LabWorks APPNOTE

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Volatile Organic Compound and Sensory Profiles of Alcoholic versus Non-Alcoholic Beer Using Immersive Twister® and TF-SPME Extraction

Megan C. Harper, Nicole Kfoury, and Jackie Whitecavage

GERSTEL, Inc., 701 Digital Drive, Suite J, Linthicum, MD 21090, USA

Keywords

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Abstract

Non-alcoholic (NA) beers have recently increased in the market as consumers look towards healthier drink options. To gain consumer acceptance, brewers must create a product that matches the flavor of the alcoholic version and is absent of off-odors. Volatile organic compounds (VOCs) are significant contributors to beer's overall flavor, and the ability to determine differences in these profiles can help brewers enhance the quality of NA beers. This study compared the VOCs of NA beers to their respective alcoholic beers. Differences in the VOC and sensory profiles were determined using immersive Twister stir bar sorptive extraction (SBSE) and thin film solid phase microextraction (TF-SPME) with the Olfactory Detection Port (ODP 4).

Introduction

Beer contains a wide range of VOCs formed during brewing, including esters, ketones, acids, and terpenes. Therefore, each beer's ingredients and brewing methods will produce unique aromas [1]. However, when beers are dealcoholized, many of these unique aromas are lost in the process. Common methods to dealcoholize beer include vacuum distillation, filtration, and modified fermentation, where the former two preserve more of the original flavor, and the latter inhibits flavor formation and requires flavor additives [2]. Studies have shown that NA beers have lower concentrations of isoamyl acetate, esters, alcohols, fatty acids, and phenolic components than alcoholic versions [3,4]. Small and large breweries only began to widely introduce non-alcoholic beers to the market around 2010 [5]. Since their introduction, these beverages have seen a swift rise in popularity. To enhance product development and further grow this market, it's vital to distinguish the varying flavor profiles between the two types of beers.

Simultaneous immersion of Twister and TF-SPME devices is a proven technique for effectively extracting volatile and semi-volatile compounds [6]. Due to the additional surface area and phase volume, extremely low detection limits are achievable compared to traditional techniques like SPME. Moreover, the TF-SPME membrane coated with the PDMS/HLB phase will extract the widest range of compounds, making it ideal for non-targeted analyses [7]. Therefore, a simultaneous extraction using PDMS Twister and PDMS/HLB TF-SPME is optimal for comparing flavor compounds found in alcoholic and NA beers.

In this study, alcoholic and NA beers were analyzed to determine differences produced by the dealcoholization processes. An immersive TF-SPME/Twister technique was used to extract and concentrate analytes from the samples. Peak areas were determined for each compound identified to compare flavor component differences in alcoholic and non-alcoholic varieties. GERSTEL's ODP 4 allows the analyst to identify which flavor compounds detected in the samples are sensory-active and differentiate between the alcoholic and NA beers. The ODP 4 can also selectively trap regions of interest while eliminating the rest of the sample matrix to aid in compound identification.

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Experimental

Instrumentation

GERSTEL MPS LabWorks Platform with ODP 4 and Agilent 8890 GC/5977B Inert plus.

Analysis Conditions

LabWorks Platform

TF-SPME	PDMS/HLB
Twister	PDMS
TDU 2	solvent vent/dry purge vent time 3.33 min 40 °C, 60 °C/min to 60 °C (3 min), 100 °C/min to 250 °C (5 min)
CIS 4	solvent vent (50 mL/min), split 50:1 -120 °C, 12 °C/sec to 275 °C (3 min)

Agilent 8890 GC

Column	30 m HP-5MS UI (Agilent) d_i=0.25 mm, d_f=0.25 µm
Pneumatics	He, P_i = 7.07 psi Constant Flow 1.0 mL/min
Oven	40 °C (1 min), 10 °C/min to 280 °C (3 min)

Agilent 5977 MSD

full scan	40 – 350 amu

Sample Preparation

Alcoholic and NA versions of a pilsner, IPA, and stout were purchased from a local liquor store. The pairs of each beer variety were of the same brand and style to ensure the comparison was equivalent.

A 10 mL aliquot of each beer was transferred to 10 mL screw-capped vials. A PDMS Twister stir bar was immersed in each sample. A PDMS/HLB TF-SPME membrane was suspended in the vials with a holder. The vials were placed on a GERSTEL Twister 20 position stir plate at room temperature. The samples were stirred at 1100 rpm for 1 hour. After extraction, the Twister and TF-SPME devices were removed, rinsed with water, and blotted dry before placing each in an empty TD tube. The TD tubes were sealed with a transport adapter and placed in a 40-position tray on the MPS LabWorks Platform system for automated analysis.

