

Article

Key Aroma Compounds in Two Bavarian Gins

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Featured Application: The sensory-analytical approach used in the present studies allows alcoholic spirits such as gin to be characterized in terms of their key aroma compounds and their corresponding influence on the overall sensory profile of the final product. This is of particular relevance in gin production, which typically does not require lengthy maturation processes, whereby insights into key aroma constituents can offer important information on how the sensory balance of the spirit can be adjusted towards the desired outcome through the use of specific botanicals.

Abstract: The characteristic, dominant flavor of gin is juniper, often within a complex aroma of other botanicals. The present study examined two gins from a distillery in the German state of Bavaria; one produced with 50 individual botanicals, the other with 15. The study focused on characterizing the aroma profiles and identifying the key aroma-active compounds of the gins. Comparative sensory evaluations of the gins revealed marked differences in their aroma profiles, with the botanical-rich gin exhibiting more *citrusy*, *orangey* and *fruity* notes than the gin containing fewer botanicals. Instrumental analyses by gas chromatography-mass spectrometry/olfactometry (GC-MS/O) using aroma extract dilution assays (AEDA) identified terpenes as the dominant key aroma compounds, specifically limonene, 1,8-cineole, linalool, estragole and *trans*-anethole, with additional contributions from aldehydes, such as nonanal, and phenylpropanoids, such as eugenol and estragole. Selected compounds were quantified using stir-bar sorptive extraction (SBSE) and stable isotope dilution analysis (SIDA) with GC-MS analysis. Further, odor thresholds and corresponding odor activity values (OAVs) of these compounds were calculated, with linalool exhibiting the highest OAV in both gins. The present analyses revealed how different botanicals alter the concentrations of key aroma compound constituents and elicit a shift in the overall aroma profile of the final spirit.

Keywords: aroma; botanicals; gas chromatography-olfactometry (GC-O); gin; juniper; odor activity value

1. Introduction

Gin has experienced a renaissance over the past decade. While 384 million liters of gin were consumed worldwide in 2010, the global consumption more than doubled in 2019 to an estimated 812 million liters [1]. Flavor is a driving parameter of consumer acceptance of alcoholic spirits like gin. Specifically for gin, the aroma stems from the use of botanicals during the distillation process, whereby individual aroma notes are imparted by constituent odor-active volatile organic compounds (VOCs) – or aroma compounds – derived from the respective botanicals. Indeed, distilleries exploit a wide range of botanicals to craft artisan versions of this spirit with unique and characteristic aromas to

appeal to consumers' tastes. Common botanicals utilized in gin production include juniper berries (*Juniperus communis* L.), coriander seeds (*Coriander sativum* L.), orange peel (*Citrus sinensis*), golden shower (*Cassia fistula*), orris root (*Iris florentina* L.), cardamom seeds (*Elettaria cardamomum* L.), angelica root (*Angelica archangelica* L.), cinnamon bark (*Cinnamomum zeylandicum*), calamus (*Acorus calamus* L.), fennel (*Foeniculum vulgare*), aniseed (*Pimpinella anisum*), lemon peel (*Citrus limon* L.), cumin (*Cuminum cyminum* L.), almond (*Prunus amygdalus* L.) and licorice root (*Glycyrrhiza glabra*) [2].

The most important botanical in gin is juniper, which imparts the predominant, characteristic flavor of gin and is a required constituent for an alcoholic spirit to carry the name gin, as specified in legal regulations (e.g., EC Regulation no. 110/2008) [3]. In the latter regulation, for example, gin is defined as a juniper-flavored spirit drink containing at least 37.5% alcohol by vol. Gin can be further sub-classified as gin, distilled gin, and London (dry) gin. While pure ethyl alcohol infused with flavoring substances and/or preparations may be sold as gin, the term distilled gin is reserved for organoleptically suitable ethyl alcohol of an appropriate quality (at least 96%vol.) that has been redistilled in the presence of selected natural botanicals; further, distilled gin produced in this manner may retain this term when it is mixed further with ethanol and/or when it is additionally flavored using substances and/or preparations. By comparison, London gin is retained exclusively for distilled gin obtained from ethyl alcohol that is limited in methanol content (max. 5 g/hL of 100%vol.) and whose flavor is introduced only through re-distillation, resulting in what is considered a higher quality spirit. London gin is often supplemented by the term dry because of its maximum permitted sugar content of 0.1 g/L.

In general, the flavor profile of a food/beverage product can be examined by human sensory evaluations, e.g., quantitative descriptive analysis (QDA) [4], to allow for individual nuances (e.g., *fruity*, *flowery*, *herbaceous*, etc.) to be described and quantified in their perceived, relative intensities. In many cases, especially in food/beverage production, knowledge of the odor-active compounds that elicit individual aroma impressions is important in order to tailor a product's flavor. The most common analytical approach to characterize the VOC constituents of a product (food or otherwise) is gas chromatography (GC), coupled to mass spectrometry (MS) [5]. While this technique offers very sensitive detection of VOCs, it does not discriminate between odorless and odorous compounds, the latter being responsible for the overall aroma of a product. To address this shortcoming, instrumental detection of compounds eluting from the GC column can be augmented with human olfactory perception, as is undertaken in GC-olfactometry (GC-O and GC-MS/O) [6]. In food science, this approach can be combined with aroma extract dilution assays (AEDA) to determine the respective contributions of individual odor-active compounds to the overall aroma of a product [7]. The combination of using human sensory and instrumental analytical approaches delivers comprehensive datasets that allow correlations to be drawn between specific aroma notes and the respective compounds eliciting these.

