discussion

Sensory properties of cider can be improved by the maturation process using different technologies. Quality parameters of matured ciders are presented in Table 1.

Table 1

Quality parameters of matured ciders

Sample	Total acids, g L <sup>-1</sup>	Solids, g L <sup>-1</sup>	Alcohol, vol %
Control	7.85±0.31 <sup>c*</sup>	0.87±0.06 <sup>b</sup>	5.58±0.23 <sup>ab</sup>
LAB	3.99±0.21 <sup>a</sup>	0.65±0.08 <sup>a</sup>	5.60±0.21 <sup>b</sup>
U_OC	7.16±0.25 <sup>b</sup>	0.85±0.05 <sup>b</sup>	5.52±0.25 <sup>a</sup>
MR_OC	6.97±0.19 <sup>b</sup>	0.84±0.06 <sup>b</sup>	5.54±0.20 <sup>ab</sup>

\* The different letters in the same column represents significant differences between values ( $p < 0.05$ ).

In cider LAB significantly ( $p < 0.05$ ) lower total acid content and higher pH was detected. Also dry matter in this sample is significantly lower than in all other samples. The alcohol content and pH are important factors affecting the growth of lactic acid bacteria and their activity. Solier et al. (2010) study shows that the low pH of the wine significantly ( $p < 0.05$ ) negative effects malolactic fermentation. Gockowiak and Henschke (2003) studies have shown that the pH from 2.9 to 3.5 have a negative impact on the viability of bacteria, but at pH 3.5 malolactic fermentation takes place successfully, regardless of the alcohol content. But the range of pH from 3.0 to 3.2 together with an alcohol content of 78.9 and 102.6 g L<sup>-1</sup> inhibited the lactic acid bacteria (Solier et al., 2010). Addition of oak chips to maturing process significantly ( $p < 0.05$ ) influenced only the content of total acids.

The total phenolic content in the analyzed ciders varied from 1028 mg L<sup>-1</sup> in the sample LAB to 1526 mg L<sup>-1</sup> in

the sample MR\_OC; the total phenolic content in the samples matured with oak chip was significantly ( $p < 0.05$ ) higher (Fig. 1.).

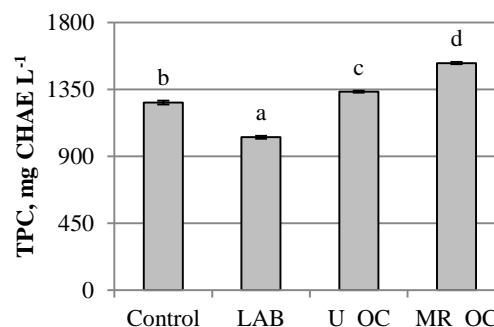


Figure 1. TPC of matured ciders

Chlorogenic acid was the most important identified phenolic compound in all samples of ciders (Tab. 2). The highest content of chlorogenic acid was identified in the control sample, but the lowest content was identified in the sample LAB. Cabrita et al. (2008) in the research on wines revealed that the content of gallic acid, ferulic acid and caffeic acid increased after malolactic fermentation, but vanillin and syringic acid remained stable. The research of Figueiredo-González et al. (2014) showed that the content of catechine and epicatechine decreased during wine maturing in oak barrels. The sample MR\_OC had significantly higher content of caffeic acid, epicatechine, ferulic acid and vanillin. At high temperature during roasting process lignin and cellulose polymers contained in oak chips degradates forming aldehydes, phenols, furfural derivatives, lactones and other compounds (Nonier et al., 2006). Similarly, Bozalongo et al. (2007) found that oak roasting increased the content of compounds created by lignin thermal degradation (vanillin, eugenol etc.).

The highest content of 4-hydroxybenzoic acid was identified in the sample U\_OC that corresponded to the findings of Cadahía et al. (2009) where the content of 4-hydroxybenzoic acid and its derivatives increased when wines were matured with French oak tree chips.

In analysed samples 22 volatiles were identified. The highest total peak area for sample U\_OC and the lowest peak are for control sample were detected. Main volatile compounds are alcohols forming 55.5% (LAB) to 69.1% (U\_OC) and esters forming 25.5% (control) to 39.1% (LAB) from total peak area of identified compounds.

The analysis of variance showed a significant difference ( $p < 0.05$ ) in all sensory properties intensity except for the intensity of fruit aroma. The addition of lactic acid bacteria caused less distinct clarity in matured ciders with the most intensive apple aroma and taste, but with the least astringent, yeast and sour taste intensity.

