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Characterizing Volatile and Nonvolatile Factors Influencing Flavor and American Consumer Preference toward Nonalcoholic Beer

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ABSTRACT: In this study, the chemical and sensory profiles of 42 different nonalcoholic beer (NAB) brands/styles already on the global market and produced through several different brewing techniques were evaluated. A trained panel (i.e., 11 panelists) performed standard-driven descriptive and check-all-that-apply analyses in triplicate to sensorially characterize the aroma and taste/mouthfeel profiles of 42 commercial NABs, a commercial soda, and a commercial seltzer water (n = 44). These beers were also chemically deconstructed using several different analytical techniques targeting volatile and nonvolatile compounds. Consumer analysis (n = 129) was then performed to evaluate the Northern Californian consumer hedonic liking of a selection (n = 12) of these NAB brands. These results provide direction to brewers and/or beverage producers on which techniques they should explore to develop desirable NAB offerings and suggest chemical targets that are indicators of specific flavor qualities and/or preference for American consumers.
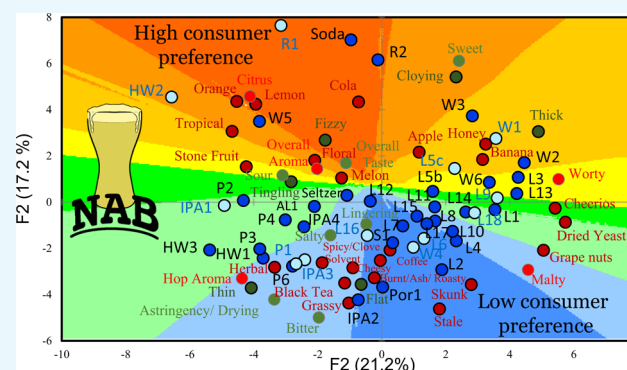
1. INTRODUCTION

In the past, in the United States, the nonalcoholic beer (NAB) or near beer category has been extremely small (accounting for ∼0.3% of total off-premise beer sales (∼$29 billion market) in 2018).[1] However, the global market for NAB is projected to double by 2024.[2] This increase is because several multinational companies and craft breweries are beginning to prioritize developing nonalcoholic, low alcohol, and alcohol-free brands to cater to nondrinkers, Millennials, and Generation Z consumers who are seeking healthier alternatives to alcohol.[3] However, despite the recent increase in interest, NABs have been produced for human consumption for over a century.[4,5] During the late 19th/early 20th century, these products were generally produced as alternatives to alcohol to prevent soldiers from getting excessively drunk or as a way for companies to possibly make nonexcisable fermented beverages.

The current legal definition of NAB depends on the country where the product is made. The legal definition of NAB, in the United States, is a beverage that contains less than 0.5% alcohol by volume (ABV),[6] while in Spain, it can be a beverage that contains less than 1.0% ABV. The term "alcohol-free" may be used only when the product contains no detectable alcohol. In general, the two main methods to produce NAB or alcohol-free beer are biological and/or mechanical, and these methods can either be used individually or in combination.[7]

Historically, biological methods have been the go-to approach because they require little advanced technology, and most breweries can implement these strategies with existing equipment. A recent survey of Czech NABs found that a majority, ∼86% of the 30 brands surveyed, are still produced by this methodology.[7] The goal of these biological methods is to limit alcohol production by either constructing an unfermentable/slightly fermentable wort via the utilization of different malting or mashing techniques and/or by arresting or restricting the fermentation from going past a certain attenuation level so that only a limited amount of alcohol is produced. However, these methods can generally result in NABs which are perceived to be too sweet because of their high residual extract and too worty because of the Strecker aldehydes extracted from the malt.[8]

Although it is not a new approach, another biological technique currently garnering a lot of attention and research focus is utilizing and finding non-Saccharomyces yeast strains that have limited or no ability to ferment maltose or

Table 1. NAB, Soda, and Seltzer Basic Nonvolatile Quality Specifications[a]

Product Code[b]	Brewery	Brewery Location	Brand Style[c]	Density (g/cm³)	Er (%w/w)	ABW (%w/w)	pH	TA (lactic %)	BU	CO₂ (g/L)	Color (EBC)	Turbidity (EBC)
AL1	25	CA, USA	Kolsch	1.0017	1.00	0.19	4.36	0.068	9.1	4.87	11.0	4.34
HW1	14	CA, USA	Hop water	0.9990	0.21	0.01	5.27	0.038	26.7	5.41	2.0	20.30
HW2	19	CA, USA	Hop water	0.9992	0.27	0.02	3.68	0.082	8.6	5.77	0.8	0.93
HW3	16	CO, USA	Hop/tea water	1.0001	0.49	0.00	5.11	0.069	18.1	5.14	10.3	3.70
IPA1	5	CT, USA	IPA	1.0180	5.20	0.34	4.31	0.216	45.4	4.79	19.3	21.95
IPA2	25	CA, USA	Red IPA	1.0044	1.76	0.33	4.13	0.125	20.7	4.54	82.0	31.20
IPA3	21	ON, CAN	IPA	1.0044	1.73	0.29	4.05	0.112	56.9	5.36	21.0	11.45
IPA4	25	CA, USA	Hazy IPA	1.0042	1.63	0.19	4.61	0.046	12.4	4.43	27.1	132.00
L1	1	HB, DE	Pilsner	1.0251	6.83	0.04	4.54	0.135	21.9	4.88	7.4	2.26
L2	7	RP, DE	Pilsner	1.0226	6.21	0.06	4.52	0.213	26.1	4.72	10.2	0.44
L3	7	RP, DE	Pilsner	1.0337	8.92	0.00	5.00	0.159	20.1	4.83	8.2	0.72
L4	26	NW, DE	Pilsner	1.0218	6.16	0.33	4.68	0.154	20.4	5.16	9.3	0.66
L5b	15	NH, NL	Lager	1.0189	5.28	0.02	4.40	0.164	14.0	4.95	7.7	0.67
L5c	15	NH, NL	Lager	1.0190	5.31	0.03	4.43	0.156	13.1	4.86	7.4	0.74
L6	2	MO, USA	Golden	1.0137	4.14	0.39	4.38	0.172	10.2	5.33	9.3	0.64
L7	27	MO, USA	Amber	1.0154	4.49	0.21	4.46	0.236	22.4	5.00	39.4	6.96
L8	2	MO, USA	Amber	1.0183	5.24	0.26	4.30	0.223	10.6	4.99	18.0	1.07
L9	20	MD, ES	Amber	1.0236	6.45	0.04	4.21	0.163	20.8	4.75	17.0	1.54
L10	20	MD, ES	Lager	1.0165	4.95	0.58	4.21	0.163	14.0	4.90	10.1	1.17
L11	12	CO, USA	Lager	1.0115	3.55	0.32	3.87	0.065	8.4	5.13	4.5	0.31
L12	24	JPN	Lager	1.0000	0.46	0.00	3.58	0.138	16.1	5.13	9.0	0.71
L13	3	HB, DE	Lager	1.0250	6.79	0.03	4.51	0.190	22.0	4.85	7.1	2.12
L14	10	HE, DE	Lager	1.0241	6.70	0.30	4.78	0.071	25.2	4.89	8.7	0.51
L15	4	MO, USA	Lager	1.0118	3.63	0.29	4.38	0.140	9.2	5.35	9.7	0.64
L16	9	NY, USA	Hoppy lager	1.0226	6.36	0.36	4.52	0.234	24.6	4.95	34.2	0.48
L17	10	HE, DE	Hoppy lager	1.0245	6.78	0.23	4.71	0.128	33.5	4.86	26.6	13.10
L18	26	NW, DE	Hoppy lager	1.0224	6.26	0.26	4.71	0.130	26.2	5.01	8.3	0.62
P1	8	OH, USA	Pale Ale	1.0016	1.03	0.32	4.74	0.112	28.4	5.17	18.8	2.32
P2	17	TX, USA	Pale Ale	1.0106	3.30	0.25	4.73	0.104	15.4	5.17	16.6	0.77
P3	21	ON, CAN	Pale Ale	1.0044	1.76	0.33	4.14	0.160	19.7	5.48	20.5	1.23
P4	5	CT, USA	Golden	1.0151	4.34	0.05	4.44	0.186	41.7	4.78	13.5	11.70
P6	21	ON, CAN	Blonde	1.0044	1.76	0.33	4.18	0.101	17.8	5.68	11.6	2.40
Por1	25	CA, USA	Porter	1.0082	2.68	0.24	4.27	0.148	19.9	4.53	179.1	72.70
R1	22	BY, DE	Wheat with lemon	1.0339	9.03	0.11	3.43	0.437	7.0	5.07	16.0	39.25
R2	26	NW, DE	Lager with lemon	1.0306	8.18	0.01	3.74	0.218	10.4	5.22	8.5	3.28
S1	27	MO, USA	Coffee Stout	1.0284	7.75	0.28	4.51	0.259	12.3	5.60	184.5	19.45
W1	18	BY, DE	Wheat	1.0325	8.64	0.00	4.31	0.185	16.6	6.24	18.6	30.40
W2	6	HE, DE	Wheat	1.0243	6.76	0.29	4.25	0.146	12.5	5.70	14.3	4.63
W3	18	BY, DE	Wheat	1.0274	7.51	0.27	4.49	0.231	10.9	6.43	21.1	46.70
W4	27	MO, USA	Wheat	1.0131	3.92	0.22	4.20	0.227	10.8	5.18	15.9	1.75
W5	27	MO, USA	Wheat with orange	1.0130	3.85	0.12	4.21	0.231	10.4	5.05	14.1	5.75
W6	13	BY, DE	Wheat	1.0240	6.64	0.20	4.43	0.147	14.8	6.65	10.5	9.87
Seltzer	23	CA, USA	Seltzer	0.9986	0.09	0.00	5.83	0.005	0.03	7.37	0.3	0.04
Soda	11	GA, USA	Soda	1.0413	10.76	0.00	2.47	0.107	3.2	7.07	53.3	0.24