Reports in the scientific literature on the aroma of gin and the related constituent aroma compounds are limited. A comparative sensory evaluation study of six different gins that focused on the attributes *juniper*, *citric*, *aniseed*, *licorice*, and *spice* reported marked differences between London dry gins and gins with different geographical typicality [8]. A study investigating the influence of distillation parameters by comparing six different gins revealed that the use of a greater amount of botanicals during distillation led to higher intensity ratings of sensory descriptors [9]. In terms of their volatile chemical constituents, several studies utilized GC-MS to examine the predominant compounds, whereby monoterpenes were the most frequently identified compounds, assigned mainly to juniper and coriander oil [9–13]. Semi-quantitative analysis of the volatile fractions of distilled gins identified 70 volatile substances, also predominantly monoterpenes, as well as sesquiterpenes, and their oxygenated derivatives [14]. While the aforementioned studies undertook comprehensive analyses of VOCs in gin, they did not differentiate between odorless and aroma-active volatiles. Only one study to date has reported on the latter, using GC-O analysis in combination with the detection frequency method [15], while others discussed the concentrations of some volatiles in relation to their odor thresholds (OTs), determined

in aqueous or 20%vol. ethanolic solutions [9,10]. Of the odor-active substances detected, 38 were subsequently identified using GC-MS and were found to be primarily mono- and sesquiterpenoids.

As outlined above, GC-O or GC-MS/O analysis with AEDA is a useful method to explore relative contributions of individual compounds to the overall aroma of a sample. This approach can be combined with stable isotope dilution analysis (SIDA) to quantify the concentrations of selected (odor-active) volatiles in a sample, as has been previously applied to other alcoholic spirits, such as Bourbon whiskey and rum [16–21]. Alternatively, the concentrations of aroma-active constituents can be determined by the addition of internal standards, as has been carried out for rum, cognac and the Chinese liquor Daqu, amongst others [22–24]. For additional insight into the relevance of individual odorants to the overall aroma, odor activity values (OAVs) can be determined, which are defined as the ratio of the concentration of an odorant in a sample to its odor threshold [16–22,24].

In terms of sampling, the extraction of volatiles in gin may be performed via liquid-liquid extraction [10,15], which was found to be most suitable for GC-O analysis using dichloromethane [15]. For GC-MS analysis of gin volatiles, headspace solid-phase micro-extraction (HS-SPME) [8,9,13,14] or stir-bar sorptive extraction (SBSE) [12] have been applied. A study comparing the application of headspace sorptive extraction (HSSE), SBSE and SPME (albeit of volatiles from Arabica coffee), observed higher recoveries using HSSE or SBSE than via SPME due to the high amount of sorbent applied [25].

Reports on quantitative studies of key aroma compounds in gin in the scientific literature are lacking. Consequently, the aim of the present work was to identify the key aroma compounds in two locally distilled gins produced using different numbers of botanicals. GC-O was employed in combination with AEDA to screen for odor-active substances and estimate their relative odor potencies. Further, SBSE sampling with subsequent GC-MS/O analysis using SIDA allowed selected aroma compounds to be quantified in the two gins. Subsequent calculations of the OAVs of these selected compounds provided indications of their relative contributions to the overall aroma of each gin. Finally, the data derived from instrumental analyses were compared with the aroma profiles of the gins, as determined via QDA by human sensory evaluations.

2. Materials and Methods

2.1. Materials

Two premium London dry gins (both 45% alc. by vol.) produced by The King Gin distillery (Neufahrn, Germany) were investigated, namely The King Gin Kini and The King Gin Gspusi (2018 vintage). According to the distillery, Kini is distilled using more than 50 botanicals and is described as *spicy, malty* and *floral*, whereas Gspusi contains 15 individual botanicals and is described as *fruity, berry-like* and *fresh*. Both bottles were stored at 6 °C after purchase and throughout the analyses.

Information on the reference aroma compounds and additional chemicals used in the analyses is provided in the Supplementary Material.

2.2. Sensory Evaluations

2.2.1. Panelists

Sensory evaluations were performed by a trained panel of seven assessors (6 female, 1 male, aged 23–56 years; Fraunhofer IVV, Freising, Germany). The panelists exhibited no known illness and had normal olfactory function at the time of the evaluation. Weekly general sensory trainings were performed based on an in-house established odor language with selected odor references comprising approximately 150 different odor qualities. Hedonic ratings of the samples were made with an expanded panel of 14 individual (12 female, 2 male, aged 21–56 years), including untrained panelists.

2.2.2. Sensory Analyses

Sensory evaluations were performed according to DIN EN ISO 13299:2016. Gin samples were presented to the panel in 140 mL transparent, lidded glass jars (J. WECK GmbH u. Co. KG, Wehr-Öflingen, Germany), each containing 15 ± 1 mL of the respective sample. The orthonasal evaluations were performed in a sensory room at 21 °C, and each sample was labelled with a random three-digit code for anonymization purposes. The aroma qualities of each sample were determined in an initial sensory session, and a subsequent consensus of the descriptors was made by the trained panel, with the attributes chosen as follows (with corresponding odorant references in parentheses): *citrus-like* (citral), *orange-like* ((R)- α -limonene), *fruity* (ethyl-2-methylbutanoate), *soapy/coriander-like* (decanal), *anise-like* (anise), *malty* (3-methylbutanal), *ethanolic/pungent* (ethanol), *eucalyptus-like* (1,8-cineole), *juniper-like* (juniper), *cola-like* (cola soft drink) and *peach-like* (γ -dodecalactone). The same descriptors were used for evaluating both gins.