Sample Introduction

Samples were desorbed in solvent vent/dry purge mode with a 50 mL/min helium flow at 250 °C for 5 minutes. Analytes were cold trapped in the CIS 4 inlet at -120 °C on a glass bead-filled liner. When desorption was complete, analytes were transferred to the column in split mode (50:1) by rapidly heating the inlet to 275 °C.

Olfactometry

When GC-O analysis was performed, the column effluent was split 2:1 between the ODP 4 and MS, respectively. The ODP transfer line was heated to 250 °C. The mixing chamber was heated to 150 °C and purged with humidified nitrogen to prevent olfactory fatigue and nasal dehydration.

Results and Discussion

Figure 1 shows the stacked view of total ion chromatograms obtained for 3 replicates of the pilsner alcoholic beer. This provides a visual of the good reproducibility obtained for the analysis. Figure 2 shows the stacked view of total ion chromatograms obtained for the alcoholic (top) and NA (bottom) pilsners. Table 1 shows the relative peak areas of the compounds identified in both, normalized to the alcoholic version. In the alcoholic pilsner, ethanol, several esters, isoamyl alcohol, fatty acids, and phenylethyl alcohol were identified. Compounds such as hexanoic, octanoic, and decanoic acids are released into the beer during maturation and add creamy, cheesy, waxy, and goat-like characteristics. These short-chain fatty acids exhibit poor peak shape on the non-polar column phase used in this study, but the peaks are reproducible and analytically useful. Esters are released in the beer during fermentation, often producing fruity notes exhibiting banana and pear-like qualities [8]. In the NA pilsner, ethanol was removed via a vacuum distillation dealcoholization process. By distilling under a vacuum, ethanol can be removed at a lower temperature, but heat is still applied. While it is gentler than a standard distillation, volatiles can still be lost in the process and other compounds, such as furans, can be produced due to Maillard reactions [3]. To compensate for the volatiles lost during the dealcoholization process, brewers will add flavorings, which are often diluted in food-grade propylene glycol or glycerin. New compounds found in the NA pilsner include propylene glycol, 4-vinylguaiacol, γ-nonalactone, 2-furanmethanol, and 5-hydroxymethylfurfural. 4-Vinylguaiacol and γ-nonalactone are common flavor compounds in beer that can be added after dealcoholization and 2-furanmethanol and 5-hydroxymethylfurfural are furans, which are common Maillard reaction products [3].

