

Aroma Composition and Polyphenol Content of Ciders Available in Latvian Market

Rita Riekstina-Dolge, Zanda Kruma, Daina Karklina

Abstract—Aroma forming volatiles are important components of fermented beverages. The aim of current research is to evaluate the volatile compounds and phenolic compounds of commercial ciders. Volatile aroma compounds and TPC of seven commercial ciders 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. Total phenol content (TPC) was determined according to the Folin-Ciocalteu spectrophotometric method and results were expressed as gallic acid equivalents. The highest volatile compounds were in apple ciders with pear flavor. The highest TPC and lower content of volatile compounds were detected in French ciders.

Keywords—cider, TPC, volatile compounds

I. INTRODUCTION

CIDER is fermented drink obtained from apple juice. Apple production is one of the largest fruit-growing sectors of Latvia. Most apples are sold fresh, but part of the apple, which does not meet the requirements of the market, has to be processed and fermented beverage production from apples could be good perspective.

Usually cider is produced using two different methods:

- 1) sparkling cider made from concentrated apple juice and/or fresh apple must, the addition of sugars and carbon dioxide being permitted, as well as the use of different stabilization processes;
- 2) "natural cider", is made according to traditional methods, which imply, among other practices (such as the prohibition of addition of sugars and CO₂), the exclusive use of juices obtained from the pressing of cider apples [1].

Different technology of the production affects the final quality of the drink. The quality of fermented drinks like cider is characterized with presence of aroma compounds in product [2], that are influenced by several factors, namely apple variety, yeast strains, fermentation conditions, the production process and fining treatments [3], [4], [5]. 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) [6], [7] and it consists mainly of esters, higher alcohols, fatty acids, aldehydes, ketones, terpenes, lactones

[8], [9]. The fermentation of apple must is a complex microbial reaction involving the sequential development of various strains of yeasts and bacteria [10]. Among these micro-organisms, yeasts are primarily responsible for alcoholic fermentation. 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. Content and composition of polyphenols present in apples are important because of their contribution to the sensory quality of fresh fruit and processed apple products. Apples are an excellent source of several phenolic compounds and also possess high total antioxidant capacity [11] found that apples had the highest soluble free phenolics when compared to 10 other commonly consumed fruits [12]. For ciders production polyphenols content and profile are an important quality indicator since they contribute related to the color, bitterness, and astringency, which provide the "overall mouth-feel" of cider [13], [14] some polyphenols such as hydroxycinnamic acids are precursors of volatile compounds that contribute to cider aroma [15]. The polyphenolic profile of apples and apple drinks is influenced by several factors: variety, climate, maturity, storage, processing [16], [17], [18]. The cidermaking steps mainly responsible for the extraction and content of the phenols in the final product are maceration, pressing, enzymatic clarification of the must prior to fermentation, centrifugation, filtration, and fining. The enzymatic clarification, centrifugation, filtration and fining of the French ciders lead to the partial elimination of procyanidins due to their ability to precipitate proteins and to interact with cell wall polysaccharides [14]. For developing cider production technology from Latvian apples, it is very important to investigate volatile compounds and TPC of commercially available ciders. The aim of this work was to identify the volatile compounds and total phenols of commercially ciders. The aim of this work was to evaluate the volatile compounds and total phenols of commercially ciders.

II. MATERIALS AND METHODS

A. Ciders

Seven commercially available ciders originating from different countries and from five companies were used. Four apple ciders and three apple ciders with pear aroma were used. A list of all ciders analyzed in this study is presented in Table 1.

B. Determination of total polyphenolic contents by the Folin-Ciocalteu method

The total polyphenolic concentration was determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method [19]. Cider was diluted with ethanol/acetic acid solution (1:20 v/v). Ethanol/acetic acid solution was prepared using acetic acid water solution (2.5%) and ethanol (98% vol.) in ratio 10:90(v/v). 0.5 ml of aliquot was mixed

Rita Riekstina-Dolge Latvia University of Agriculture, Faculty of Food Technology, Jelgava, LV-3001, Latvia (phone: 0037163005644; fax: 0037163022829; e-mail: rita.riekstina@llu.lv)

Zanda Kruma Latvia University of Agriculture, Faculty of Food Technology, Jelgava, LV-3001, Latvia (phone: 0037163005644; fax: 0037163022829; e-mail: zanda.kruma@llu.lv)

Daina Karklina, Latvia University of Agriculture, Faculty of Food Technology, Jelgava, LV-3001, Latvia (phone: 0037163005637; fax: 0037163022829; e-mail: daina.karklina@llu.lv)

