Ethyl carbamate and aroma compounds in distilled spirits from different stone fruits

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The purpose of this study was to find a possible correlation between ethyl carbamate (EC) content and aroma compounds in stone fruit spirits, which might result from a too harsh treatment of the stones. Possible markers should be identified, which would allow to correlate high EC levels with (negative) flavour compounds. Therefore, a total of 60 distilled spirits from different stone fruits (21 mirabelle, 20 plum, 19 cherry) was analysed on their contents of EC by HPLC-FLD and aroma compounds by headspace-trap GC/MS. The content of EC ranged from not detectable to 2,121 μ g/l with a mean of 192 μ g/l and a standard deviation of 332 μ g/l. The GC/MS analysis revealed that esters, alcohols and aldehydes made up the main part of the aroma fractions. Statistical correlation analysis showed that the ester fraction increases with increasing EC content. Yet, sensory analyses did not show any correlation between EC content and specific flavour compounds or the order in the sensory evaluation, respectively. Therefore, high EC levels could not be correlated with certain (negative) aroma compounds, thus high EC content cannot generally be regarded as a reliable marker for bad flavour quality of distilled spirits or vice versa.

Keywords: stone fruit spirits, ethyl carbamate, fruit flavours, aroma compounds, carcinogenic, fermented foods

Ethylcarbamat und Aromaverbindungen in Spirituosen aus verschiedenen Steinobstarten. Ziel dieser Studie war es, eine mögliche Korrelation zwischen dem Gehalt an Ethylcarbamat (EC) und Aromaverbindungen in Steinobstbränden zu finden, die durch eine zu wenig schonende Behandlung der Steine erzeugt werden könnten. Es sollten mögliche Marker identifiziert werden, die es ermöglichen, hohe EC-Werte mit (negativen) Aromaverbindungen zu korrelieren. Dazu wurden insgesamt 60 Destillate aus verschiedenen Steinobstarten (21 aus Mirabelle, 20 aus Pflaume, 19 aus Kirsche) auf ihren Gehalt an EC (HPLC-FLD) und Aromaverbindungen (Headspace-Trap GC/MS) analysiert. Der Gehalt an EC reichte von "nicht nachweisbar" bis zu 2.121 μ g/l mit einem Mittelwert von 192 μ g/l und einer Standardabweichung von 332 μ g/l. Die GC/MS-Analyse ergab, dass Ester, Alkohole und Aldehyde den Hauptanteil der Aromafraktionen ausmachen. Die statistische Korrelationsanalyse zeigte, dass der Esteranteil mit steigendem EC-Gehalt zunimmt. Sensorische Analysen zeigten jedoch keine Korrelation zwischen dem EC-Gehalt und bestimmten Aromaverbindungen bzw. der Rangordnung in der sensorischen Bewertung. So konnten hohe EC-Gehalte nicht mit bestimmten (negativen) Aromaverbindungen korreliert werden, daher kann ein hoher EC-Gehalt nicht allgemein als

zuverlässiger Marker für eine schlechte sensorische Qualität von Spirituosen angesehen werden oder umgekehrt.

Schlagwörter: Steinobstbrände, Ethylcarbamat, Fruchtaromen, Aromaverbindungen, karzinogen, fermentierte Lebensmittel

Ethyl carbamate (EC), also referred to as urethane, belongs to the chemical class of carbamates, which are characterised by the functional group -NH-CO-O-. EC is found in wine, beer and spirits as well as in bread, yogurt and others (Xia et al., 2017).

Historically, EC was first synthesized in the 19th century as a colourless, odourless crystal, with a salty and slightly bitter taste. EC has distinct commercial applications as co-solvent for pesticides, in cosmetics, and in drug synthesis (Ghanayem, 2007). In medicine, in the early 1940s, EC was used as a hypnotic in humans in dosages of up to 1 g per day and person, as an anesthetic in animals and as an antineoplastic agent for treating myeloma. Furthermore, EC is added to cosmetic or pharmaceutical products or is utilised in dentistry (NTP, 2016).