[a]12 NABs highlighted in blue were used for consumer analysis. Abbreviations: real extract (Er), bitterness unit (BU), titratable acidity (TA), and alcohol by weight (ABW). [b]NABs were classified into the following general categories: ale/lager (AL), hop water (HW), India pale ale (IPA), lager (L), pale ale (P), porter (Por), radler (R), stout (S), and wheat (W). [c]Generally, the brand styles were descriptors used on the packaged product.

maltotriose.[9,10] The aim is to use these non-*Saccharomyces* yeasts in combination with unique fermentable substrates/adjuncts to harness fermentation and to generate secondary metabolites, which can result in NABs that have more complex and preferable fruity flavors while also mitigating ethanol production.[3] However, the utilization of non-*Saccharomyces* yeast strains can also have unwanted flavor consequences, such as the production of lactic acid, and can still result in the production of ethanol, which must be considered and accounted for during production.

Mechanical methods are also employed to remove ethanol from alcoholic beer and can be split into two general techniques: thermal and membrane filtration.[7,11,12] Thermal technologies, such as vacuum distillation and falling film evaporators, rely on the differing vapor pressures of ethanol and water. Generally, heat is applied to alcoholic beer to evaporate and remove the ethanol. Most of the current systems are also run under vacuum to reduce the thermal load on the product. Although, NABs produced using these techniques are often found to contain significantly lower levels of aroma volatiles and have less fruity, more caramelly/bready aroma profiles, and taste/mouthfeel profiles which are thin, bitter, and sour.[7,13] However, aroma/volatile extracts can be blended with these products if local regulations allow.

Membrane technology, such as reverse osmosis or nano-filtration, is another mechanical approach that utilizes semipermeable membranes and pressure or concentration differences to filter out ethanol.[14] Although these techniques can be performed under much cooler temperatures than the

thermal techniques, they currently are more expensive to operate and maintain. These methods also lead to the reduction or concentration of flavor active molecules and have a significant outcome on the resulting aroma and flavor profiles.

Botanicals such as citrus juice/peel or hops can also be added in the cellar to biologically or mechanically made NABs. These additions can have a significant impact on the resulting aroma and flavor of these products.[15,16] In competitive NAB markets (e.g., Germany), most breweries use a combination of these approaches to design NABs that have some of the main characteristics of their flagship alcoholic brands.[17]

While the method chosen by a brewer has a direct influence on the resultant chemicals driving the aroma and taste/mouthfeel profiles of NABs, very few studies[13,15] have investigated how the broad range of chemical profiles produced by these different methodologies influence consumer preference, and none have examined the preference of Americans toward NABs. If the goal is to market these products for everyday consumption to American consumers, understanding the chemical indicators of preference in these products is critical.

Therefore, the main goals of this project were to (1) use a trained panel (i.e., 11 judges) to perform descriptive analysis (DA) to sensorially characterize the aroma and taste profiles of a range of different commercial NABs (*n* = 42) and to chemically deconstruct these samples to gain an understanding of the flavor and chemical profiles of the products in this market space and (2) use consumer analysis to evaluate the

preference of Northern California consumers ($n = 129$ consumers) toward a selection ($n = 12$) of these NABs to identify if specific chemical qualities [e.g., bitterness units (BUs), hop acid concentrations, carbonation level, residual extract, titratable acidity (TA), and volatile concentrations such as higher alcohols, staling aldehydes, hop volatiles, etc.] are indicators of specific aroma or taste qualities and/or preference for American consumers.

2. MATERIALS AND METHODS

2.1. Collection Details and Sample Handling Protocol of NABs, Soda, and Seltzer. For sensory and chemical analyses, a soda, a seltzer, and a diverse set of 42 NABs, comprising several different styles and made using several different production methodologies, were obtained from six different countries (Table 1). The soda and seltzer water were evaluated in order to serve as controls and to compare how different or similar the chemical and sensorial profiles of NABs were to these products. Owing to the propensity of NAB aroma and flavor to be impacted by staling reactions, most of the 42 NABs analyzed for this study were either donated or purchased directly from breweries, shipped straight to the University of California Davis (UC Davis) at the end of August/beginning of September 2019, and then stored at −2 °C until chemical and sensorial analysis in September/October 2019. A total of 11 NABs (i.e., IPA3, L5b, L7, L11, L14, P3, P6, R1, S1, and W4) were obtained from the local Total Wine and More (Bethesda, MD, U.S.); one was purchased from the local Whole Foods Market (Austin, TX, U.S.) (i.e., HW1), and the soda, as well as the seltzer were acquired from the local Safeway Inc. (Pleasanton, CA, U.S.). Notably, two different packaging types for L05 were also evaluated (i.e., L05c—can and L05b—bottle). Directly after purchase, these samples were stored under the same conditions mentioned above. For each sample, the package codes were matched to ensure all of a given product originated from the same batch. All the products were <3 months old at the time of evaluation and/or were evaluated before the best buy date labeled on the package.

For consumer analysis, based on the initial sensory and chemical data, 12 NABs representing the most diverse flavor and chemical profiles were selected from the list of 42 NABs (colored in light blue in Table 1; HW2, IPA1, IPA3, L5c, L6, L9, L16, L18, R1, W1, and W4). Although most of these samples were from different production batches, they were procured, stored, and evaluated under similar circumstances to what was mentioned above. It was assumed the batch to batch variation on the sensory and chemical analyses would be smaller compared to changes upon storage. This is assumption is also supported by some basic data analysis discussed in Section 2.4.2.

2.2. Sensory: Descriptive and Check All That Apply Analyses. Descriptive and check-all-that-apply (CATA) analyses were performed based on published methodology by a trained panel to characterize the sensory profiles (i.e., aroma, taste, and mouthfeel) of the 42 NABs, soda, and seltzer.[18] These sensory analyses were performed in the J. Lohr Wine Sensory Room at the UC Davis over four weeks in September/October 2019. For the panel, 11 judges (6 females and five males) who had self-identified that they consumed beer on a regular basis, had a high preference for beer, and were available to participate in all of the study time slots were recruited from students, staff, and friends of UC Davis. The protocol for this

study (IRB project number 1468002-1) was exempted by the internal regulatory board.

Panel participants were trained over five 60 min training sessions. Over four of these sessions, the panel blindly and randomly evaluated all the samples (in sets of 11) to establish, by consensus, the sensory terms, and corresponding reference standards (Table S1), which best described the differences between the samples. While the panelists could use their own vocabulary to describe the samples, panelists were also given the second edition Beer Flavor Map by DraughtLab (Flavor LAB, LLC) to help guide term generation for DA. Because of the number of different NAB styles being evaluated, 41 different terms were generated to describe the aroma, taste, and mouthfeel of the samples. Therefore, to make the evaluation less fatiguing on the judges, DA was only performed on terms which the panelists decided they could scale over most of the samples (i.e., five aroma terms and seven taste/mouthfeel terms, colored light red and green in Table S1, respectively). For terms more unique to specific products, CATA was used (i.e., 23 aroma terms and 6 taste/mouthfeel terms, colored dark-red and green in Table S1, respectively). It is important to note that the way CATA was performed in this study was an unorthodox approach. In that, CATA was performed by trained panelists (i.e., reference standards were developed for each term), and it was performed in a replicated fashion ($n = 3$). The results from this analysis are discussed in Section 3.1. The final training session was used as a practice to guide the panelists through the testing environment.