The cider samples matured with unroasted and medium-roasted oak chips showed higher bitter taste intensity and lower sour taste intensity (Figure 2).

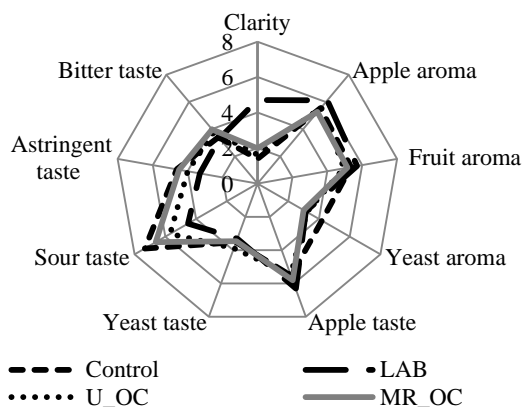
Table 2

**Individual phenolic compounds in ciders (mg L<sup>-1</sup>)**

Compounds	Control	LAB	U_OC	MR_OC
Chlorogenic acid	191.25±9.56bd	167.08±7.96a	190.69±9.08bd	188.41±6.28b
Caffeic acid	6.10±0.29 b	4.55±0.23 a	6.96±0.33 b	23.83±0.88c
Syringic acid	7.03±0.25 b	6.38±0.24 a	8.20±0.30c	6.20±0.31 a
Gallic acid	0.10 ±0.01	n.i.	0.60±0.02	0.38±0.01
Vanillin	0.44±0.02 a	0.82±0.03 b	0.86±0.04 b	1.61±0.07 c
Hydroxybenzoic acid	0.30±0.02 b	0.17±0.01 a	0.86±0.03d	0.35±0.01 c
Catechin	2.82±0.12 d	3.26±0.13 c	0.78±0.03 a	1.18±0.06 b
Epicatechine	0.27±0.01d	0.08±0.01 b	0.05±0.01 a	3.16±0.14 c
Sinapic acid	0.11±0.01	0.31±0.01	n.i.	2.21±0.08
Ferulic acid	0.63±0.02 a	1.33±0.06 d	0.72±0.04 b	3.67±0.17 c

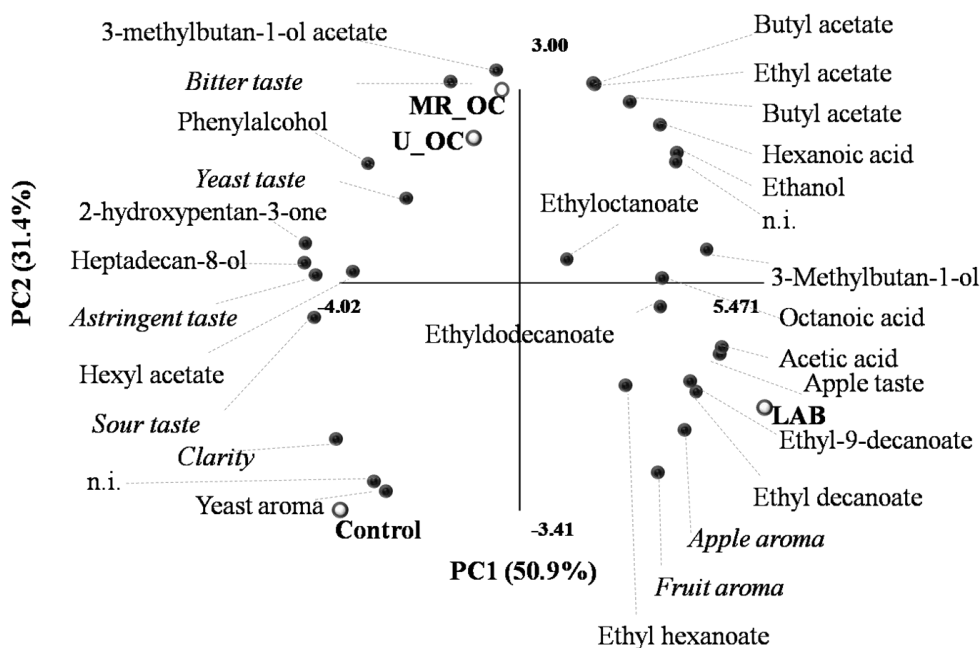
n.i. – not identified

\* The different letters in the same row represents significant differences between values (p<0.05).