The samples were comparatively evaluated by rating the intensity of these individual odor qualities. Additionally, hedonic ratings of each sample on a scale from 0 (strong disliking) via 5 (neutral) to 10 (strong liking) were made; untrained assessors were included for the hedonic ratings. Analysis of variances (ANOVA) was performed on the mean intensity ratings across the panel to examine the significance in differences between the sensory profiles (significance level, $p < 0.05$).

2.3. Isolation of Volatiles

Volatiles were extracted by liquid-liquid extraction using dichloromethane (DCM), as has been previously reported as a suitable extraction approach for gin volatiles [15]. Each sample was extracted three times in succession using 50 mL sample in 50 mL DCM. The three extracts were then combined and subsequently washed three times with 50 mL sodium chloride (NaCl) solution (1 mol/L) each, then dried with anhydrous sodium sulfate and filtered. The volatile compounds in the extract were subsequently separated from the non-volatile constituents by solvent-assisted flavor evaporation (SAFE) [26]. The distillate thus obtained was washed with NaCl solution (1 mol/L, 3×50 mL), dried over anhydrous sodium sulfate, filtered and concentrated at 50 °C to ~ 3 mL by a Vigreux column (50 cm \times 1 cm i.d.), and finally to ~ 150 μ L by micro-distillation [27]. A blank sample was prepared by applying the identical work-up procedure outlined above with 50 mL DCM.

2.4. Aroma Extract Dilution Analysis

The flavor dilution (FD) factors of the individual odorants were determined by AEDA with GC-O. The aroma distillate (cf. Section 2.3) was diluted stepwise (1:2; v/v) with DCM, resulting in solutions corresponding to FD factors in a 2^n series up to 2048. The blank sample was analyzed in the same manner as the extract.

2.5. Gas Chromatography-Olfactometry

Analysis of each sample extract by GC-O was made in triplicate, each performed by a different trained assessor. The GC-O system comprised a Trace GC Ultra (Thermo Fisher Scientific GmbH, Dreieich, Germany) using two different capillary columns, namely a DB-FFAP and a DB-5 (both 30 m \times 0.32 mm, film thickness 0.25 μ m; J & W Scientific, Agilent Technologies GmbH, Waldbronn, Germany). The system further incorporated a pre-column (uncoated, deactivated fused silica capillary, 2–3 m \times 0.32 mm) and a Y-splitter after the main column, splitting the effluent in a 1:1 volume ratio between a flame ionization detector (FID) and an olfactory detection port (ODP) via two uncoated, deactivated fused silica capillaries (0.5 m \times mm). The FID signal was recorded by a SE 120 chart recorder printer (BBC Georz Metrawatt, Nuremberg, Germany). Aliquots (2 μ L) of the undiluted distillate and the solutions were injected manually using the cold on-column technique (40 °C). After 2 min, the temperature was raised at 7 °C/min to 230 °C (DB-FFAP) and 250 °C (DB-5), and held for 5 min. The flow rate of the helium carrier gas was 2.5 mL/min. The temperatures of the FID and the ODP

were set to 250 °C and 235 °C, respectively. Linear retention indices (RIs) of the odor-active (aroma) compounds were calculated as described in the literature [28].

2.6. Gas Chromatography-Mass Spectrometry/Olfactometry

Analyses of sample extracts via GC-MS/O were performed with a Trace GC Ultra and a Trace DSQ MS (both Thermo Fisher Scientific GmbH, Dreieich, Germany) using DB-FFAP and DB-5 columns (cf. Section 2.4). Aliquots (2 µL) were injected by a multipurpose autosampler (MSP 2; Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) using the cold on-column technique (40 °C). After 2 min, the temperature was raised at 8 °C/min to 235 °C and held for 5 min for the DB-FFAP column, and to 250 °C and held for 10 min for the DB-5 column. The flow rate of the helium carrier gas was 2.3 mL/min. At the end of the capillary column, the effluent was split between an ODP and the MS using deactivated fused silica capillaries (0.5 m × 0.2 mm). Mass spectra were generated in electron ionization (EI) full scan mode (m/z range 35–250, 70 eV). Compound identification was made by comparing the odor qualities, mass spectra and RIs on the DB-FFAP and DB-5 capillary columns with those of the respective reference compounds.

2.7. Quantitation of Selected Aroma Compounds by Stir-Bar Sorptive Extraction, Stable Isotope Dilution Analysis and Gas Chromatography-Mass Spectrometry

The gin samples were diluted individually 1:10 and 1:100 (v/v) in water, depending on the concentrations of the respective odorants, as estimated in a preliminary experiment. Subsequently, the isotopically labelled analogs (dissolved in 60% ethanol; amounts depending on the concentrations of the analytes) were added and the suspension was stirred at room temperature for 2 h. A Twister® (polydimethylsiloxane sorbent, 10 mm length, 0.5 mm coating thickness; Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) was added to the diluted sample and stirred at room temperature for 1 h before removal.

The SBSE Twister® bar was placed in a thermo-desorption unit (TDU) liner. Desorption commenced at a temperature of 40 °C (initial time: 0.5 min), then increased to 250 °C at a rate of 120 °C/min, before being held at 250 °C for 5 min. The extracted volatiles were transferred to the cold-injection-system (CIS), cooled at –100 °C using liquid nitrogen, then transferred onto the GC system (cf. Section 2.6) by thermal desorption. After an initial 0.1 min at 40 °C, the oven temperature was raised at 12 °C/min to 240 °C and held for 10 min. The column flow of the helium carrier gas was adjusted to 50 mL/min. Mass spectra were generated in full scan mode (m/z range 35–250, EI 70 eV).

Response factors were calculated for each odor-active compound by analyzing binary mixtures of defined amounts of the labeled standard and the unlabeled analyte at three different mass ratios (1+3, 1+1, and 3+1 v/v; see Table S1 in Supplementary Material) under identical conditions (cf. Section 2.6). Data evaluation was performed using Thermo Xcalibur 2.2 SP1.48 (Thermo Fisher Scientific GmbH, Dreieich, Germany).