Prior to testing, judges were instructed to smell through labeled aroma standards. For a warmup, before each testing session, the judges had to randomly identify a selection of the aroma standards coded with three-digit numbers. To prevent fatigue, this same exercise was only completed with the taste and mouthfeel standards on a weekly basis. During testing, 60 mL of the sample was evaluated at ∼14.1 °C by the judges in 5 oz. Belgian beer tasting glasses (Libbey, OH, U.S.) covered with plastic lids (Dart, MI, U.S.) and coded with randomized three-digit numbers that differed for each judge. The samples were evaluated ∼30 min after opening the container, and the judges assessed the samples in individual, ventilated, and light-isolating tasting booths under red light.

The testing was performed over three replications in a randomized and balanced Latin Square order designed by FIZZ (version 2.47B, Biosystèmes, Couternon, France). Scaling for DA was performed on a 12.5 cm line scale anchored by wording "less intense" to "more intense". Judges first scaled the DA aroma terms for each beer (both ortho- and retronasal) (Table S2). The judges were then instructed to check all the other attributes from the list of CATA aroma terms, which best described the unique aroma quality of the NABs (Tables S3 and S4). Following this, the panelists scaled the basic taste and mouthfeel attributes (Table S5) and again checked the attributes from a list of CATA mouthfeel terms, which best described the NABs (Table S6). Panelist responses were collected on Chromebook tablets using Qualtrics (UT, USA). The judges were instructed first to evaluate the aroma of the samples, then evaluate the taste/mouthfeel of the samples, and then to cleanse their palates with carbonated (20 psi) 0.1% pectin during a forced 30 s break between samples. A total of 11 samples were evaluated in one session with a forced 3 min break after six samples. Upon the completion of the three testing replications, three additional sessions were performed in a randomized fashion to collect hedonic data and to scale

how close the judges thought the products were to beer, soda, and sparkling water/flavored water. At the end of each session, judges were given food and were compensated with a gift card upon the completion of the study.

2.3. Sensory: Consumer Preference Analysis. As outlined in Section 2.1, based on the initial sensory and chemical data, 12 NABs with some of the most diverse chemical and flavor profiles were selected out of the 42 NABs for consumer analysis. Consumer analysis was performed in the J. Lohr Wine Sensory Room at the UC Davis over twelve 1 h sessions on a Saturday and Sunday in November 2019 (i.e., six sessions per day) with a 30 min break between sessions to allow for set up for the next session. A total of 144 consumers of beer and/or individuals interested in the assessment of beer flavor were recruited from students and staff of UC Davis and/ or from the Davis community (i.e., 12 consumers per session). During testing, 60 mL of the sample was served at ~14.1 °C to the judges in 5 oz. Belgian beer tasting glasses (Libbey, OH, U.S.) covered with plastic lids (Dart, MI, U.S.) and coded with randomized three-digit numbers that were the same for each consumer. The samples were evaluated ~30 min after opening the container, and the judges assessed the samples under red light in individual, ventilated, and light-isolating tasting booths. The serving presentation of the samples between judges was based on a randomized and balanced Latin Square order designed by FIZZ (version 2.47B, Biosystèmes, Couternon, France).

The consumer testing session was broken into four general parts. First, consumers were introduced to the testing environment and were served six samples to evaluate. Consumers were asked to rate (using a nine-point hedonic scale) how much they liked the overall aroma, taste, and mouthfeel of the sample and then to individually evaluate how much they liked just the aroma and just the taste and mouthfeel for each sample (again using a nine-point hedonic scale). For each sample, the consumers were also given an option to freely profile what they liked or disliked about the sample, asked to rate how similar the sample was to beer, soda, and sparkling water/flavored water (using a nine-point similarity scale), and whether or not they would purchase the sample. Consumers were then forced to wait for 30 s between the samples and instructed to rinse with carbonated (20 psi) 0.1% pectin water to cleanse their palates. The consumers were then prompted to fill out a demographics survey (i.e., 13 questions, ~2−3 min). Following this, the consumers evaluated another six samples in a way identical to the first set. To finish testing, the consumers filled out an additional survey (i.e., 16 questions, ~3−5 min), which gauged their general preferences around purchasing and consuming NABs. Consumer responses were collected on Chromebook tablets using Qualtrics (UT, U.S.). At the end of each session, the consumers were given food, were compensated with a gift card, and were instructed not to talk about the study upon leaving the testing facility.

2.4. Nonvolatile and Volatile Analyses of NABs. Simultaneous to sensory analysis and consumer analysis, several standard and published methods were used to chemically deconstruct both the nonvolatile and volatile profiles of the NABs.

2.4.1. Nonvolatile Beer Analyses. The basic nonvolatile quality specifications are reported (Table 1). These quality specifications were measured in duplicate for the samples evaluated in both the sensory and consumer studies. The

density, real extract (Er), and alcohol content by weight (ABW) of decarbonated samples were measured using a DMA 4100M and Alcolyzer Plus (Anton Paar, VA, USA). The pH and TA were determined using an Orion Versa Star Pro advance electrochemistry meter with an Orion ROSS Ultra refillable pH/ATC triode (8157BNUMD) (Thermo Fisher Scientific, MA, U.S). The standard protocol outlined by the American Society of Brewing Chemists (ASBC) was followed to analyze TA.[19] In brief, 0.1 M NaOH was used to titrate the decarbonated samples at 25 °C to pH 8.2, and the TA was reported as percent lactic acid.

BUs and color were determined by both the ASBC and European Brewing Congress (EBC) standard protocol, respectively, using a Hach DR6000 spectrophotometer (Hach, CO, U.S.).[19] BUs were determined by adding 10 mL of cold carbonated beer (~2 °C), 20 mL of 2,2,4-trimethylpentane (TMP, isooctane) and 1 mL of 3 N HCl to a 50 mL centrifuge tube and shaking for 15 min with a mechanical wrist action shaker. The tubes were then centrifuged for 15 min at 5000 rpm, and the absorbance of the TMP (top) layer was measured at 275 nm and multiplied by 50 to report the BU value. For the color analysis, the absorbance of decarbonated beer at 25 °C was measured at both 430 and 700 nm. To report the EBC color value, the absorbance at 430 nm was multiplied by 25. Samples with excessive turbidity were filtered and remeasured. To determine the CO_2 content, samples were brought to 25 °C in a water bath and then measured using a Haffmans Inpack 2000 CO_2 calculator (Pentair Haffmans, Zürich, CH). Turbidity of decarbonated samples at 25 °C was measured using a Hach 2100AN turbidimeter (Hach, Loveland, CO).

Hop acids (Table S7) were determined using reversed-phase high-performance liquid chromatography (HPLC) operated under conditions outlined in the European Brewing Congress (EBC) standard method 9.47.[20] Isohumulones, reduced isohumulones (rho, tetra, and hexa), and humulinones were quantified at a wavelength of 270 nm using the international calibration standards ICS-I4, ICS-T3, ICS-R3, ICS-H2, and ICS-Hum1, while humulones (cohumulone, n-/ad-humulone, and total humulones (cohumulone + n-/ad-humulone) were quantified at a wavelength of 340 nm using the international calibration extract ICE4. All standards were purchased from Labor Veritas AG, Zürich, CH.