Several studies directly relate EC to carcinogenic and other harmful actions to human health (Nettleship and Henshaw, 1943; Baffa Júnior et al., 2011; Forkert et al., 2007). In animal studies EC showed cancer-causing potential in dosages from 100 to 2,000 mg/kg body weight in lung, blood vessels, and liver (Nayaran and Kumar, 2012; Schlatter et al., 2010). A two-year study on rodents revealed that there might be a potential health risk for humans due to cancer-promoting properties of EC (Vázquez et al., 2017). The IARC (World Health Organization's International Agency for Research on Cancer) has classified EC in group 2A of cancerogens (IARC, 2010) and the European Union classified EC in category 1B, thus, "potentially cancerogenic" (EU, 2018). Glyphosate and acrylamide have also been classified in the same categories (IARC, 2020).

Currently, no legal limit has been defined in Germany, yet. Recommendation (EU) 2016/22 points out that using GMP during stone fruit spirit production a maximum of 1 mg/l EC in the respective products can be easily undercut. Thus, when a limit of 1 mg/l EC is exceeded, the respective product is no longer regarded as safe according to European Community regulation Nr. 178/2002.

The precursors of EC arise from many factors and steps in the production process, from raw material to the conditions during the storage period of the spirits. Generally, EC is formed by a reaction of nitrogen containing compounds, such as urea, citrulline, carbamoyl phosphate, or hydrogen cyanide, with ethanol. The main sources of these substances are amino acids or their decomposed derivatives naturally occurring in fermented food. The main source for hydrogen cyanide is amygdalin.

Amygdalin has been detected in several fruit tree kernels such as plum (4,100 mg/100 g), apricot (2,394 mg/100 g), cherry (2,306 mg/100 g) and apple (739 mg/100 g) (Lucas and Sotelo, 1983). Amygdalin is enzymatically cleaved through the enzyme mixture emulsin contained in many stone fruits, leading to two molecules glucose, one molecule benzaldehyde, and one molecule hydrogen cyanide (Kuroki and Poulton, 1986).

The hydrocyanic acid in the free or conductive form oxidizes to hydric acid cyanate, which then reacts with ethyl alcohol to EC. This process is strongly favored by exposure to UV light (Christoph et al., 2006; Zacaroni et al, 2015). The presence of copper salts prevents the formation of free hydric acid and, thus, the formation of EC through the formation of copper complexes. Copper is widely used to make alembic stills and has function as a copper catalyst during the distillation to deplete hydrogen cyanide or can be added as copper compounds to the mash before the distillation to reduce formation of EC. However, mainly a lack of GMP contributes to a higher incidence of this contaminant in the beverage. This is due to a stronger strain on the kernels and

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a higher extraction of amygdalin as already discussed above (Lawrence et al., 1990). Copper can also be naturally found in fermented products or is artificially added to a fermented product to reduce undesired sulphurous off-flavours (Gössinger et al., 2006). Analysis of food products on EC is usually performed with GC-based methods or HPLC systems after derivatisation (Fig. 1). Generally, sample extraction and subsequent GC/MS or GC/MS-MS are regarded as references for the analysis of EC in alcoholic beverages (Lachenmaier, 2005; Xu et al., 2012; Xian et al., 2016).



Fig. 1: Reaction scheme of ethylcarbamate with xanthydrol

Beside ethanol, aroma compounds are the second most important group of compounds defining the quality of alcoholic beverages. Their flavour is affected by a very large number of compounds present in low concentrations but with large impact on the bouquet of the respective product.

In mirabelle spirits aldehydes play an important role, especially hexanal, heptanal, and nonanal. Further distinctive aroma compounds are propanal, valeric acid, and ethyl nonanoate. The peach-like flavour of mirabelles is linked to the lactone (R)-(+)- γ -decalactone. Compared to other stone fruit spirits, mirabelle spirit does contain higher concentrations of linalool, 3-methyl-3ene-1-ol, hexyl-2-methyl butanoate and cinnamic acid ethylester (Bauer-Christoph et al., 1997; Ledauphin et al., 2010).

Cherry fruit spirits are mainly defined by benzaldehyde, which is a result of the decomposition of amygdalin. In addition to benzaldehyde, terpenoids such as linalool, ethylester, and aromatic organic acids play an important role. Furthermore, eugenol and vanillin have been identified in cherry spirits. Research conducted by Nikicevic et al. (2011) showed that mainly esters could be detected in the analysed cherry spirits. Especially, ethyl octanoate and ethyl hexanoate have to be named in this context, which can be found in distilled spirits from apples and apricots as well. An additional group of esters are acetic esters including isoamyl acetate and 2-phenyl acetate as the most important representatives. With regard to terpenoids, linalool plays a distinctive role in cherry fruit spirits (Nikicevic et al., 2011; Serradilla et al., 2012). According to the research conducted by Schehl et al. (2005), the kernel content of the fermenting mash has only little effect on the final aroma composition of cherry spirits as opposed to plums.