TABLE I
 CHARACTERIZATION OF CIDER SAMPLES

Abbreviation	Name of cider	Producer	Country	Alcohol vol.	Characterization
1.	Cidre Bouche Brut De Normandie	SAS Calvados Christian Drouin	France	4.5	Brut
2.	Cidre Bouche Doux Lieblich	Jean de Loret	France	2	Sweet
3.	Lucky Dog Apple	AS Latvijas balzams	Latvia	5	Sweet
4.	Up cider apple cider natural	Harvall Ltd	Finland	4.7	Sweet
5.	Lucky Dog Pear	AS Latvijas balzams	Latvia	5	Sweet,
6.	Up cider pear cider natural	Harvall Ltd	Finland	4.7	Sweet
7.	Sun cider pear	ISC Kalnapilio Tauro grupa	EU	4.5	Sweet

with 0.25 ml Folin-Ciocalteu reagent, after 3 minutes 1 ml 20% Na₂CO₃ and 3.25 ml distilled water were added. Samples were heated for 10 min. at 70 °C and kept for 30 minutes at 18 ±2 °C temperature. The absorbance was measured at 765 nm using a spectrophotometer JENWAY 6300. Total phenols were expressed as gallic acid equivalents (mg L⁻¹). Each determination was performed in triplicate and results are expressed as mean ± SD.

C. Determination of volatile aroma compounds

Volatiles from ciders were extracted using solid phase microextraction (SPME). 5 g of sample 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±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) was used. Working conditions were as follows: 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 40-300. 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.

D. Statistical analysis

Each determination was performed in triplicate and results are expressed as mean ± SD. Analysis of variance was performed by ANOVA procedure and p<0.05 was considered as statistically significant.

III. RESULTS AND DISCUSSION

A. Volatiles compounds of ciders

Aroma is very important characteristic of ciders and the highest total peak area of identified volatile compounds in samples 5, 6 and 3 were identified (Fig.1.).

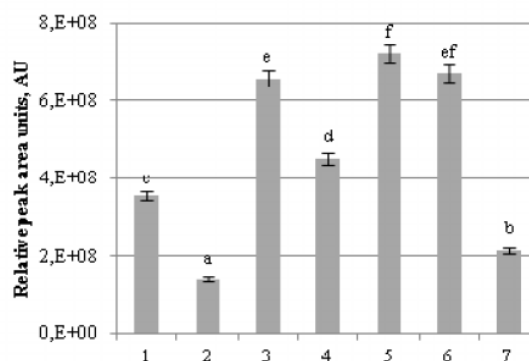


Fig. 1 Relative peak area units of volatile compounds in cider samples

In typical French ciders (samples 1 and 2) significantly lower amount of volatiles were detected. Possibly it could be explained that in some commercial ciders additionally flavouring substances were added, that are very volatile and easy absorbs to the SPME fiber. Esters, alcohols, acids and terpenes were the most abundant classes in cider (Fig.2.).