2.4.2. Volatile Beer Analyses. To evaluate if the samples used for both DA/CATA sensory and consumer analysis were dissimilar owing to production differences (such as batch-to-batch variation), analysis of variance (ANOVA) was used to compare all the measured basic quality results (data not shown). TA was the only quality specification that was significantly different between the samples used for these different evaluations. Therefore, it was assumed that the volatile analyses would also not be significantly different and were only performed on the samples used for the DA/CATA sensory. Duplicate measurements were run for each sample, and the average of these values was used for all the related multivariate statistical modeling. Selected hop aroma compounds (i.e., terpenes, oxygenated terpenes, etc.; Table S8) and aldehydes were evaluated using headspace solid phase microextraction and *gas chromatography*−tandem mass spectrometry (HS-SPME−GC-MS/MS) using published methodology.[21,22] Some other lower-boiling point volatile compounds (i.e., esters, alcohols Tables S9 and S10) were measured using HS−GC-flame ionization detector (FID), operated under
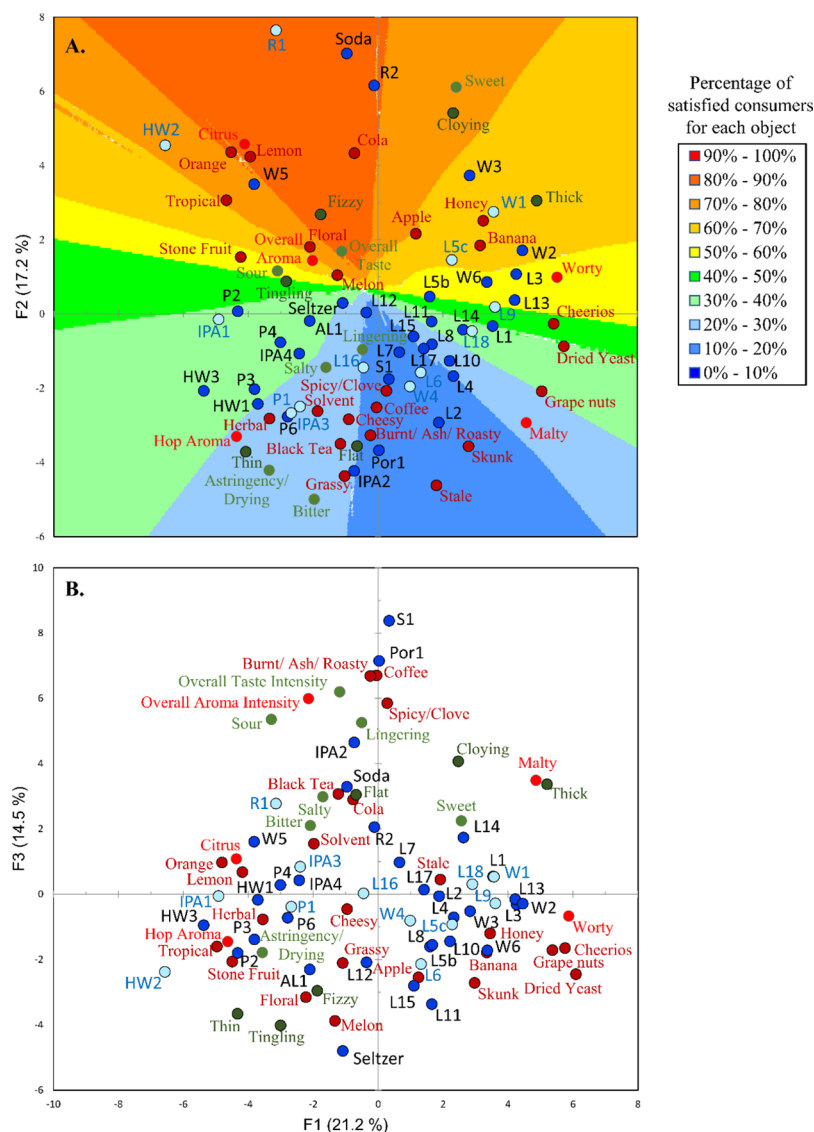
Figure 1. PCA biplots of the aroma attributes (DA—light-red circles and CATA—dark-red circles, Table S1) and taste/mouthfeel attributes (DA—light-green circles and CATA—dark-green circles, Table S1) which were statistically different amongst the NABs, soda, and seltzer (light- and dark-blue circles, Table 1). The samples in dark blue were just evaluated by the trained panel, whereas the 12 samples in light blue were also evaluated by consumer analysis. The (A) biplot of PC1 and PC2 explained 38.4% of the variation in the data, while (B) biplot of PC1 and PC3 displayed an additional 14.5% of the variation in the data set. External preference mapping, using the PCA factor scores for only the 12 samples evaluated via consumer analysis, was used to generate and project a contour plot representing the percentage of satisfied consumers for each product into the biplot of PC1 and PC2 (using F-ratios to find the best vector model, $p < 0.05$, contour plot threshold % = 100). The region in dark blue represents a low percentage of satisfied consumers, whereas the dark-red region represents a high percentage of satisfied consumers.

conditions outlined in the EBC standard method 9.39.[20] Dimethyl sulfide was measured using HS−GC-*pulsed flame photometric detector* (PFPD), operated under conditions outlined by the Mitteleuropäische Brautechnische Analysenkommission (MEBAK) 2.23.1.1 standard method.[23]

2.5. Statistical Analysis. ANOVA, three-way ANOVA (including the factors: judge, sample, and replication, as well as corresponding two-way interactions), multiple comparison analysis (Fisher's least significant difference, $p < 0.05$), CATA analysis (i.e., Cochran's Q test, $p < 0.05$), Pearson correlation analysis, principal component analysis (PCA), external preference mapping (PREFMAP), multiple linear regression, and graphical construction were carried out using XLSTAT 2020.1.1 (Addinsoft, NY, USA). For cases in which significant effects were observed for both the sample term and

an interaction term including sample in the three-way ANOVAs, a pseudo-mixed model was used with R-3.2.2.[24] To determine the significance of the sample term, a new F-value was calculated with the mean sum of squares using the significant interaction as the error term for the sample term (data not shown).

These tests and graphs were used to gauge the judges' effectiveness in generating descriptive data, to evaluate the significant differences in the aroma, taste, and mouthfeel profiles between the different NABs, to assess the associations between the chemical and sensory data, and to relate the consumer preferences to the chemical and sensory results to determine indicators of American consumer preference toward the different NABs.

3. RESULTS AND DISCUSSION

3.1. DA and CATA Results. When accounting for significant panelist and replication interaction terms, all the terms used for DA were found to be significantly different for at least one of the samples (ANOVA $p < 0.05$, Tables S2 and S5). For at least one of the three replications, significant sample differences were observed for all the terms evaluated using replicated CATA (Cochran's Q test $p < 0.05$, Tables S3, S4, and S6), and a majority (i.e., 66%) of the CATA terms were significant over all three of the replications. Another 14% of the terms were significant for at least two of the replications. To compare this data alongside the DA data, the results for each of the CATA terms were summed over the three replications (i.e., if a term had been selected by all 11 judges over all three of the replications this would have resulted in a max total of 33 per CATA term).

PCA was then performed using the correlation matrix ($n − 1$) of the least square means from DA and the sums for the CATA terms to generate biplots to map the sensorial profiles of the products (Figure 1). PC1 accounted for 21.2% of the variation and was defined by more malty and worty aroma (aroma DA—light-red circle) on the right side of the plot, while the left side was defined by citrus, (aroma DA—light-red circle), stone fruit, tropical, floral, herbal, and hoppy aroma characteristics (aroma CATA—dark-red circles). In general, the products (W—wheats and L—lagers) on the right side of the plot had aromas that were malty and worty in character, while products (P—pale ales, IPA—India pale ales, and HW—hop waters) on the left were perceived to be more citrus, stone fruit, tropical, floral, herbal, and hoppy in character.

PC2 explained 17.2% of the variation (Figure 1A), and the products (R—radler and soda) in the top of the biplot were characterized as citrusy in aroma and were sweeter, cloying, and thick. In contrast, the products in the bottom of the biplot were more bitter, astringent/drying, and thin in character. Interestingly, PC2 also helped to differentiate between the aroma profiles of the products within the same style classifications. For example, the lagers and wheats (L2, W4, L6, etc.) on the bottom right side of the biplot were more malty, stale, thin, and bitter as compared to lagers and wheats (W3, W1, L5c, W6, etc.) in the top right side of the biplot which were sweeter and had more honey and fruit-like (i.e., banana and apple) aromas. In comparison, the pale ales, IPAs, and hop waters (P1, P6, HW1, IPA3, etc.) on the bottom left side of the biplot had aromas which were more herbal and black tea in character, whereas the pale ales, IPAs, and hop waters (P2, HW2, IPA1, etc.) in the top left of the biplot were more citrus, tropical, floral, and stone fruit in character. PC3 (Figure 1B) accounted for another 14.5% of the variation and was distinguished at the top by overall aroma and taste intensity, as well as the products that had aromas which were more the burnt/ash/roasty, coffee, and spicy/clove in character (S—stouts and Por—porter), while products at the bottom were more thin and tingling (seltzer). The differences between the aroma and taste/mouthfeel profiles of the varying NAB products are further highlighted when PCA is performed using only the correlation matrix of the aroma attributes (Figure S1) or just on the correlation matrix of the taste/mouthfeel characteristics (Figure S2).