2.8. Orthonasal Odor Thresholds

Orthonasal OTs of the key aroma compounds were determined in 45%vol. ethanol in water, and these values were subsequently used to calculate their respective OAVs, as described in the literature [29].

3. Results and Discussion

3.1. Sensory Evaluations

The aroma profiles of the two gins are plotted in Figure 1. Both gins were dominated by an *ethanolic* odor (not depicted), perceived with intensities of 7.0 and 5.3, respectively, for Gspusi and Kini; a lack of significance between these ratings do not allow conclusions to be drawn as to its higher rating in the gin with fewer botanicals, although it might be speculated that the use of many botanicals

elicits a more complex aroma of the gin that acts to reduce the ethanolic impression. Since the present study focuses on key aroma (impressions) in relation to the use of botanicals, however, the ethanolic impression will not be considered in the ensuing discussion (and therefore, this impression has been omitted from the aroma profile plot of Figure 1).

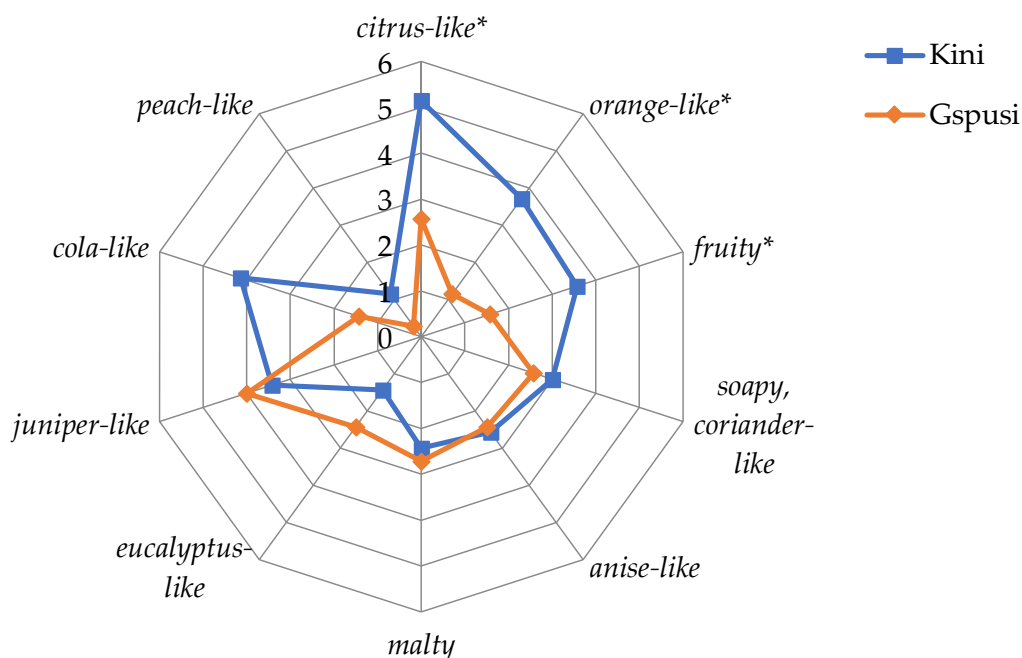


Figure 1. Comparative aroma profiles of the two gins, The King Gin Kini and Gspusi, perceived via orthonasal evaluation. Kini was produced with 50 botanicals, while Gspusi contained 15. Attributes marked with asterisks correspond to significant differences between the gins ($p < 0.05$), as determined by analysis of variance (ANOVA). Note that the intensity scale has been adjusted to cover the range of rated intensities (maximum rated intensity of 5.1), rather than the full range available (10). The ethanolic odor impression (5.3 for Kini, 7.0 for Gspusi) has been omitted from the profile to focus only on the aroma impressions related to the botanicals.

Kini exhibited an aroma profile that was dominantly *citrus-like* (5.1), *orange-like* (3.7) and *fruity* (3.6), with these aroma notes being rated with significantly higher intensities than in Gspusi (rated at 2.6, 1.1 and 1.6, respectively). The strong *cola-like* impression in Kini was only weakly perceived in Gspusi (respective ratings of 4.1 and 1.4), although the difference was not statistically significant due to the higher variance in the individual ratings across the panel. Based on the most intensely rated attributes, the aroma profile of Gspusi was dominantly *juniper-like* (4.0), which might be attributed to the use of fewer botanicals and an associated emphasis of this aroma note; *juniper-like* in Kini was rated with similar intensity (3.4), but this was less pronounced than some of the other aroma impressions (as discussed above). A *peach-like* note was rated with higher intensity for Kini than for Gspusi (1.1 and 0.3, respectively), whereas a *eucalyptus-like* impression was rated higher for Gspusi than for Kini (2.4 and 1.4, respectively), albeit in both cases without statistical significance. The aroma notes *soapy/coriander-like*, *anise-like* and *malty* were each rated with similar intensities in both gins (within the range 2.4–3.0).

In terms of the hedonic evaluations, both gins were rated positively, albeit with Kini being rated higher than Gspusi (6.5 and 5.1, respectively). Although this difference was not statistically significant, the trend nevertheless indicates a more pleasant overall aroma of Kini compared with Gspusi. This might be explained by the richer and more complex aroma of the former compared to the latter, or the less intense ethanolic note in Kini, or a combination of both. It should be noted, however, that like the attribute ratings, the hedonic evaluation was made orthonasally and not retronasally,

and preferences in flavor during consumption of the gins might be different. In a study that compared orthonasal and retronasal profiles of Chilean Pisco spirit, however, no significant differences were observed between these two methods of assessment [30].