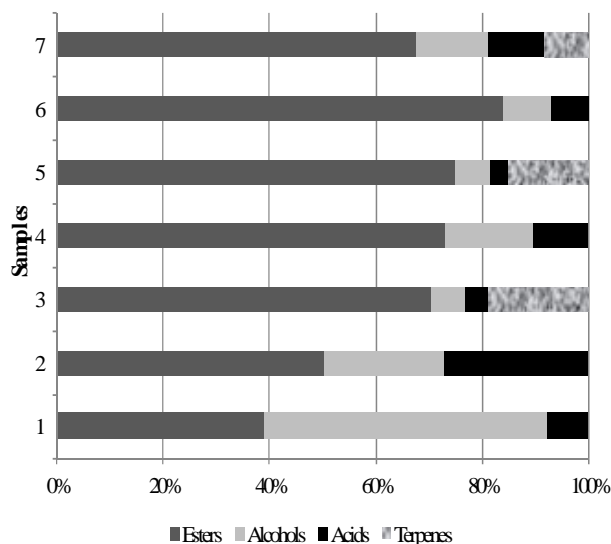


Fig. 2 Classes of main volatile compounds (%) in cider samples

TABLE III
TERPENES, ALCOHOLS AND ACIDS COMPOUNDS (% ± STANDARD DEVIATION) AS MEASURED
BY SPME-GC-MS IN CIDERS SAMPLES

Compounds	Cider samples						
	1	2	3	4	5	6	7
Terpenes							
limonene	n.d.	n.d.	18.79 ± 0.67	0 ± 0	15.08 ± 0.54	n.d.	8.22 ± 0.29
Alcohols							
1-butanol, 3-methyl	21.85 ± 0.78	5.61 ± 0.2	5.3 ± 0.19	3.82 ± 0.14	5.13 ± 0.18	4.3 ± 0.15	8.65 ± 0.31
1-hexanol	5.72 ± 0.2	7.61 ± 0.27	n.d.	11.07 ± 0.4	n.d.	3.35 ± 0.12	1.23 ± 0.04
8-heptadecanol	15.49 ± 0.55	7.55 ± 0.27	n.d.	n.d.	n.d.	n.d.	n.d.
phenylethyl alcohol	10.04 ± 0.36	2.05 ± 0.07	1.13 ± 0.04	1.65 ± 0.06	1.32 ± 0.05	1.37 ± 0.05	3.96 ± 0.14
Acids							
octanoic acid	2.32 ± 0.08	3.9 ± 0.14	1.38 ± 0.05	2.04 ± 0.07	1.26 ± 0.05	1.2 ± 0.04	2.65 ± 0.09
sorbic acid	n.d.	n.d.	1.08 ± 0.04	7.09 ± 0.25	1.19 ± 0.04	5.07 ± 0.18	4.95 ± 0.18
decanoic acid	5.46 ± 0.19	23.04 ± 0.82	1.95 ± 0.07	1.3 ± 0.05	1.09 ± 0.04	0.73 ± 0.03	2.92 ± 0.1

n.d. – not detected

TABLE II
ESTERS COMPOUNDS (% ± STANDARD DEVIATION) AS MEASURED
BY SPME-GC-MS IN CIDERS SAMPLES

Compounds	Cider samples						
	1	2	3	4	5	6	7
Butanoic acid, ethyl ester	n.d.	n.d.	2.38 ± 0.07	11.84 ± 0.37	n.d.	n.d.	n.d.
1-butanol, 3-methyl acetate	4.14 ± 0.13	4.06 ± 0.13	13.01 ± 0.41	n.d.	25.84 ± 0.81	34.51 ± 1.08	17.22 ± 0.54
Hexanoic acid, ethyl ester	9.93 ± 0.31	6.98 ± 0.22	1.13 ± 0.04	1.61 ± 0.05	1.22 ± 0.04	0.78 ± 0.02	5.01 ± 0.16
Acetic acid hexyl ester	3.13 ± 0.1	22.3 ± 0.7	34.05 ± 1.06	6 ± 0.19	28.54 ± 0.89	36.03 ± 1.13	5.5 ± 0.17
3-hexen-1-ol, acetate	n.d.	n.d.	11.69 ± 0.37	2.54 ± 0.08	3.47 ± 0.11	n.d.	n.d.
Octanoic acid ethyl ester	5.2 ± 0.16	3.48 ± 0.11	3.11 ± 0.1	12.75 ± 0.4	1.61 ± 0.05	6.36 ± 0.2	19.93 ± 0.62
Butanoic acid, 3-hexyl ester	n.d.	n.d.	n.d.	5.33 ± 0.17	n.d.	n.d.	n.d.
2,4-hexadienoic acid, ethyl ester	n.d.	n.d.	n.d.	8.35 ± 0.26	n.d.	3.44 ± 0.11	n.d.
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, acetate	n.d.	n.d.	3.47 ± 0.11	17.38 ± 0.54	7.61 ± 0.24	n.d.	5.16 ± 0.16
Decanoic acid ethyl ester	9.5 ± 0.3	10.78 ± 0.34	n.d.	7.23 ± 0.23	n.d.	2.86 ± 0.09	14.59 ± 0.46
Phenylethylacetat	n.d.	n.d.	1.53 ± 0.05	n.d.	6.64 ± 0.21	n.d.	n.d.
Acetic acid 2-phenylethyl ester	7.22 ± 0.23	2.64 ± 0.08	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. – not detected

Mainly samples were dominated by ester compounds, except sample 1 that dominate by alcohol compounds. Terpenes were identified only in samples 3, 5 and 6. Totally twenty volatile compounds were indentified in analysed commercial cider samples (Table 2 and Table 3). The major volatile compounds quantified in analysed samples were acetic acid hexyl ester, 1-butanol, 3-methyl acetate and limonene. Peng, Yue, and Yuan [20] reported that key aroma components in cider are ethyl acetate, acetic acid isobutylester, isopentylalcohol acetate, ethyl caprylate, ethyl 4-hydroxubutanoate, isopentylalcohol, 3,4,5-trimethyl-4-pentanol, nonyl alcohol, 3-methylthio-1-propanol.