While lager type NABs still make up a majority of the NABs produced, it is apparent from these results that NABs can be and are designed to have a wide range of different aroma, taste,

and mouthfeel profiles. As mentioned, using a trained panel to perform standard-driven replicated DA in combination with standard-driven replicated CATA is unique to this study. These results show that this approach successfully differentiated the products by both the more common attributes shared between the products, as well as for the attributes which were more specific to certain samples. Therefore, this approach may be useful to collect additional statistically relevant sensory data while also minimizing panelist fatigue for future studies on large sample sets in which a lot of terms are needed to describe differences in aroma, taste, and mouthfeel profiles.

3.2. Consumer Preference Results. As outlined in Section 2.3, the results in Section 3.1 were used to select 12 products for consumer analysis, which had some of the most diverse sensorial profiles out of the 44 products tested by the trained panel (highlighted in light blue in Table 1 and also in light-blue circles in Figure 1). As mentioned in Section 2.2, at the end of DA, additional sessions were used to collect hedonic data from the trained panel on all 44 of the products. Although it is not standard research practice to collect hedonic responses from trained DA panelists. This additional data was collected to see how the responses from the panelists would differ from the consumer population because some commercial brewers also use small panels for the research and development of new products.

For the consumer panel, consumers who were under 21, did not self-identify as an American consumer, and/or had not been a U.S. resident for >5 years were removed and not considered in the following data analysis. This resulted in a final data set generated by 129 consumers; 66 males and 63 females. The outcomes from the demographics and preference surveys (Figures S3 and S4 and Table S11) showed that the general makeup of the resulting consumer panel comprised individuals who were regular beer drinkers, a majority of whom were under 35 (~66%) and had a B.S. equivalent or more advanced degree (~76%). The top four reasons for consumers' interest in trying NAB were health, a thirst-quenching alternative to alcohol, abstaining from alcohol for a while, and flavor. While these results support current market research/trends indicating that lifestyle trends might be driving consumers to search for alternative products, flavor is a critical consideration driving consumer interest toward NAB.[3] Given that 43% of the consumers had not tried NAB prior to the survey, this supports that there is room to grow and develop in this category in the U.S.

ANOVA was used to assess the overall, aroma, and taste/mouthfeel liking for the samples using both the consumer panel and the trained panel data (Table S12). Significant differences were observed in the means between the products. Overall, the most preferred products (i.e., HW2 and R1) were only liked slightly by the consumers. The consumers were indifferent (i.e., 5—neither liked nor disliked) toward most of the products, and they disliked IPA3 and P1 moderately. When considering just consumer aroma preference, the aroma of HW2 was liked very much by the consumers, followed by the aromas of IPA1, R1, and L16, which were liked slightly, whereas they neither liked nor disliked the aromas of P1, W1, IPA3, W4, and L9. In contrast, when focusing just on taste/mouthfeel liking, the most preferred products (i.e., HW2 and R1) were liked slightly, whereas the least preferred products (i.e., IPA3 and P1) were disliked moderately. Similar to the results from the trained panelists, aroma and taste/mouthfeel liking were highly correlated with the overall liking of the
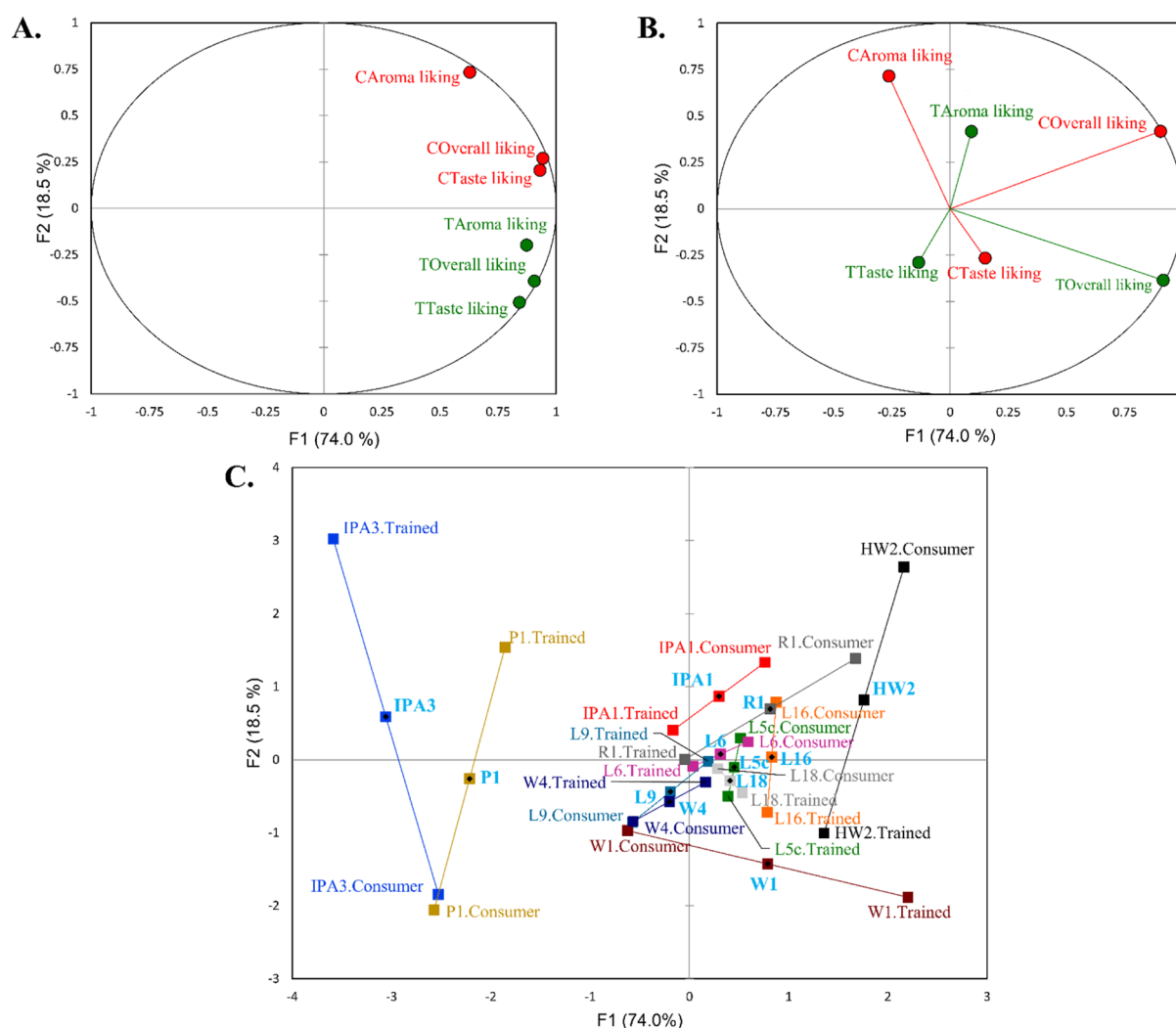
Figure 2. Multiple factor analysis was used to compare the least square means of the hedonic ratings for overall, aroma, taste/mouthfeel liking for each product from both the trained and consumer panel. (A) Correlations between the overall, aroma, taste/mouthfeel liking ratings for the trained and consumer panels, (B) partial axes plot (i.e., the principal components of the individual PCAs for each panel), and (C) coordinates of the projected points for the 12 NABs for both the consumer and trained panel data.

products for the consumer panelists (Pearson correlation coefficients $p < 0.05$, Tables S13 and S14). Therefore, both factors should be viewed as essential considerations in the design of NABs and key indicators of consumer preference for these products.

To relate the consumer preference data to the sensory data collected by the trained panel, external preference mapping PREFMAP was performed with the overall, aroma, and taste/mouthfeel liking scores, as well as the PCA factor scores from only the 12 samples evaluated via consumer analysis to generate a vector model.[25] The results were then displayed as a contour plot representing the percentage of satisfied consumers (i.e., satisfaction was defined as the percentage of consumers giving a hedonic rating higher than the mean hedonic rating for the set of 12 samples) in the biplots of PC1 and PC2 [Figure 1A (overall liking), S1 (just aroma liking), and S2 (just taste/mouthfeel liking)]. The regions in dark blue represent a low percentage of satisfied consumers, whereas the dark-red regions represent a high percentage of satisfied consumers. Again, the 12 products which were evaluated by consumer analysis are also highlighted by light-blue circles in these plots.