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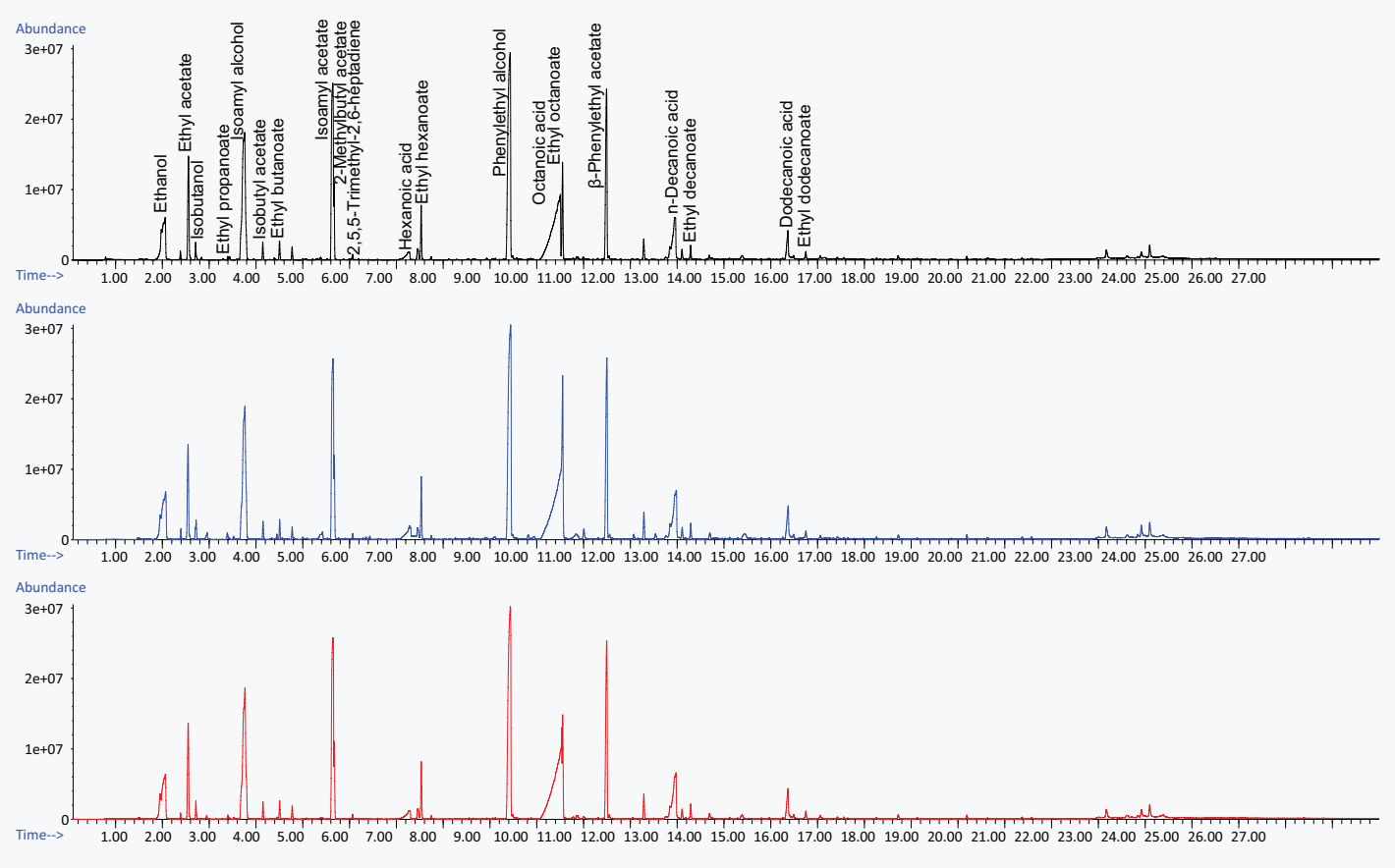

Figure 1: Stacked view of pilsner alcoholic replicates.

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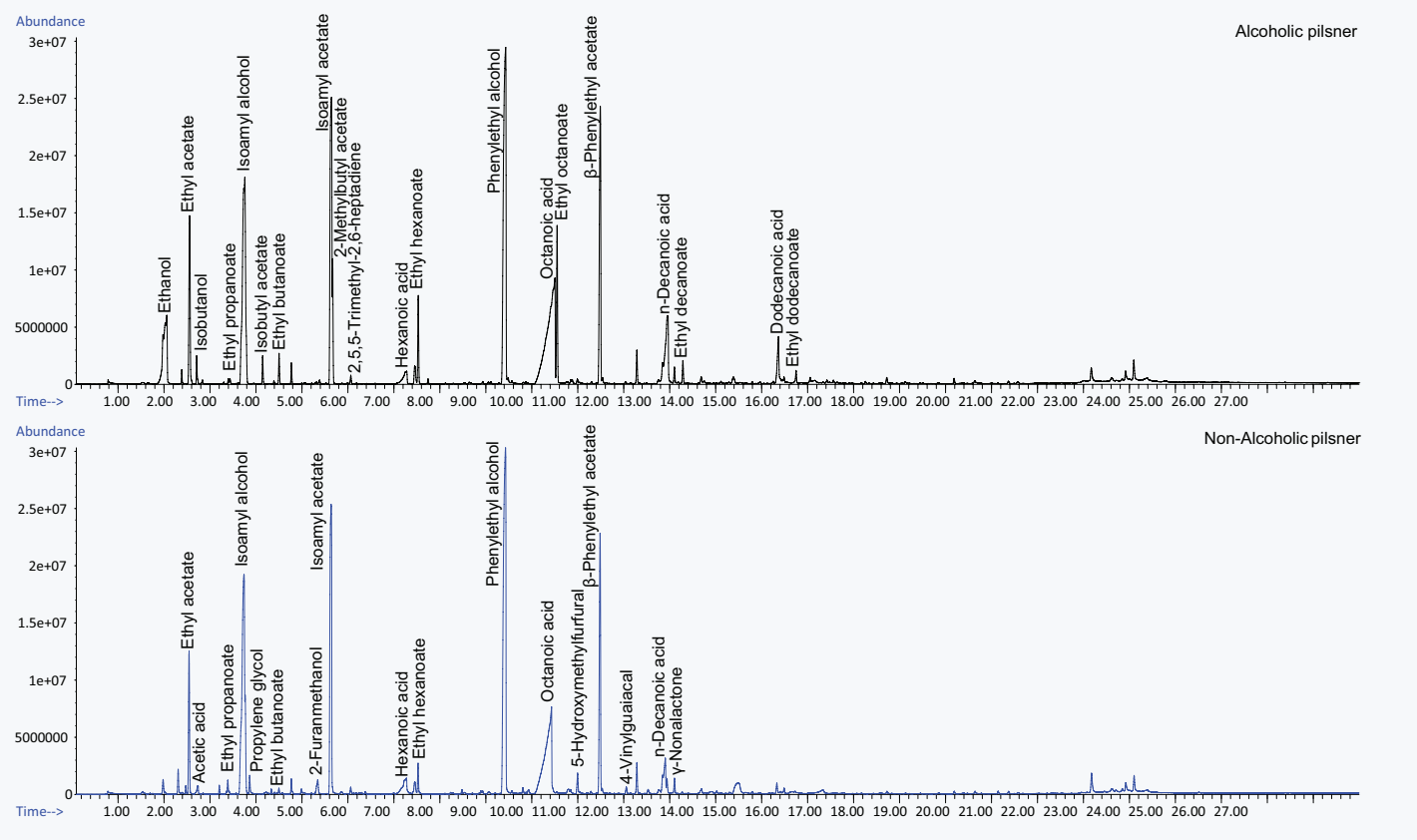