3.2. Identification of Aroma Compounds

Overall, 48 aroma-active compounds were detected in the two gins, of which 38 were identified, as listed in Table 1. In AEDA, the FD factors relate to the last dilution in which an individual odorant is still perceivable during GC-O analysis, and these thereby indicate the most potent odorants in a sample [31]. It is worthy of note that compounds perceived at FD 2048 might still be perceivable at the next AEDA dilution, namely FD 4096 (not performed in the present study), hence the representation as ≥ 2048 to indicate that these compounds might be present at higher dilutions. The compounds in Table 1 are listed in order of decreasing FD factor with respect to their presence in Kini; as such, the most potent odorants (those with the highest FD factors) in Kini appear at the top of the table, whilst those in Gspusi appear throughout the table, although primarily also at or near the top.

Comparing the FD factors between the two gins, Kini contained the most compounds – eight in total – at the highest FD factor of ≥ 2048 , compared to two in Gspusi. Common compounds present at the highest FD factor of ≥ 2048 in both gins were (*Z*)-rose oxide and linalool, with *flowery*, *rose-like* and *flowery notes*, respectively. The additional FD ≥ 2048 compounds in Kini were *trans*-anethole, eugenol, limonene, δ -carene, octanal and coumarin, exhibiting odor impressions ranging from *anise-like*, *clove-like*, *orange/lemon peel-like*, *citrus-like*, *eucalyptus-like*, *soapy*, *coconut-like* and *cinnamon-like*.

The second highest FD factor of 1024 was represented by three compounds in Kini, and two compounds in Gspusi. Specifically, α -pinene, γ -terpinene and an unknown compound were present at FD 1024 in Kini, with odor impressions *rosiny* and *conifer-like*, *turpentine-like* and *soapy*, and *musty*, respectively. By comparison, the two compounds at FD 1024 in Gspusi were myrcene and (*E,E*)-2,6-nonadienal, with odor qualities of *earthy*, *metallic* and *geranium-like*, and *fatty* and *cucumber-like*, respectively. Kini and Gspusi respectively contained nine and five compounds at FD 512.

Previous studies have similarly identified terpenes, terpenoids and aldehydes in gin, specifically α -pinene, camphene, sabinene, δ -carene, myrcene, limonene, 1,8-cineole, γ -terpinene, *p*-cymene, (*Z*)-rose oxide, linalool, citronellal, citral, geraniol, as well as hexanal, octanal, nonanal and decanal via GC-O or GC-MS analysis [10,14,15], and estragole and eugenol by high performance liquid chromatography (HPLC) analysis [32]. A number of compounds detected in the present study have not been previously reported for gin and are reported here for the first time, specifically (*E*)-2-hexenal, 1-octen-3-one, 2-acetyl-1-pyrroline, 1-(*R*)-fenchone, trimethylpyrazine, 1-octen-3-ol, (*Z*)-2-nonenal, (*E,E*)-2,6-nonadienal, (*E,Z*)-2,6-nonadienal, (*E*)-2-decenal, (*E,E*)-2,4-decadienal, *trans*-anethole, 2-methoxyphenol, *p*-anisaldehyde, thymol, myristicin, coumarin and vanillin. The number and nature of aroma compounds present in gin closely reflect the number and type of botanicals used in their production. A richer diversity of aroma compounds, therefore, would be expected to be present in gins produced with a wide range of botanicals, as is the case for the Kini gin in this study, and to a lesser extent the Gspusi gin.

Table 1. Aroma-active compounds identified in distillates obtained from two different Bavarian gins, The King Gin Kini and Gspusi, and present with flavor dilution (FD) factors ≥ 4 in at least one of the gins. Compounds are listed in order of decreasing FD factor in relation to their presence primarily in Kini, then Gspusi.