Regarding plum spirits, during fermentation most of the esters are formed. Especially, ethyl butyrate, ethyl lactate, ethyl octanoate and ethyl hexanoate have to be named in this context. Further characteristic aroma compounds are nonanal, benzaldehyde, and damascenone, linalool and α terpineol (Balcerek et al., 2017; Jurica et al., 2016; Milicevic et al., 2012; Popović et al., 2019; Vyviurska et al., 2017). Depending on plum variety, technology, and product age flavour compo-

sition of plum spirits can differ significantly. Research conducted by Schehl et al. (2005) revealed that fermentation with or without kernels can have a significant effect on the final aroma compositions. Tešević et al. (2005) found that with increasing age of the spirit the concentration of benzaldehyde, cinnamic acid ethyl ester, salicylic acid ethyl ester, and benzoic acid ethyl ester increased.

Since high EC concentrations in distilled spirits are an inevitable consequence of a harsh treatment of the kernels, this might also have an impact on the final aroma of the distilled spirits, and, moreover, on sensory aspects. Thus, the aim of this research was to analyse EC contents and aroma profiles of different stone fruit spirits and reveal possible correlations between EC, aroma profile, and, moreover, sensory quality.

Materials and Methods

A total of 60 distilled spirits was taken from the 2019 competition of the Landesverband der Klein- und Obstbrenner Nord-Württemberg e. V. (Regional Association of the Small and Fruit Distillers North-Württemberg, Owen/Germany) and all had been awarded a bronze, silver, or gold medal, respectively, within this competition.

All chemicals were of analytical grade and were taken from: Alfa Aesar (Kandel/Germany; EC and xanthydrol), Honeywell/Riedel de Haen (Seelze/Germany; acetonitrile), Sigma Aldrich (St. Louis, Missouri/USA; 2-heptanone).

HPLC analysis

HPLC analysis with fluorescence detection was performed as described elsewhere (Zhang et al., 2014). Briefly, all samples were diluted to a final ethanol content of 20 %vol. Then, 600 μ l of the diluted sample were mixed with 400 μ l of a 20 mM 9-xanthydrol solution in 1-propanol. Furthermore, 100 μ l of 1,5 M hydrochloric acid were added. The liquid was thoroughly mixed and then

incubated at 30 °C for 30 min. Afterwards the solution was filtrated through a 0,22 μ m filter of regenerated cellulose (neolab migge, Heidelberg, Germany) and 20 μ l of the filtrated solution were injected onto the HPLC. All samples were derivatised in duplicate.

The HPLC system was an UltiMate 3.000 system (Dionex, Germering, Germany) consisting of a quaternary pump, autosampler, column oven, and a fluorescence detector with 238 nm excitation and 300 nm emission wavelengths. The column was a LiChrospher 100 RP-18 (250 mm x 4,6 mm; 5 μ m particle size, Wicom, Heppenheim, Germany) with a pre-column of the same material and maintained at 40 °C. The flow was set to 0,8 ml/min. Eluent A was 20 mM sodium acetate in double distilled water adjusted to pH 7,2 with acetic acid. Eluent B consisted of gradient grade acetonitrile. The gradient was as follows: 0 – 29 min 55 % A, 45 % B; 30 – 42 min 40 % A, 60 % B; 45 – 55 min 55 % A, 45 % B.

External quantification was achieved injecting EC standard solutions with concentrations of 25, 50, 75, 100, and $250 \mu g/l$ EC in 20 %vol. ethanol.

GC/MS analysis

For GC/MS analysis 200 μ l sample were diluted with double distilled water to a final volume of 100 ml. 5 ml of this solution were transferred into a 10 ml headspace vial (neolab, Heidelberg, Germany) and 10 μ l internal standard (2-heptanone) were added. The vial was sealed with a PTFE/Butyl (grey) septum with aluminium cap crimp top (PerkinElmer, Rodgau-Jügesheim, Germany), thoroughly mixed, and then placed into the headspace apparatus.