Figure 2: Stacked view of alcoholic (top) and NA (bottom) pilsners.

Table 1: Compounds identified in both the alcoholic and NA pilsners and the relative peak areas for n=3.

Compound	m/z	Alcoholic Pilsner	NA Pilsner
Ethyl acetate	43	100	77.5
Ethyl propanoate	57	100	263.9
Isoamyl alcohol	55	100	103.8
Ethyl butanoate	71	100	19.3
Isoamyl acetate	43	100	93.4
Hexanoic acid	60	100	99.1
Ethyl hexanoate	88	100	33.4
Phenylethyl alcohol	91	100	102.3
Octanoic acid	60	100	65.7
β-Phenylethyl acetate	104	100	84.8
n-Decanoic acid	73	100	35.4

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Figure 3 shows the stacked view of total ion chromatograms obtained for the alcoholic (top) and NA (bottom) IPAs. IPAs are made with more hops during the brewing process, thus generating additional flavor compounds in the resulting chromatographic profiles. In the alcoholic IPA, ethanol, esters, fusel oils, fatty acids, phenylethyl alcohol, and terpenes were identified. In the NA IPA, the beer was fermented to less than 0.5% alcohol by volume to retain the integrity of the alcoholic IPA's flavor profile, fermentation technique, and ingredients. However, differences remained in the resulting chromatogram. Fusel oils and some of the esters, terpenes, and fatty acids remained in the non-alcoholic IPA, but ethyl butanoate, isobutyl acetate, hexanoic acid, and citronellol could not be accurately identified. However, new compounds like isomaltol, α-terpineol, and 5-hydroxymethylfurfural were identified. Like the pilsner, these differences are all characteristic of NA varieties and significantly impact the resulting flavor. Table 2 shows the relative peak areas of the compounds identified in the alcoholic and NA IPAs, normalized to the alcoholic version. All compounds exhibited reduced peak areas in the NA IPA except hexadecanoic acid which showed a significantly higher peak area.