RI ^a		Compound ^b	Odor Quality ^c	FD ^d Factor	
DB-FFAP	DB-5			Kini	Gspusi
1333	1114	(Z)-rose oxide	flowery, rose-like	≥ 2048	≥ 2048
1534	1102	linalool	flowery	≥ 2048	≥ 2048
1821	1289	trans-anethole	anise-like	≥ 2048	512
2151	1361	eugenol	clove-like	≥ 2048	512
1182	1025	limonene	orange peel-like, lemon peel-like	≥ 2048	256
1138	1014	δ -carene	citrus-like, eucalyptus-like	≥ 2048	128
1280	1002	octanal	citrus-like, soapy	≥ 2048	128
2445	1441	coumarin	coconut-like, cinnamon-like	≥ 2048	16
1013	945	α -pinene	rosiny, conifer-like	1024	512
1349	n.d. ^e	unknown	musty	1024	8
1247	1063	γ -terpinene	turpentine-like, soapy	1024	4
1157	993	myrcene	earthy, metallic, geranium-like	512	1024
1567	1153	(E,E)-2,6-nonadienal ^f	fatty, cucumber-like	512	1024
1382	1104	nonanal	soapy, citrus-like	512	512
1080	800	hexanal	grassy	512	256
1670	n.d. ^e	unknown	fatty, musty, nutty	512	256
1799	1327	(E,E)-2,4-decadienal	fatty, deep-fried	512	64
1487	1233	decanal	soapy	512	32
2017	1258	p-anisaldehyde	sweet woodruff-like, almond-like	512	8
1401	1022	trimethylpyrazine ^f	earthy, musty	512	4
1494	1145	(Z)-2-nonenal ^f	fatty, cardboard-like, green	256	64
1321	932	2-acetyl-1-pyrroline ^f	popcorn-like, roasty	256	2
1441	987	1-octen-3-ol	mushroom-like	128	1
1703	n.d. ^e	unknown	fatty, nutty	128	1
1291	979	1-octen-3-one	mushroom-like	64	128
1058	954	camphene	fruity, solvent-like	64	64
1110	978	sabinene	eucalyptus-like	64	16
1615	n.d. ^e	unknown	flowery, caramel-like, citrus-like, honey-like	64	16
1852	1087	2-methoxyphenol	smoky, smoked ham-like	64	<1
1198	1038	1,8-cineole	eucalyptus-like, menthol-like	32	4
1774	n.d. ^e	unknown	earthy, musty	32	4
2200	n.d. ^e	unknown	leather-like	32	4
1142	n.d. ^e	unknown	fruity	32	<1
2178	1297	thymol	thyme-like, rosemary-like	32	<1
1259	1029	p-cymene	oregano-like	16	256
1393	1098	1-(R)-fenchone	eucalyptus-like, menthol-like, musty	16	64
1210	854	(E)-2-hexenal	fruity, apple-like, banana-like	16	1
1469	1161	citronellal	citrus-like	16	4
1669	1240	citral	flowery, citrus-like	16	4
2539	1399	vanillin	vanilla-like	16	4
1597	n.d. ^e	unknown	cucumber-like, fatty, citrus-like	16	<1
1656	1198	estragole	anise-like, fennel-like, licorice-like	16	<1
2266	1532	myristicin	raw carrot-like, clove-like	16	<1
1390	n.d. ^e	unknown	citrus-like, green	8	512
1572	1195	(E,Z)-2,6-nonadienal ^f	cucumber-like	8	8
1830	1255	geraniol	flowery	<1	32
1230	n.d. ^e	unknown	fatty	<1	16
1629	1261	(E)-2-decenal ^f	coriander-like, fatty, waxy	<1	16

^a RI: (linear) retention index. ^b Odorants were identified by comparison of their odor quality and intensity, RI on DB-FFAP and DB-5 capillaries, as well as mass spectra (electron ionization mode at 70 eV) with data of respective reference compounds. ^c Odor quality perceived at the odor detection port (ODP) during gas chromatography-olfactometry (GC-O). ^d Flavor dilution (FD) factor determined in aroma-extract dilution assays (AEDA) via DB-FFAP capillary. ^e n.d.: not determined. ^f No unequivocal mass spectrum (electron ionization mode, 70 eV) was obtained; identification is based on the remaining criteria given in footnote b.

Comparing the sensory data with the volatile constituents identified, the high ratings of *citrus-like*, *orange-like* and *fruity* impressions for Kini are in good agreement with the aroma compounds exhibiting citrusy notes detected at high FD factors, specifically δ -carene, limonene (also described as orange peel-like), octanal, citronellal, and citral (each perceived with at least four times higher FD than

in Gspusi). Further *citrus-like* impressions are associated with the aldehyde nonanal (also *soapy*), which was present in both gins at the same FD (512), and an unidentified odorant (also described as *green*) with an RI 1390 on the DB-FFAP column that was similarly present at FD 512 in Gspusi but only at FD 8 in Kini. The strong *fruity* impression of Kini reported by sensory analysis might reflect the presence of the fruity smelling odorants camphene, (*E*)-2-hexenal, and an unidentified compound at RI 1142 on DB-FFAP, whereby the first compound was present at identical FD in both gins, whereas the other two were higher (at least twice as high) in Kini than in Gspusi. The *soapy, coriander-like* impression was rated similarly in both gins, yet the soapy compounds octanal and γ -terpinene were present at higher FD factors in Kini (FD \geq 2048 and FD 1024, respectively) than in Gspusi (FD 128 and FD 4, respectively). Similarly, the intensity ratings of the *anise-like* and *eucalyptus-like* notes in the two gins were not significantly different, yet the anise-like compounds estragole and *trans*-anethole were present at higher FD factors several times higher in Kini. In general, differences in the FD factors of individual aroma compounds of the two gins are likely associated with the use of diverse individual botanicals during gin production. Additionally, however, it is a well-known phenomenon that complex odorant mixtures may have suppressive, synergistic or additive interactions, leading to unpredictable effects. As such, although the presence of individual aroma compounds at high FD factors certainly elicit strong odor impressions, specific notes of complex mixtures cannot be solely represented by their single constituents [33–35].

Overall, terpenes and terpenoids were found to be the dominant odor-active compounds in the Kini and Gspusi gins. These classes of compounds are mainly formed in plants via the mevalonate pathway [36] and are well known aroma-active constituents in a large variety of essential oils from different botanicals used in gin production (Table S2), including juniper, citrus fruits and ginger. Comparing the gins, approximately 40% of the substances identified had higher FD factors in Kini than in Gspusi, which can be explained by the greater number and variety of botanicals used in the production of the former. By comparison, the saturated and unsaturated short chain aldehydes, including odorants such as hexanal, (*E*)-2-hexenal and (*E,E*)-2,6-nonadienal, are mainly formed by the degradation of fatty acids. Oleic, linoleic and linolenic acid are especially susceptible to autoxidation, which leads to the formation of unsaturated aldehydes and ketones, and subsequently saturated aldehydes [37]. In relation to the present study, FD factors of related compounds were either similar for both gins or higher for Kini with the exception of (*E*)-2-decenal. Citrus fruits are most likely the main source of the aldehydes found in the two gins, so differences in their respective potencies might be a reflection of different amounts used in the production of Kini and Gspusi.