The compound 1-butanol is a primary volatile compound of apples. Its concentration does not depend on processing technology and is cultivar and maturity dependent [21], [22]. The biosynthesis of higher alcohols is considered to be linked to amino acid metabolism. Higher alcohols are formed as by-products of anabolic and catabolic metabolism [23]. 3-methylbutanol is fusel alcohol is derived from isoleucine and leucine [24] and fusel alcohols 3-methyl-1-butanol and is important flavor compound in yeast-derived beverages [25]. Esters give fruitlike flavors and aromas to beverages. The alcohol 3-methylbutanol (12,148.51– 36,238.69 µg/L) is considered the most quantitatively important alcohol in Chinese ciders, while

1-hexanol (5.39–472.12 $\mu\text{g/L}$) is considered a characteristic volatile compound of fruits other than apples and is produced during the enzymatic oxidation process of linoleic acid [26]. Limonene were identified in samples 3 and 5 and its aroma in literature is described as lemon and mint [27].

B. Total phenols of ciders

The content of total phenols in ciders varied between 6.54 mg L^{-1} to 79.13 mg L^{-1} (Fig. 1). These results are in accordance to Berrueta, Gallo and Vicente [28] who reported polyphenol concentrations ranged from 0.28 to 217 mg L^{-1} in the ciders from French apples, and from 21 to 512 mg L^{-1} in ciders from Galician apples. Alonso-Salces et al. [29] observed total polyphenol content of Basque ciders 200–1200 mg EC L^{-1} and it is approximately, one-half of the content in French ciders (300–3800 mg EC L^{-1}).

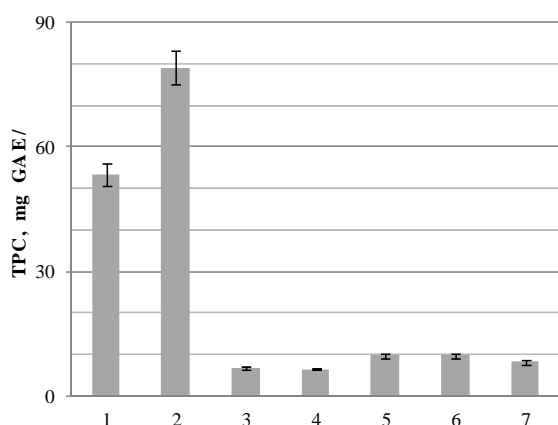


Fig. 3 Total phenol content in cider samples

Our previous study showed the content of total phenols in cider from Latvian apples varied between 27.78 mg L^{-1} to 92.32 mg L^{-1} and it was 1.1 to 2.3 times lower than in corresponding apple juice. Higher content of TPC were in ciders from cider apples between 69.90 mg L^{-1} to 92.32 mg L^{-1} [30]. Results of current research showed the highest content of total phenols was in commercial French ciders and ranged between 53.29 mg L^{-1} to 79.13 mg L^{-1} . Variation of cider TPC could be explained by the different raw materials (apple varieties, yeast strains, added addition) and by preparation technologies and conditions.

IV. CONCLUSION

Cider quality is related with taste and aroma, and it is influenced by the volatile compounds and phenols. Amount of volatile and phenols compounds are dependent on production technology. The highest TPC and lower volatile compounds were detected in French ciders.

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Rita Riekstina-Dolge lecturer and PhD student in Latvia University of Agriculture, Faculty of Food Technology, was born in Latvia, Sigulda in 1971. Main topics of research : cider production, quality parameters of apples. She has participated in different projects. At present R. Riekstina-Dolge is participant in the project „Sustainable use of local agricultural resources for development of high nutritive value food products (Food)” within the National Research Programme “Sustainable use of local resources (earth, food, and transport) – new products and technologies (NatRes)” (2010.-2013.)

Zanda Kruma, Dr.sc.ing., leader researcher at the Latvia University of Agriculture, Faculty of Food Technology, was born in Latvia, Aizpute in 1980. In 2008 she defended PhD thesis and obtained doctoral degree in food science. Main topics of research: biologically active substances in foodstuffs, food aroma analysis. She has 22 scientific publications and participated in 6 different projects.

Daina Karklina, Dr. sc. ing., professor at the Latvia University of Agriculture, Faculty of Food Technology, was born in Latvia, Riga in 1950. Scientific interests – Functional properties of food products, production of healthy qualitative and safety food products. She has 133 scientific publications and participated in 16 different projects. At present D. Karklina is participant in the project „Sustainable use of local agricultural resources for development of high nutritive value food products (Food)” within the National Research Programme “Sustainable use of local resources (earth, food, and transport) – new products and technologies (NatRes)” (2010.-2013.)