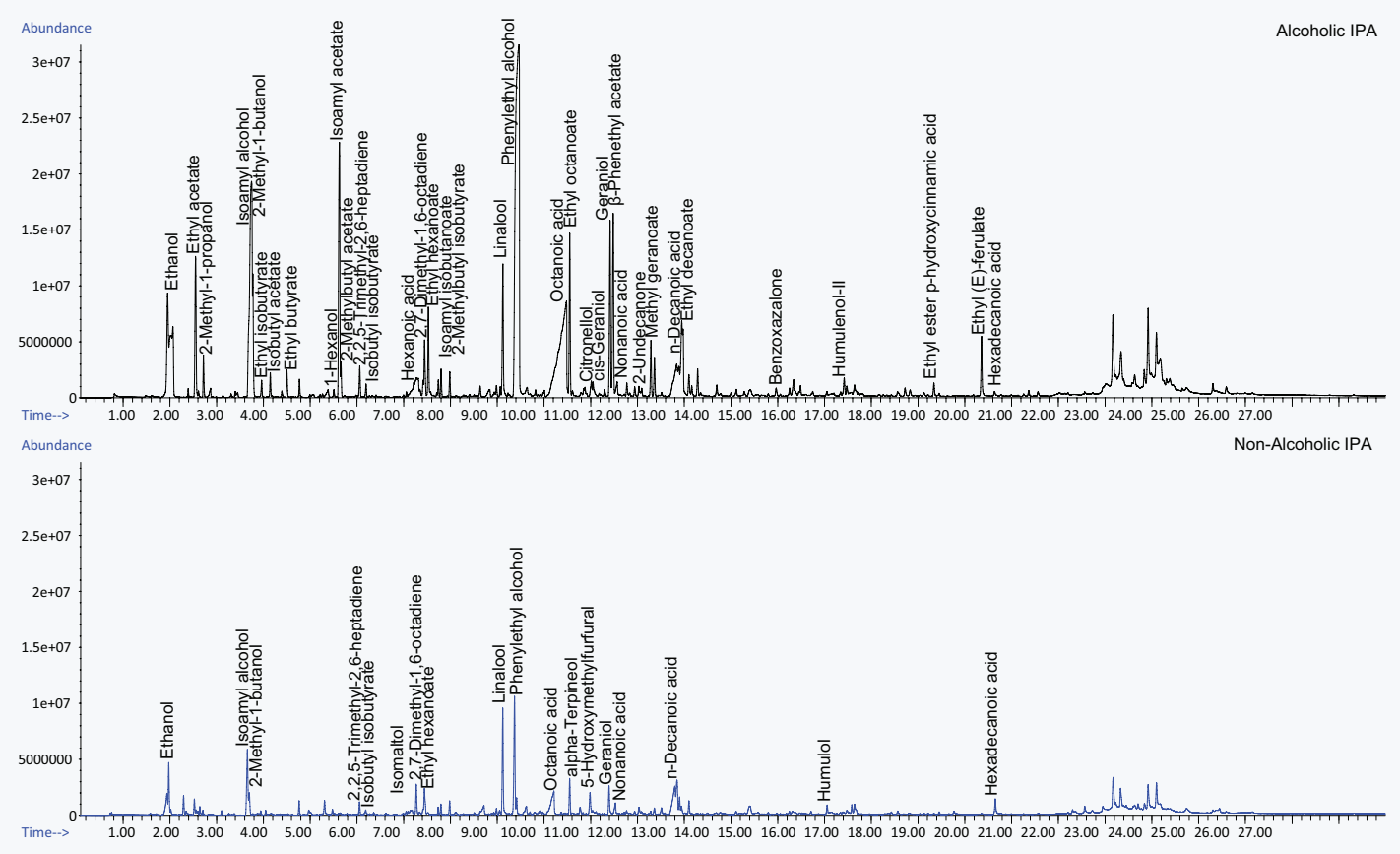

Figure 3: Stacked view of alcoholic (top) and NA (bottom) IPAs.

Table 2: Compounds identified in both the alcoholic and NA IPAs and the relative peak areas for n=3.

Compound	m/z	Alcoholic IPA	NA IPA
Ethanol	45	100	16.9
Isoamyl alcohol	55	100	12.1
2-Methyl-1-butanol	57	100	16.0
2,2,5-Trimethyl-2,6-heptadiene	69	100	45.2
Isobutyl isobutyrate	71	100	37.5
2,7-Dimethyl-1,6-octadiene	69	100	49.7
Ethyl hexanoate	88	100	1.5
Isoamyl isobutanoate	43	100	47.6
2-Methylbutyl isobutyrate	43	100	41.9
Linalool	71	100	84.1
Phenylethyl alcohol	91	100	12.4
Octanoic acid	60	100	12.5
Geraniol	69	100	16.7
Nonanoic acid	60	100	98.9
n-Decanoic acid	73	100	8.8
Hexadecanoic acid	73	100	647.2