These contour plots make it easier to visualize the sensorial indicators of consumer preference. For example, consumers were less satisfied with L16, L6, and W4 in the bottom right portion of the biplot. These products were more thin and bitter and characterized by malty, skunk, and stale aromas. However, a higher percentage of consumers were satisfied with W1, L5c, and L9. Comparatively, these NABS were more sweet, cloying, and thick and had aroma profiles that were more honey- and fruit-like (i.e., banana and apple). Schmelzle, et al.[15] observed that German consumers preferred NABs (i.e., mostly lager types) which had slightly fruity/apple aromas but did not prefer NABs with malty/honey aromas and bitter taste. Also, consumers were less satisfied with P1 and IPA3 in the bottom left portion of the biplot (Figure 1A). These NABs had aroma profiles that were more hoppy, herbal, grassy, cheesy, and black tea in character and had taste/mouthfeel profiles which more bitter, astringent/drying, and thin. In comparison, a higher percentage of consumers were satisfied with IPA1 and HW2. These products were less bitter and had aroma profiles, which were more hoppy, citrusy, stone fruit, tropical, and floral in character. The citrusy and sweet radler (R1) at the top of the biplot was most preferred by consumers.
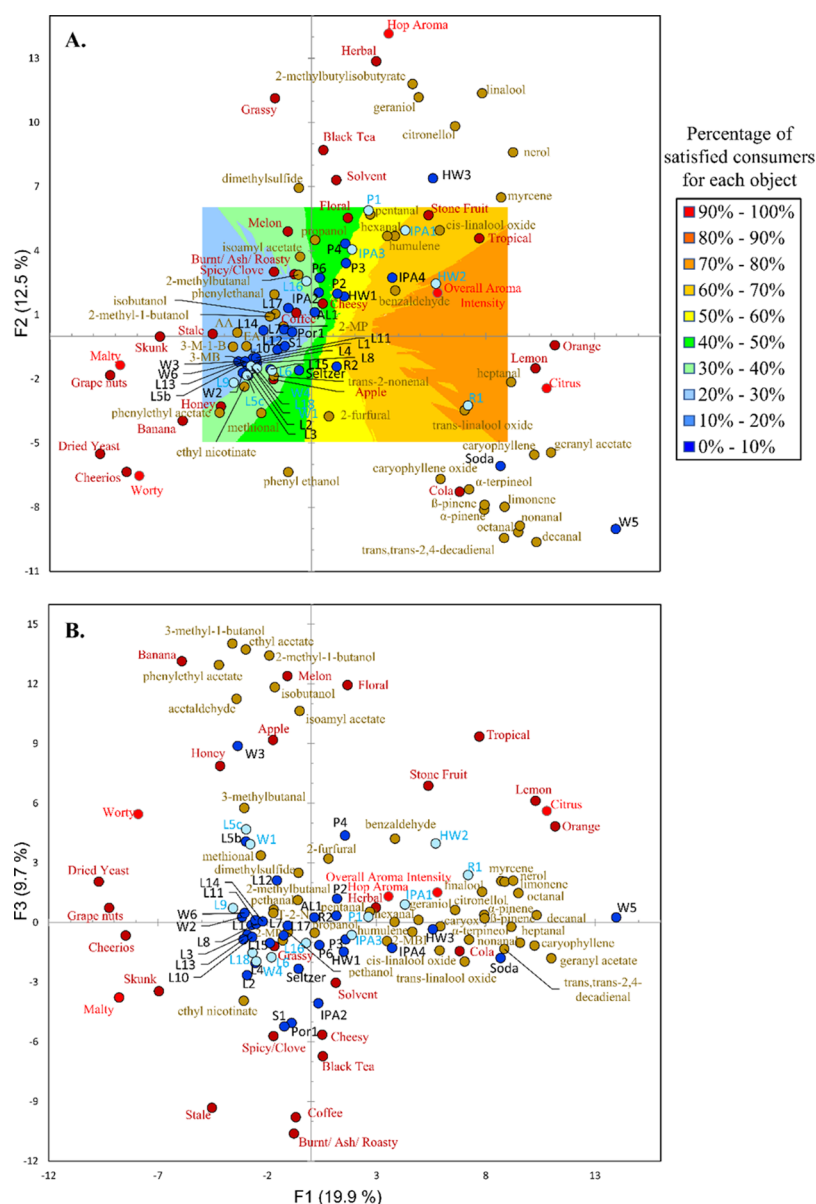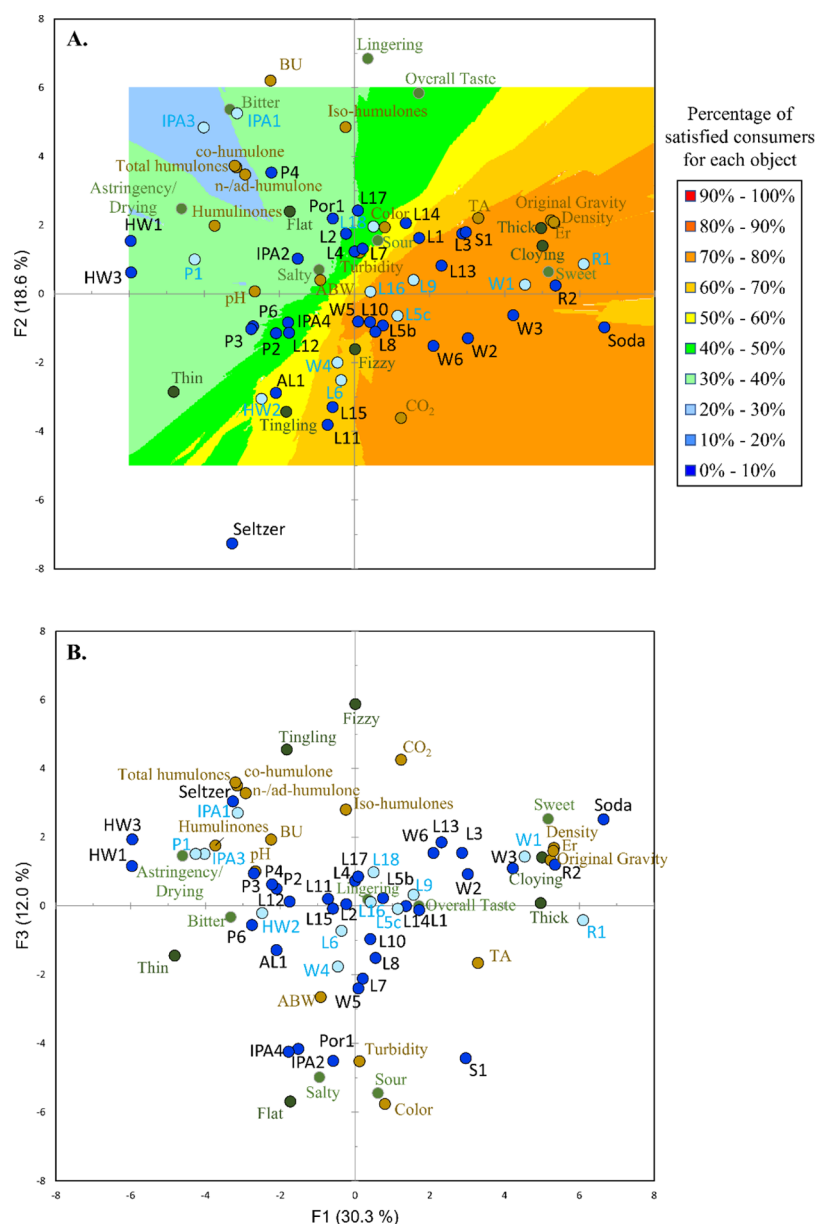
Figure 3. PCA biplots of the aroma attributes (DA—light-red circles and CATA—dark-red circles, Table S1) which were statistically different amongst the NABs, soda, and seltzer (light- and dark-blue circles, Table 1), as well as the volatiles (light-brown circles, Tables S8−S10). The samples in dark blue were just evaluated by the trained panel, whereas the 12 samples in light blue were also evaluated by consumer analysis. The (A) biplot of PC1 and PC2 explained 32.4% of the variation in the data, while the (B) biplot of PC1 and PC3 displayed an additional 9.7% of the variation in the data set. External preference mapping, using the PCA factor scores for only the 12 samples evaluated via consumer analysis, was used to generate and project a contour plot representing the percentage of satisfied consumers for each product into the biplot of PC1 and PC2 (using F-ratios to find the best vector model, $p < 0.05$, contour plot threshold % = 100). The region in light blue represents a low percentage of satisfied consumers, whereas the dark-orange region represents a high percentage of satisfied consumers.