The GC/MS apparatus consisted of a TurboMatrix 40 headspace-trap sampler, a Clarus 600 GC, and a Clarus 600C MS (PerkinElmer, Rodgau-Jügesheim, Germany). The conditions of the GC/MS apparatus are given in Table 1. Identification of substances was based on their respective MS spectra. For quantification only the peak area was used without further calibration through external standards. The importance of each compound for the total aroma was calculated as follows: the peak area was divided by the sensory threshold as published by Burdock (2010). Table 1: GC conditions for aroma analysis

Headspace Trap			GC		MS	
Temperature	Oven	80 °C	Software	TurboMass 5.4.2.1617	Solvent Delay	5 min
	Needle	100 °C	Column	Elite-624, 30 m, ID 0.25 mm, 1.4 μm	Scan	m/s 39 - 225
	Transferline	105 °C	Temperature Program	40 °C/5 min, 20 °C/min – 220 °C, 3 min hold, 20 °C/min – 240 °C, 3 min hold	timing	21 min
	Trap High	300 °C	GC run time	21 min		
	Trap Low	40 °C	Gas	Не		
	Thermostat.	20 min				
	Pressurise	1 min				
	Trap load	1.2 min				
Timing	Desorption	0.5 min				
	Dry Purge	5 min				
	Trap Hold	10 min				
	GC cycle	22 min				
Pressure	Desorption	20 psi				
	Vial pressure	25 psi				
	Column pressure	20 psi				
Option	Dry Purge					
	Outlet Split	yes				
	Operating mode	trap				

Sensory analysis

Sensory analysis was performed in the sensory lab of the State Research Institute for Viticulture & Pomiculture in Weinsberg under air-conditioned and artificial lighting environment. Samples were preserved and tempered before sensory analysis at 20 °C. The panelists were selected based on their experience in sensory evaluation activities and familiarity with the product group. The sensory panel consisted of 13 semitrained employees of the institute, who assessed the samples according to the DLG-5-points scheme. The samples were also scored by using a modified version of quantitative descriptive analysis. Sensory attributes were selected during a preliminary focus group session using stone fruit spirits widely differing in their sensory characteristics.

After this the descriptive terms benzaldehyde and fruit intensity were both chosen for the description of aroma and flavour. The 60 samples were provided in 14 flights of 4 or 5 samples. The samples were presented to the judges coded and in randomized order within each flight. Sensory attributes for aroma were evaluated in a monadic presentation order. Sensory attributes for flavour were evaluated in a serial presentation order. Intensity ratings were scored on an unstructured anchored scale with the terms "lowest rating" at the lower end and "highest rating" at the higher end. The samples were handed to the judges in standardized glasses. Those were rinsed manually with pure water after every flight. Also the panelists rinsed their mouth with mineral water before testing the samples as necessary. Panel sessions were conducted once within three consecutive days.

Data was recorded using CASA (Computer Aided Sensory Analysis) and Fizz Acquisition Software (Biosystems, Couternon, France; version 2.51 a 86;). Results were calculated using Fizz Calculations (version 2.50 c 04).

Statistical analysis

Statistical calculations were performed using XLStat (Addinsoft, New York, USA; version 2020.5.1, build 1043). An ANOVA was performed

on the respective datasets using Tukey's $HSD_{0,05}$ test.

Results

EC

Results of the HPLC analysis on EC are given in Figure 2. The results show that the contents of EC in the distilled spirits ranged from not detectable to 2,121 µg/l with a mean of 195 µg/l and a standard deviation of 332 µg/l. The highest amounts could be found in spirits from cherries with a mean concentration of 287 µg/l. The lowest amounts were found in plums with a mean concentration of 124 µg/l. This is in good accordance with data from other research groups (Zacaroni et al., 2015; Lee et al., 2018). Only two samples exceeded the limit of 1 mg/l EC: a cherry spirit containing 2,121 µg/l and a mirabelle spirit containing 1,069 µg/l. This corresponds to 3.3 % of the totally analysed lot.