The compounds eugenol and estragole, detected in both gins, are phenylpropanoids that are formed in plants via the shikimate biosynthetic pathway with phenylalanine as an intermediate [38,39]. Phenylpropanoids are also a common component of essential oils, besides terpenes, and represent the majority of naturally occurring phenolic phytochemicals or their precursors [38]. Botanicals with high amounts of phenylpropanoids were used in the production of Kini (botanicals listed in Table S2 in the Supplementary Material), which might explain the presence of these compounds in this gin, especially eugenol, which was present at the highest FD factor (\geq 2048).

3.3. Quantitation of Selected Aroma Compounds

Seven individual compounds, listed in Table 2, were chosen for quantitation in the gins via SBSE and SIDA. The selection of compounds for quantitation was made based on their presence at high or differing FD factors in the two gins.

Table 2. Concentrations of selected aroma-active compounds in The King Gin Kini and Gspusi gins, as determined via stir-bar sorptive extraction (SBSE) and stable isotope dilution analysis (SIDA), in comparison to values obtained from the literature.

Aroma Compound	Concentration [mg/L]						Literature
	Kini			Gspusi			
	Mean ^a	n ^b	Range ^c	Mean ^a	n ^b	Range ^c	
myrcene	0.82	3	0.75–0.91	2.71	4	2.55–2.88	2.28 ^d –11.09 ^e
limonene	3.83	3	3.69–4.06	7.03	4	6.46–7.89	1.22–17.21 ^e
1,8-cineole	7.53	5	6.89–8.29	0.26	5	0.25–0.26	n.a. ^f
nonanal	0.61	4	0.35–0.79	0.22	4	0.21–0.25	n.a. ^f
linalool	28.53	5	26.5–30.9	0.93	4	0.81–1.05	1.90 ^d –36.99 ^e
estragole	2.94	3	1.19–3.46	0.16	5	0.15–0.17	n.a. ^f
trans-anethole	4.70	5	4.29–5.29	1.26	5	1.23–1.29	n.a. ^f

^a Mean value calculated from *n* determinations; ^b Number of determinations; ^c Concentration range from *n* determinations; ^d Determined by headspace solid-phase micro-extraction gas chromatography-mass spectrometry [8]; ^e Determined by headspace solid-phase micro-extraction gas chromatography-mass spectrometry [14]; ^f Concentration of this compound in gin were not available in the literature.

Most of the selected aroma compounds quantified were generally present at higher concentrations in Kini than in Gspusi. Especially noteworthy were the *eucalyptus-like* smelling 1,8-cineole and the *flowery* smelling linalool, which were at concentrations almost 30 times higher in Kini than in Gspusi. Similarly, the *anise-like* or *fennel-like* smelling estragole was 20 times higher in Kini. These quantitative data appear to contradict the semi-quantitative results of AEDA, which showed only minor differences in intensities of 1,8-cineole and estragole between the two gins, and no difference for linalool. On the other hand, the AEDA data are in agreement with the sensory evaluations, whereby no significant differences in the attributes *anise-like* or *eucalyptus-like* between the gins were observed (*flowery* was not evaluated). Contradictory results like these have been previously reported and discussed in the literature [34]. Studies have shown that concentration does not linearly correlate with OAV of individual compounds when these are present at higher concentrations. Moreover, odor thresholds are significantly influenced by the presence of other odorants in the matrix, as previously mentioned in relation to synergistic or additive effects. Nevertheless, the OAV model is generally valid for odor-active compounds present at lower concentrations [34] and provides an indication of the importance of an odorant to the overall aroma, as explored in the next section.

The concentrations of the selected aroma compounds determined in the two gins in the present analysis are generally in good agreement with values reported elsewhere for gin, demonstrating that the present methods yield concentrations in comparative ranges to other approaches. Myrcene, for example, exhibited slightly lower or equivalent mean concentrations to those reported in the literature, with 0.82 mg/L and 2.71 mg/L here for Kini and Gspusi, respectively, compared to values ranging between 2.28 mg/L [8] and 11.09 mg/L [14] elsewhere. This was similarly the case for linalool, with mean concentrations of 0.93 mg/L in Gspusi and 28.53 mg/L in Kini compared to 1.9 mg/L [8] and 36.99 mg/L [14] reported in other studies. By comparison, limonene was found here to be fully within the range of the literature values, with mean concentrations of 3.83 mg/L and 7.03 mg/L for Kini and Gspusi, respectively, compared to between 1.22 mg/L and 17.21 mg/L reported in the literature [14].

As highlighted in the introduction, besides the common use of juniper, different gins are produced with a highly diverse number and type of botanicals to create their unique or characteristic individual flavor profiles. Accordingly, the exact composition of constituent aroma compounds vary across distilleries and for different gins depending on which botanicals are used. Further, the amount of each botanical used, as well as how each is processed and added during the production process, will affect the concentrations of individual aroma constituents. Many botanicals contain common aroma compounds, albeit in varying amounts, thus individual compounds can be rarely attributed solely to a single botanical. As such, the omission of any particular botanical during gin production

does not automatically lead to the absence of a specific constituent aroma compound in the gin, which might be imparted by another botanical ingredient. Further, in the present study, although a list of botanicals used in each gin was kindly provided by the distillery, the quantities implemented are proprietary information. As such, a detailed and comprehensive comparison of the quantitative or semi-quantitative (FD factor) differences of individual aroma compounds within each gin type or between the two gins in relation to the botanical ingredients cannot be made here.

3.4. Odor Activity Values

As mentioned above, OAVs are defined as the ratio of the concentration of an odorant in a sample to the odor threshold of that odorant, and these give an indication of the relative importance of an individual odorant to the overall aroma. The OAVs were determined for the seven quantified aroma compounds (see Table 2) using experimentally determined thresholds of the individual aroma compounds in 45%vol. ethanolic solutions (Table 3), with the exception of linalool for which a published literature value was used [20].