Multiple factor analysis was applied to investigate the relationship between the overall, aroma, and taste/mouthfeel liking ratings of the trained and consumer panels (Figure 2). Most of the preference ratings between the consumer and trained panels for the 12 samples were highly correlated except for the aroma preferences of the consumers (Figure 2A and Table S13). This likely resulted because of the differences in aroma preference for W1 between the consumer and trained panel, which is highlighted by the large distance between the consumer and trained panel in the projected point plot (Figure 2C). When comparing the least square means, W1 was one of the most preferred sample by the trained panel, while the consumer panel seemed indifferent about this sample.

However, when looking at the percent satisfied consumers from the external preference mapping, it is clear most of the consumers were satisfied with this sample. Overall, the least preferred products (e.g., IPA3) are on the left of the plot, while the most preferred products (e.g., HW2) are on the right of the plot. In contrast to the trained panel, the consumer panel had a much higher preference toward NABs that had aroma profiles driven by botanicals (i.e., citrus juice/peels or dry hopped) such as citrus, lemon, orange, stone fruit, tropical, melon, and floral characteristics and not a strong preference toward NABs which were worty, malty, and grape nut in character (Tables S13 and S14). Other studies have also shown that hop aroma generally makes NABs more preferable.[15,16] The trained panel

Figure 4. PCA biplots of the taste/mouthfeel attributes (DA—light-green circles and CATA—dark-green circles, Table S1) which were statistically different amongst the NABs, soda, and seltzer (light- and dark-blue circles, Table 1), as well as the nonvolatile factors (light-brown circles, Tables 1 and S7). The samples in dark blue were just evaluated by the trained panel, whereas the 12 samples in light blue were also evaluated by consumer analysis. The (A) biplot of PC1 and PC2 explained 48.9% of the variation in the data, while the (B) biplot of PC1 and PC3 displayed an additional 12.0% of the variation in the data set. External preference mapping, using the PCA factor scores for only the 12 samples evaluated via consumer analysis, was used to generate and project a contour plot representing the percentage of satisfied consumers for each product into the biplot of PC1 and PC2 (using F-ratios to find the best vector model, $p < 0.05$, contour plot threshold % = 100). The region in dark blue represents a low percentage of satisfied consumers, whereas the dark-red region represents a high percentage of satisfied consumers.

had a preference toward NABs, which were sweeter and had aroma profiles that were worty, cheerio, dried yeast, banana, and honey in character.

In general, it is not an accepted practice to use a trained panel to collect hedonic data because the number of panelists is not high enough to collect preference data relevant to the broader consumer base, the familiarity of the judges with the products could bias their ratings, and the attention of the judges during DA should be solely on deconstructing the unique aroma and taste/mouthfeel profiles of the samples.[18,26] However, in this study, the trained panel was made up of judges who were regular beer consumers and who were highly

interested in the consumption of beer/NAB. They were also not employees interconnected to the products and were not told about the hedonic evaluations until after the DA testing sessions had finished. One must be very careful in applying this type of methodology for future studies, and it should not be used in place of consumer testing. Yet, consumer testing is extremely expensive and becomes more complicated to implement as the number of samples to test increases. Therefore, this approach could be a useful tool for companies with a limited budget to help select what products should even be evaluated by consumer analysis. For example, because of the lack of access to fresh products at the time of the consumer

A.

B.
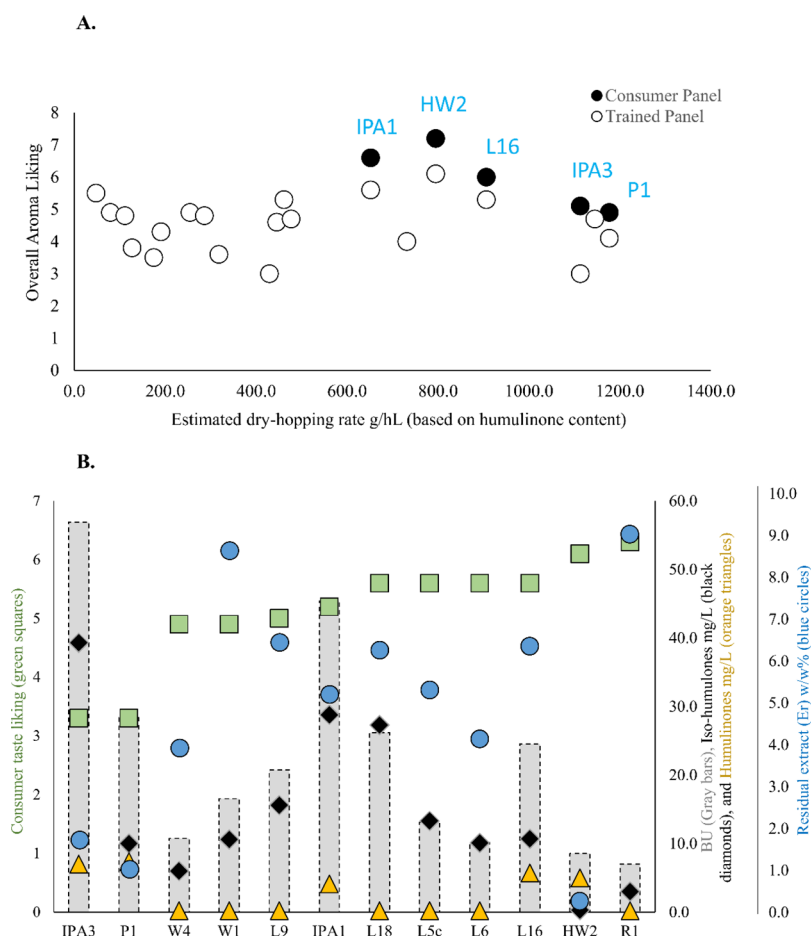
Figure 5. (A) Overall aroma liking for the consumer (black circles) and trained panels (white circles) over the different products containing humulinones based on estimated dry hopping rates using data from Lafontaine and Shellhammer[36] and (B) BUs (gray bars), iso-humulones (black diamonds), humulinones (orange triangles), and residual extracts (blue circles) of the 12 different NABs evaluated by the consumer panel sorted by the least square means for taste/mouthfeel liking (green squares).

analysis, stout and/or porter styles were not selected to be analyzed in the consumer panel. Given that the trained panel disliked these styles very much and that these products sit in a space in which consumers were extremely dissatisfied (Figure 1), there is at least some evidence to suggest that the chemical and sensorial profiles of these NAB styles may not be preferable to Northern Californian consumers.

3.3. Identifying Volatile Indicators of Consumer Preference for NABs. To understand the role of volatile and nonvolatile factors in driving consumer preference for the different NABs, PCA was run on the correlation matrix of just the aroma attributes and volatile compounds (Figure 3), as well as on the correlation matrix of just the taste/mouthfeels and the nonvolatile factors (Figure 4). External preference mapping was then performed with the aroma (Figure 3) and taste/mouthfeel (Figure 4) liking scores and the PCA factor scores from the 12 samples evaluated via consumer analysis from each of these plots to generate vector models. As in Section 3.2, the results were displayed as contour plots representing the percentage of satisfied consumers.

The volatiles driving the aroma profiles of the highly preferred botanically driven NABs [i.e., the blended citrus juice radler (R1) or the dry-hopped hop water (HW2) and IPA (IPA1)] were largely monoterpenes, terpene alcohols, esters, and aldehydes (Figure 3A and Table S15). These volatiles were also significantly negatively correlated to the worty, malty,

cheerio, grape nut, dried yeast, and banana aromas, indicating that they mask/suppress these aromas. The compounds most positively correlated to citrus, lemon, and orange aromas were limonene, octanal, decanal, geranyl acetate, nerol, linalool, α-terpineol, and benzaldehyde. These observations are in agreement with other studies that have shown these volatiles to be important indicators of citrus fruit aroma,[27,28] as well as for lemon-lime carbonated beverage character.[29] As explained, in Section 3.1 the hop-forward NABs were either mostly perceived as citrusy, tropical, stone fruit, or floral (IPA1 and HW2) or they mostly perceived as herbal, black tea and grassy (P1 and IPA3). The main indicators of tropical, stone fruit, or floral aromas were benzaldehyde, linalool, nerol, citronellol, myrcene, geraniol, and 2-methylbuytl isobutyrate. Again, other studies have found these compounds to be important indicators of hop-derived tropical, stone fruit, or floral in beer.[30−32] Interestingly, benzaldehyde was the only volatile which was significantly correlated with consumer aroma liking (Table S13). The herbal, black tea, and grassy aroma characteristics were more positively correlated with the presence of hexanal, pentanal, and dimethyl sulfide. There is also prior evidence showing aldehydes, such as hexanal, are responsible for green, grassy characteristics in hoppy beer and wort.[15,33] It should be mentioned that polyfunctional thiols are another class of potent odorants that have been shown to be important volatile indicators of tropical and citrus flavor in

beer.[32,34] Future studies should investigate the influence these compounds have on NAB aroma profiles and consumer preference.