Fig. 2: EC content ($\mu g/l$) of the analysed stone fruit spirits

ANOVA using Tukey's HSD_{0,05} test did not reveal any statistical significance between all three fruits, although mean EC content in cherry spirits was more than twofold higher than in plum spirits (Table 2). Furthermore, no statistical significance was found between the regions, where the fruit spirits had been produced. Despite a lacking statistical significance, the mean values differ quite considerably (Table 3). According to statistics, the region with the highest mean concentration on EC was East Württemberg, located around the city of Heidenheim. From anecdotal evidence, a relatively high number of distilleries

in this district still do not use copper catalysts, which otherwise would help with the depletion of hydrogen cyanide and, thus, reduce EC formation potential. A further narrowing of the production region, i. e. district county of origin, did not show a significant impact on EC content of the stone fruit spirits. It can be, thus, concluded that the above mentioned problem cannot be pinned to a specific county or even producer, but rather seems to be of a wider relevance in the whole region as such.

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Most interestingly, samples with higher medal range also showed higher contents on EC (Table 4). Although this result was not statistically significant, the difference in EC content between Bronze and Gold medal is yet quite distinct. This might lead to the supposition that a stronger strain on the fruits and, moreover, kernels to increase flavour extraction in general will, depending on the fruit, ultimately also increase EC formation due to higher amygdalin extraction (Schehl et al., 2005).

Table 2: Results of the ANOVA (p<0.05) on ethyl carbamate content (μ g/l) and fruit type

Fruit	Ethyl carbamate (µg/l)
Cherry	287 a
Mirabelle	181 a
Plum	124 a
Pr > F (Model)	0.306
Significant	No

Table 3: Results of the ANOVA (p<0.05) on ethyl carbamate content (µg/L) and production region

Region	Ethyl carbamate (µg/l)
Ostwürttemberg	318 a
Stuttgart	196 a
Heilbronn-Franken	180 a
Nordschwarzwald	153 a
Rhein-Neckar-Odenwald	77 a
Donau-Iller	71 a
Pr > F (Model)	0.931
Significant	No

Table 4: Results of the ANOVA (p<0.05) on ethyl carbamate content (µg/I) and medal received in the competition

Medal	Ethyl carbamate			
	(µg/l)			
Gold	233 a			
Silver	224 a			
Bronze	136 a			
Pr > F(Model)	0.598			
Significant	No			

Aroma

The percentage aroma profile of the distilled spirits is given in Figure 3. The main part of the aroma compounds is made up by esters, followed by alcohols, ethers, and aldehydes. This is in good accordance with the data published by Sliwinska et al. (2015).



Fig. 3: Percentual content of aroma compounds in stone fruit spirits (% total area in GC/MS analysis)

Principal component analysis (PCA) could not achieve a complete separation of the fruits based on their flavour profile. A large overlapping of all fruits could be found, especially for plum and mirabelle (Fig. 4). This is not amazing, since both plants are botanically closely related (plum: *Prunus domestica* subsp. *Domestica* vs. mirabelle: *Prunus domestica* subsp. *Syriaca*). Yet, cherry showed a quite good separation from the two other fruit spirits.

In total, 77 % of the variance could be explained by the first two factors. The most important variables in this regard are as follows: mirabelles are mainly separated from the other fruits by octanal, heptanal, ethyl butanoate, ethyl nonanoate, and ethyl acetate (Fig. 5). These compounds lead to rather citrus, honey, fruity, and fatty impressions. Especially, the orange-like nonanal is a characteristic compound, which separates mirabelles from plums (Bajer et al., 2017). Plums show a correlation to isoamyl acetate and ethyl hexanoate. These two compounds have a clear fruity aroma with banana and pineapple characteristics, respectively. It can be supposed that the fruits were of progressed ripening stage, since no green flavours were detected in relevant concentrations in these samples. Quite a number of substances is responsible for the separation of cherry fruit spirits from the other two groups (Fig. 5). Especially, the ethyl esters ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl pentanoate, and pentanal and 1,1-diethoxyethane have to be named in this context. While the ethyl esters contribute to fruity characteristics, the other two compounds show rather pungent and green flavours.

Most interestingly, benzaldehyde had only an impact on the separation of cherries from the other two groups. This is quite astonishing, since benzaldehyde is regarded a general character impact compound of stone fruit kernel aroma (Vyviurska et al., 2017).

Correlation analysis did not reveal any correlation between EC and any of the analysed aroma compounds apart from a very slight positive correlation between EC and pentanal (0.257), the latter having a pungent aroma (detailed results not shown).