Table 3. Odor detection thresholds (OT_D) of seven selected aroma compounds as determined experimentally in 45%vol. ethanolic solutions, as well as the associated odor activity values (OAVs) calculated based on the concentrations of the individual compounds in the gins.

Compound	OT _D [μg/L 45% Ethanol]		OAV	
	Range	Mean	Kini	Gspusi
myrcene	77–479	101	8	27
limonene	647–10113	2804 ^a	1	3
1,8-cineole	37–35795	635	12	<1
nonanal	81–1263	254	2	<1
linalool	-	24 [20] ^b	1189	39
estragole	837–13082	2841	1	<1
<i>trans</i> -anethole	189–7392	748	7	2

^a Odor threshold was determined for (*R*)-limonene; ^b Odor threshold was determined for a racemate mixture in ethanol/water (40/60 by vol.).

Among the selected aroma compounds quantified, the *flowery* smelling linalool had the highest OAV and thereby contributed most to the overall aroma in both gins. Further, with an OAV of 1189 in Kini, this compound was more pronounced than in Gspusi (OAV 39), although its FD factor was identical in both gins (with the highest value of FD ≥ 2048 ; see Table 1), indicating its overall dominant contribution. The *eucalyptus-like* 1,8-cineole had the second highest OAV in Kini (OAV 12), but was not prominent in Gspusi (OAV <1), followed by the *earthy, metallic, geranium-like* smelling myrcene at OAV 8 in Kini, but this was more dominant in Gspusi, at OAV 27. This observation is reflected in the AEDA data, whereby 1,8-cineole exhibited a higher FD factor in Kini (FD 32 vs FD 4 in Gspusi), and conversely, myrcene was higher in Gspusi (FD 1024 vs FD 512 in Kini). The *anise-like trans*-anethole exhibited OAVs of 7 and 2 in Kini and Gspusi, respectively, indicating its higher contribution to the overall aroma of the former compared to that of the latter; again, the AEDA data reflect this observation, with respective FD factors of ≥ 2048 and 512 for Kini and Gspusi. OAVs of the other compounds ranged from <1 to 3 in the two gins, reflecting varying minor contributions to the aroma. Although these OAVs provide indications of the contributions of individual odorants to the overall aroma of a sample, the significance of their impact on the odor profile cannot be inferred from these data, but rather require additional recombination and omission experiments [40]. Using the latter approach it has been shown that odor qualities of a less intense aroma compound can be entirely suppressed in the presence of a more intense odorant [34], thereby highlighting the complexity of odor interactions and how these affect perception.

4. Conclusions

The production of gin involves the use of juniper and any number of additional botanicals, each of which imparts its characteristic aroma attributes to the gin through transfer of constituent odor-active compounds. Through use of specific botanicals, the aroma character of the resulting alcoholic spirit can be affected to reflect desired traits. Examining gin via sensory evaluations in combination with instrumental analyses, such as GC-MS/O, specifically via AEDA, allows the perceived aroma notes to be compared with the individual aroma compounds. In the present work, two commercial London dry gins produced with different varieties of botanicals were studied. Overall, 48 aroma-active compounds were detected in the gins, of which 38 could be unequivocally identified. The compounds (*E*)-2-hexenal, 1-octen-3-one, 2-acetyl-1-pyrroline, 1-(*R*)-fenchone, trimethylpyrazine, 1-octen-3-ol, (*Z*)-2-nonenal, (*E,E*)-2,6-nonadienal, (*E,Z*)-2,6-nonadienal, (*E*)-2-decenal, (*E,E*)-2,4-decadienal, *trans*-anethole, *p*-anisaldehyde, thymol, 2-methoxyphenol, myristicin, coumarin and vanillin are reported here for the first time in gin. Sensory analysis revealed that the *citrus-like*, *orange-like* and *fruity* aroma notes were the dominant attributes in The King Gin Kini gin, which was produced with 50 botanicals, compared to The King Gin Gspusi gin, produced with 15 botanicals. The semi-quantitative AEDA data agreed with the aforementioned dominant sensory impressions. Further, the data indicated importance dominance of the *flowery*-smelling compounds (*Z*)-rose oxide and linalool and the *spicy*-smelling compounds eugenol (*clove-like*), *trans*-anethole (*anise-like*) and coumarin (*cinnamon-like*), with the latter substances being more important in Kini. Quantitative analysis and calculation of OAVs for seven representative odorants revealed linalool to dominate both gins, followed by 1,8-cineole in Kini and myrcene in Gspusi (albeit only in relation to the selected compounds).

The increasing popularity of gin with consumers in recent years has led to a growth in the market, with the emergence of new craft gin distilleries and a substantial diversity of available gins. Part of this growth can be attributed to its favorable production, whereby the flavor of the final product is primarily driven through use of different botanicals, rather than lengthy maturation processes, e.g., as required in whisky manufacture. As such, gin can be produced on relatively short timescales and its flavor can be readily – and substantially – adjusted by varying the ingredients. As these ingredients are the key driver of aroma profiles of artisan gins, the methods outlined in the present work to characterize constituent aroma compounds in relation to sensory profiles provide craft gin distillers with a useful tool to understand gin flavor in relation to key aroma compounds, and to thereby adjust the botanicals implemented to achieve the desired impressions in the final product.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2076-3417/10/20/7269/s1>, Table S1: Selected ions (*m/z*) of analytes, stable isotopically labeled standards, and response factors. Table S2: Aroma-active compounds and their occurrence in botanicals used in the production of the gins investigated in the present study. Botanicals marked grey were only used for the production of Kini.

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