Figure 4 shows the stacked view of total ion chromatograms obtained for the alcoholic (top) and NA (bottom) stouts. Stouts are commonly made with roasted barley making them darker in appearance and creamier and dessert-like in flavor. In the alcoholic stout, fusel oils, esters, fatty acids, phenylethyl alcohol, tryptophol, and 4-vinylguaiacol were identified. Tryptophol is a sleep-inducing secondary alcohol produced during the fermentation process, and 4-vinylguaiacol is a natural product found in coffee beans offering smoky, spicy, vanilla-like organoleptic properties [9]. The NA stout exhibited all the same compounds with the addition of propylene glycol and acetophenone. Table 3 shows the relative peak areas of the compounds identified in both the alcoholic and NA stouts, normalized to the alcoholic version. Fewer differences were seen between the stouts because the manufacturer used a mild, cold filtration, dealcoholization approach to eliminate ethanol. While the absence of heat in the filtration process prevents the formation of Maillard reaction products, compounds may be lost in the filtration process. The lost compounds were added back by the brewer, to ensure the taste is comparable to the alcoholic version. Although the esters exhibited reduced peak areas in the NA stout, several compounds showed comparable and, in some cases, higher peak areas.

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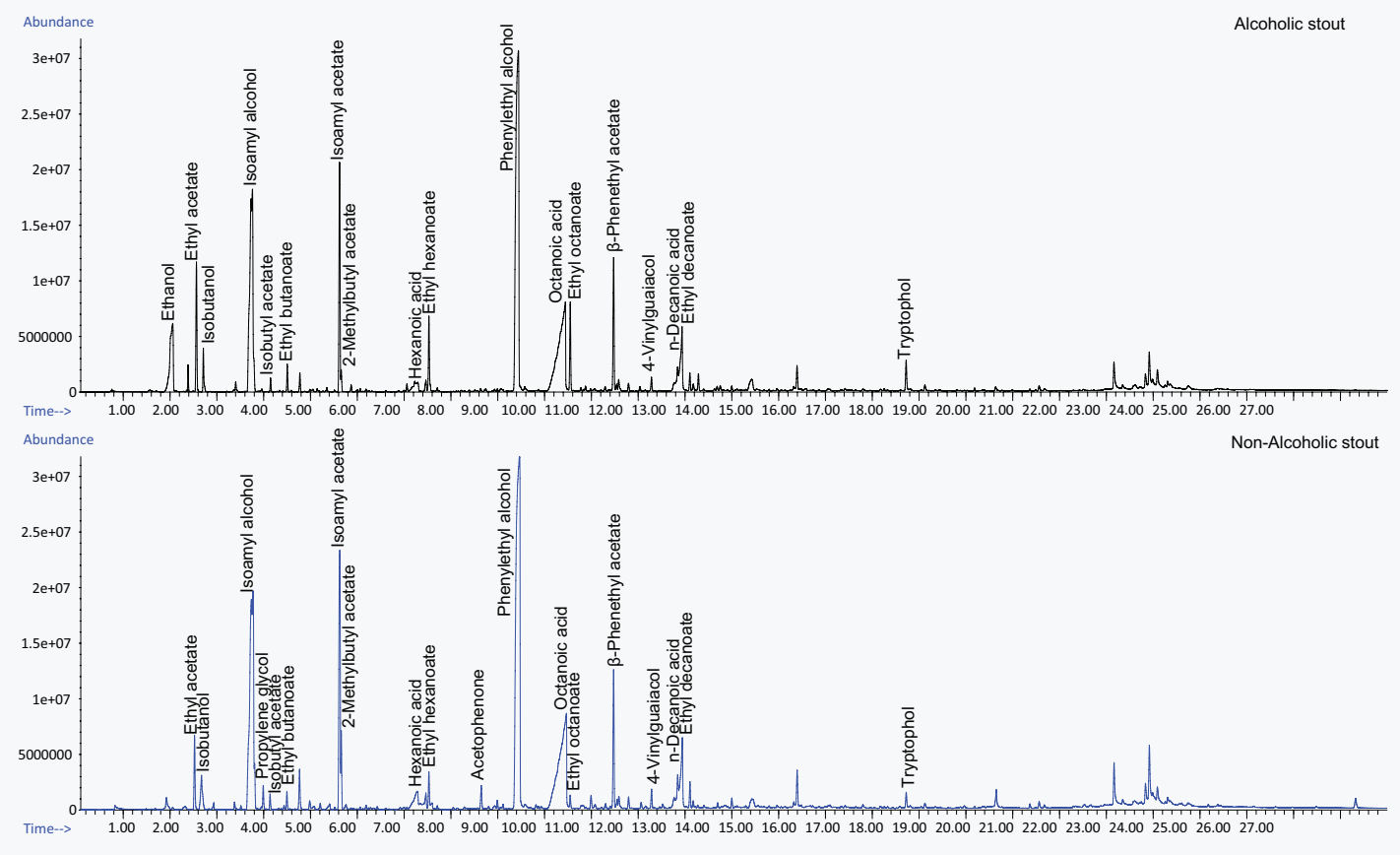

Figure 4: Stacked view of alcoholic (top) and NA (bottom) stouts.