Fig. 4: PCA of stone fruit spirits based on their aroma profile



Fig. 5: PCA of stone fruit spirits based on their aroma profile, only the most influencing factors are depicted

Sensory analysis

A good correlation was found between benzaldehyde content and sensory evaluation. Correlation factors for benzaldehyde as measured by GC/MS and benzaldehyde aroma and flavour in sensory were 0.577 and 0.500, respectively.

An ANOVA performed on sensory data and competition medal showed that a significant difference could be found between gold and bronze medal with regard to benzaldehyde aroma and flavour (Table 6). Similar results were found for the ANOVA on sensory data and fruit type and medal, respectively (Table 5). Especially, plum and mirabelle spirits showed high sensory rankings on benzaldehyde aroma and flavour, when they received gold or silver medals in the competition.

The results of our sensory panel were in good accordance with the respective medal in the competition, although silver medallists were slightly better rated than gold medallists (Table 6). Mirabelle spirits with silver medals received the best overall ranking, whereas cherry spirits with bronze medals were downgraded in our sensory panel.

	aroma benzal- dehyde	aroma fruit intensity	DLG 5 points	flavour benzal- dehyde	flavour fruit intensity
Mirabelle	1.963 a	2.452 a	2.970 a	1.851 ab	2.100 b
Plum	2.339 a	2.345 a	2.916 a	2.174 b	1.953 ab
Cherry	1.976 a	2.105 a	2.699 a	1.605 a	1.628 a
Pr > F(Model)	0.032	0.555	0.204	0.033	0.078
Significant	Yes	No	No	Yes	No

Table 5: Results of the ANOVA (p<0.05) on different sensory attributes for the stone fruit spirits

Table 6: Results of the ANOVA (p<0.05) on different sensory attributes for the medal received in the local competition

	aroma benzalde- hyde	aroma fruit intensity	DLG 5 points	flavour benzal- dehyde	flavour fruit intensity
Gold	2.638 b	2.412 a	2.901 ab	2.118 a	2.000 a
Silver	1.869 a	2.349 a	3.011 b	1.782 a	1.998 a
Bronze	1.885 a	2.177 a	2.686 a	1.793 a	1.720 a
Pr > F(Model)	0,032	0.555	0.204	0.033	0.078
Significant	Yes	No	No	Yes	No

Discussion

One supposition for this project was that a harsh treatment of the fruit kernels will inevitably also increase EC content. A harsh treatment of fruit kernels is generally regarded as one major bad precondition for stone fruit distilled spirits. Yet, according to our results high concentrations of EC cannot be used as a reliable marker for a generally bad product. Products with high EC concentrations were not automatically downgraded in sensory analysis.

Furthermore, no correlation could be found between EC and benzaldehyde. Although high EC concentrations are an inevitable consequence of harsh kernel treatment, benzaldehyde concentrations were not increased concurrently. These might lead to the following suppositions: Firstly, EC was at least partially formed from other precursors than amygdalin, such as urea. Secondly, copper catalysts might have bound some of the cyanide, but its depletion was not very effective. From anecdotal evidence, some of the distilleries do not use copper catalysts at all, which might explain the higher EC contents in some of the spirits. Thirdly, given the fact that one mol cyanide and one mol benzaldehyde arise from the depletion of amygdalin, their molar proportion in the spirits should be identical. Yet, no stringent correlation could be found between EC and benzaldehyde. This would support the above mentioned supposition that either EC is also formed from other precursors than amygdalin or cyanide is not completely depleted by copper catalysts.

Conclusions

Only two of 60 samples exceed the EC limit of 1 mg/l. Statistical analysis could not reveal a correlation between aroma compounds and EC content. Given the fact that benzaldehyde and cyanide are released from fruit kernels in equimolar quantities it can be concluded that high benzaldehyde contents, i. e. high strain on the kernels, do not ultimately lead to high EC contents in the respective distilled spirits. Furthermore, no correlation could be found between sensory performance and EC content. Thus, high EC content cannot be generally regarded as a reliable marker for bad flavour quality of distilled spirits and vice versa.

Author contributions

MPN planned the project, calculated the statistics, and wrote the manuscript, LS performed the measurements, CS edited the manuscript, TF adapted the HPLC method and edited the manuscript, AE aided with the GC/MS analysis, SH programmed the software for sensory, DH assisted with sensory evaluation and edited the manuscript.

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