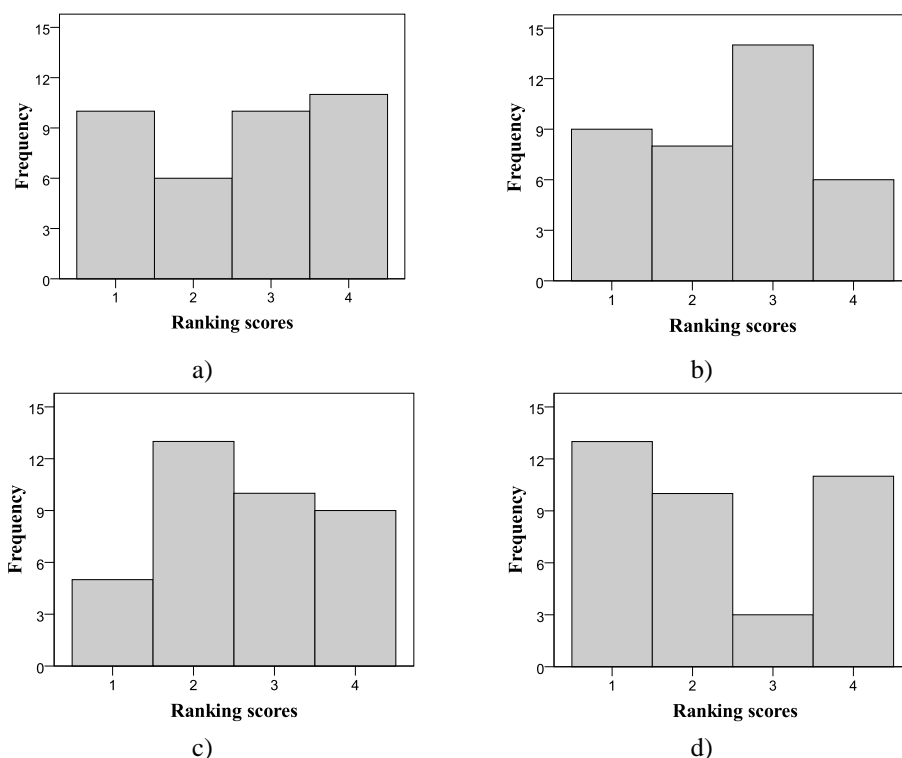


**Figure 2. Spider plot for sensory properties of matured ciders**

Principal component analysis of volatile compounds and sensory properties showed that differences can be explained by three factors: the first two of them accounted for 82% of the total variable set (Fig. 3). The results showed that different cider maturing technologies influenced the content of volatile compounds in ciders. The control sample was characterized by the most intensive yeast aroma. The LAB sample was characterized by the most intensive fruit, apple aroma and the most intensive apple taste. The dominating volatile substances in the LAB sample were acetic acid, ethyl-9-decanoate, ethyl decanoate, octanoic acid, ethyl hexanoate; on the whole sweet, oily, fruit (grape), flower aromas, dominated in this sample; less dominant aromas were stale, bitter, soap and wax.



**Figure 3. PCA of volatiles and sensory properties of matured ciders**



**Figure 4. Histograms of matured cider ranking**  
a) control, b) U\_OC, c) MR\_OC, d) LAB

The ranking test results are presented as histograms showing frequency of each ranking score (1 – the highest rank; 4 – the lowest rank) evaluated by panellists (Figure 4).

Preference ranking test results showed that the assessors preferred cider LAB.

### Conclusions

Addition of lactic acid bacteria *Oenococcus oeni* during cider maturing process changed ciders' chemical properties reducing the total content of acid, soluble solids, phenols, content of volatile compounds. Ciders matured with unroasted chips and medium-roasted chips have lower total content of acids and higher total phenolic content. Maturing with oak chips as well as addition of lactic acid bacteria influenced the ratio of volatile substances, i.e., content of esters and volatile acids increased, but content of alcohol decreased. Ciders matured by adding lactic acid bacteria *Oenococcus oeni* have the most intensive apple aroma and taste, the lowest astringent, yeast and sour taste intensity. The cider sample matured by unroasted and medium-roasted oak chips showed higher intensity of bitter taste and lower intensity of sour taste. The ranking test sensory results showed that the highest rank was awarded to the sample that had matured by adding lactic acid bacteria *Oenococcus oeni*.

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