Table 3: Compounds identified in both the alcoholic and NA stouts and the relative peak areas for n=3.

Compound	m/z	Alcoholic Stout	NA Stout
Ethyl acetate	43	100	50.3
Isobutanol	43	100	163.2
Isoamyl alcohol	55	100	288.5
Isobutyl acetate	43	100	109.9
Ethyl butanoate	71	100	61.7
Isoamyl acetate	43	100	108.1
2-Methylbutyl acetate	43	100	185.7
Hexanoic acid	60	100	103.2
Ethyl hexanoate	88	100	46.5
Phenylethyl alcohol	91	100	120.5
Octanoic acid	60	100	111.6
Ethyl octanoate	88	100	12.9
β-Phenethyl acetate	104	100	101.9
4-Vinylguaiacol	150	100	125.0
n-Decanoic acid	73	100	114.1
Tryptophol	130	100	46.1

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To further investigate the differences in the IPAs, sensory data was obtained. The IPAs were chosen as the alcoholic and NA varieties were the most different in terms of the number of compounds identified and overall aroma complexity. First, two panelists tasted the samples side-by-side to identify sensory differences. In the NA IPA, there was a key flavor described as fatty acid/onion that was not detected in the alcoholic variety. The two panelists then evaluated the IPAs at the ODP 4, and the combined odor descriptors are shown in Table 4. The sensory data are comparable for some retention time regions, but there remained several areas where the dealcoholization process produced clear differences. Specifically, the odor region described as sweaty, body odor, and red onion between 9.32 and 9.40 minutes, bolded in red in Table 4, was only detected in the NA IPA and was representative of the key odor detected in the sensory panel. However, it did not correlate with an identifiable peak at the MS.

Table 4: ODP report for alcoholic and NA IPAs from two panelists.

Start RT [min]	Stop RT [min]	Alcoholic IPA	NA IPA
2.72	2.79	musty, sulfur, fecal	musty
3.99	4.04	fishy, musty	musty
4.26	4.38	musty, sulfur, vegetal	sulfur, vegetal
4.92	4.99	malty	malty
5.2	5.26	fruity, tropical, berry	fruity, bubblegum
5.82	5.89	fruity, fermented	n.d.
5.98	6.04	n.d.	fruity, musty
6.04	6.06	skunky	skunky
6.17	6.27	skunky	skunky, burnt, coffee
6.41	6.57	musty, fruity, fermented, fatty acid, sweaty, cheesy	fatty acid, sweaty, fruity, fermented, cheesy
6.58	6.69	fruity, tropical, red fruit	fruity, bubblegum, tropical, berry, sweat, fermented
6.78	6.84	musty, fatty acid, sweaty	n.d.
6.95	7.04	grainy, yeasty	grainy, yeasty, corn chip
7.00	7.07	banana	n.d.
7.55	7.61	potato, earthy	potato, earthy
7.64	7.68	skunk	skunky, roasted, coffee
8.11	8.25	fruity	fruity, mango, tropical, burnt, rancid beer
8.45	8.47	fruity	fruity
8.68	8.72	earthy	earthy, mushroom
8.74	8.82	sweaty, floral	cotton candy, berries, floral
8.96	8.99	fruity, grass	n.d.
8.91	9.13	sweaty, fatty acid, body odor	sweaty, fatty acid, body odor
9.32	**9.40**	**n.d.**	**sweaty, body odor, red onion**
9.68	9.71	fruity	fruity
9.82	9.89	floral, rose	floral
9.9	9.93	earthy, grainy	grainy, vegetal
9.99	10.20	cotton candy, fruity	cotton candy, fruity
10.2	10.3	n.d.	chemical, plastic, skunky
10.37	10.53	sweaty, fatty acid	sweaty, fatty acid
10.42	10.47	n.d.	earthy, grainy
10.57	10.63	floral, fruity, fruit loops	floral, mint, fruit loops
10.73	10.83	n.d.	earthy, vegetal
10.77	10.9	maple, fruity, aldehydic	caramel, mint

Table 4 (cont.): ODP report for alcoholic and NA IPAs from two panelists.

Start RT [min]	Stop RT [min]	Alcoholic IPA	NA IPA
10.88	10.98	floral, powdery	floral, rose
11.30	11.35	floral, chemical, swimming pool	n.d.
11.46	11.5	n.d.	earthy
11.61	11.69	musty, papery, plastic, waxy	papery, cardboard
11.84	11.87	grainy	grainy
11.99	12.04	n.d.	vegetal
12.41	12.45	mint	n.d.
12.71	12.78	floral	floral, anise, spice
12.8	12.85	n.d.	floral, powdery
12.85	12.95	floral, soapy, lemon, citronella	floral, perfumy, soapy, fruity
13.28	13.32	n.d.	brown spice
13.44	13.5	musty, skunky, chemical	chemical, burnt
13.57	13.6	n.d.	woody
13.72	13.76	floral, fruity, grape	woody, cardboard
13.78	13.87	brown spice, clove	brown spice
14.55	14.6	n.d.	woody
14.72	14.78	fruity, juicy, strawberry	fruity
14.88	14.91	musty, barn, hay	musty, barnyard, hay

Note: n.d. = not detected

To identify this key odor region, more mass on column was achieved by trapping multiple extractions at the ODP. Five extractions, over a series of injections, of the NA IPA were used to trap the odor region between 9.32 and 9.40 minutes onto a single Tenax TA thermal desorption tube. Upon reintroduction of the trapped region, a small peak was visible. Figure 5 shows the zoomed-in overlay of the NA IPA chromatogram with the fivefold trapped region used for compound identification. In the trapped chromatogram, the small peak was identified as 3E-hexenoic acid. A standard of 3E-hexenoic acid was analyzed to confirm that the retention time, mass spectrum, and odor matched that of the compound in the sample.

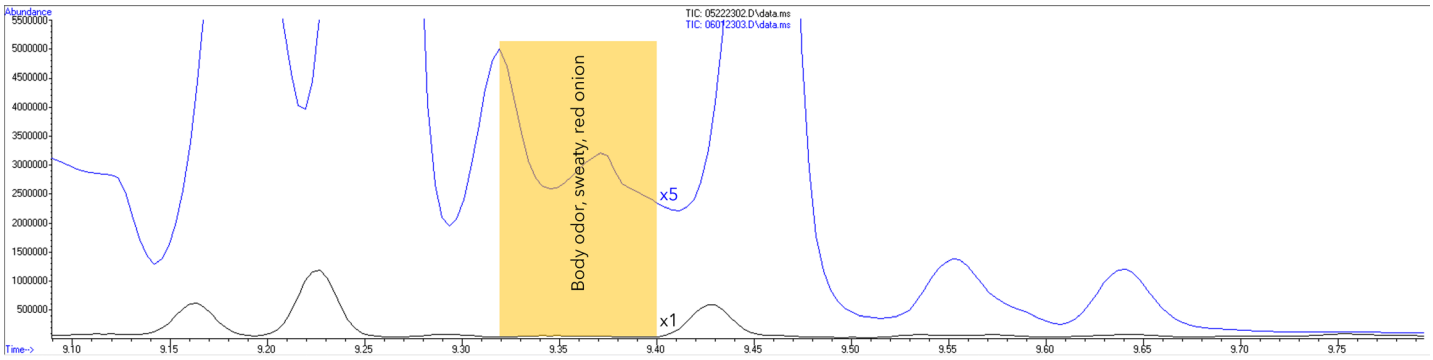

Figure 5: Overlay of non-alcoholic IPA extracted 5x, trapped, and reintroduced vs. a 1x extraction.

Conclusion

Immersive extraction using the GERSTEL Twister and TF-SPME membranes allows for efficient and optimal pre-concentration of essential flavor compounds from alcoholic and NA beer varieties. The extraction devices' ability to sorb the broadest range of analytes and deliver the most mass on column with reproducible results provided a simple means to obtain detailed flavor profiles of beer. The data provided offers a clear depiction of variations in peak areas, the depletion of esters, and the presence of new compounds, such as carrier solvents and furans, within the non-alcoholic (NA) counterparts. These distinctions arise due to the implementation of three distinct dealcoholizing methods. The ODP 4 and its selective trapping capabilities allowed for the increased mass on column while removing the rest of the sample matrix to produce an identifiable peak for a key sensory-active component. Having access to this kind of information is extremely valuable when it comes to enhancing and advancing product quality and development.

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