The hopping method used to create NAB has a direct impact on the resulting aroma and taste/mouthfeel profile (which will be discussed in Section 3.4).[35] One of the preferred methods brewers use to impart hop aroma but not hop bitterness is dry hopping. Essentially, dry hopping can be defined as the "cold" extraction of volatile and nonvolatile components out of hops into the product. One factor influencing the aroma profile extracted from hops is the amount of hops added during dry hopping.[36] In the current study, humulinones were significantly correlated with hop aroma intensity. Humulinones are oxidized humulones, which are naturally present in the hops, and studies have found that they are generally only extracted and present in dry hopped beers.[36−38] Interestingly, ~48% of the NABs tested in this study contained humulinones. Therefore, the relationship between the dry hopping rate and humulinone content reported by Lafontaine and Shellhammer[36] was used to approximate the dry hopping rates used to create the different dry hopped NABs in this study and the impact that this may have had on the overall aroma liking for the trained and consumer panels (i.e., dry hopping rate (g/h L) = 158.68 × humulinone content, Figure 5A). Similar to this study, as the dry hopping rate increased above 800 g/h L, the aroma profiles of the NABs went from more citrus in character to more herbal/tea in character. Interestingly, the aroma profiles of the NABs dry-hopped ~600−800 g/h L were most preferred by the consumer panels and may indicate that this may be an optimal rate to preferentially extract volatiles driving citrus flavor. In contrast, dry-hopping rates above this level may lead to the extraction volatiles, leading to more herbal/tea characteristics (i.e., hexanal, pentanal, and dimethyl sulfide).

The type of hop varieties and/or products (i.e., pelletized, supercritical CO_2 extract, etc.) also has a big impact on the resulting hop aroma and flavor imparted.[39] Given that the production details of these products are unknown, it is impossible to know exactly what varieties or products were used to produce these different NABs. However, the essential oils and volatile profiles of some hop varieties (i.e., Citra, Cascade, Centennial, Mosaic, etc.) have been shown to impart more citrusy aroma, while the oils and profiles of other hop varieties (i.e., Hallertauer Mittelfrüh, Saazer, etc.) have been shown to impart more spicy and floral aromas.[35,40] Interestingly, blending multiple varieties during dry hopping also allows for volatiles that may be unique to certain hop varieties to be mixed, resulting in synergistic effects which increase tropical fruity flavor and aroma intensity.[30,32]

For lager- and wheat-type styles, consumers were the least satisfied with L16, L6, and W4 characterized by malty, skunk, and stale aromas, while W1, L5c, and L9 were preferred by a higher percentage of consumers, and these NABs were more honey- and fruit-like (i.e., banana and apple). The volatiles positively correlated with malt flavor were ethyl nicotinate, 3-methylbutanal, phenyl ethanal, and *trans*-2-nonenal. Honey-like aroma was positively correlated with 2-furfural, methional, *trans*-2-nonenal, and acetaldehyde. While apple character was positively correlated with 3-methylbutanal and 2-furfural, and banana character was positively correlated with phenylethyl acetate, 3-methyl-1-butanol, 2-methyl-1-butanol, isoamyl acetate, isobutanol, ethyl acetate, acetaldehyde, and 3-methylbutanal. Again, because the production methodologies of all the products are unknown and that it is likely some of the products are made using a mix of production methodologies, it is hard to know exactly what methodologies are responsible for these flavors.

However, studies have shown that non-*Saccharomyces* yeasts, such as *Cyberlindnera mrakii*, *Pichia kluyveri*, and *Torulaspora delbrueckii*, have been shown to produce fruity nonalcohol beers NABs which have high ester concentrations (e.g., isoamyl acetate, phenylethyl acetate, etc.).[3,41] Given that the consumers were more satisfied with fruity lager and wheat NAB styles, this might be evidence that the use of non-*Saccharomyces* yeast might be the key to the development of volatiles driving preferable aromas in these styles. The fermentation substrate(s) selected to produce NAB (e.g., malted barley, wheat, oats, etc.) should also be an important consideration because it will also influence the aroma and flavor resulting from fermentations with these yeasts.[42] It should be noted though that some of these strains/fermentations resulted in NABs with ABVs >0.05%. Therefore, further processing would be needed to reduce the ethanol content below the legal limit in the U.S. There is evidence that NABs with a slightly fruity aroma can also be produced by mechanically removing ethanol from fully attenuated beers and then blending this product back in with a normal beer to hit a desired ABV specification.[15] Furthermore, NABs produced with inhibited or interrupted fermentation can have strong wort-like aromas and sweet tastes, but certain fermentation periods and/or botanical (i.e., citrus and hop) aroma might reduce/mask these aromas to make these products more preferable.[15]

3.4. Identifying Nonvolatile Indicators of Consumer Preference for NABs.

There are also clear relationships with the analytical factors driving the differences in taste/mouthfeel profiles and consumer liking (Figure 4). For example, the NABs, which had a low percentage of satisfied consumers, had a high sensorial bitterness, which was highly correlated with high BUs and moderately correlated with high isohumulone and humulinone concentrations (i.e., the top left in Figure 4A, Tables 1, S14, and S16). In comparison, the NAB consumers were more satisfied with were generally sweeter and had higher residual extracts (i.e., the middle right in Figure 4A). Consumers were also more satisfied with NABs that had higher CO_2 concentrations which made them fizzier and more tingling (i.e., the bottom right in Figure 4A). Also notable is that TA and sour taste were positively correlated and negatively correlated with pH. The general distributions (Figure S5) of the nonvolatile factors across the different NAB, soda, and seltzer products show that there are wide distributions in how these products are designed.

Bitterness, which was highly correlated with BU and moderately correlated with isohumulones and humulinones, was one of the main factors negatively influencing the overall and taste liking for the consumer and trained panels (Figures 4 and 5B and Table S14). As mentioned in Section 3.3, the type of hopping method has a significant influence on the resulting aroma and flavor of a product. The main technique used to impart hop-derived bitterness is kettle hopping. During wort boiling in the kettle, humulones in the hops are extracted and undergo an isomerization dependent on time and temperature, which results in the formation of isohumulones (i.e., the main driver of hop-derived bitterness and the BU in beer and NAB).[33,36] However, extracts containing isohumulones or reduced isohumulones are also sold and can be added to adjust bitterness and/or foam stability in the cellar.

The BU is the standard way to measure isohumulones in beer and NAB by the brewing industry, but because it is not a selective method, other ultraviolet-absorbing compounds influence the BU value.[38,43] BUs were highly correlated with isohumulones but only moderately correlated with humulinones, indicating that the main driver of bitterness in the NABs were isohumulones. However, Hahn, et al.[43] recently used HPLC to show both isohumulones and humulinones were drivers of bitterness in U.S. craft beers. Humulinones are ~2/3 as bitter as isohumulones, and because humulinones are extracted during dry hopping, their impact on the taste profile should also be considered. Given this, to generate NABs with preferable taste profiles, brewers could reduce the amount of hops added during kettle hop additions (i.e., iso-humulones) and increase the amount of hops added during dry-hopping (i.e., humulinones) to promote hop aroma and reduce hop bitterness. Across all the different products, the means of BU and concentrations of isohumulones and humulinones were ~18, ~12, and ~1.5 mg/L, respectively (Figure S5).

Multiple linear regression analysis (using stepwise model selection, probability for entry = 0.05 and probability for removal = 0.1) was used to further understand how the different nonvolatile factors (in Table 1), as well as all the possible third-order interactions, influenced bitterness perception for the 44 different products. The best-fitted model (i.e., bitter = $2.19 - 0.21 \times$ Er (w/w %) $+ 0.18 \times$ BU, $R^2 = 0.83$) showed that BU and Er had the most significant impact on bitterness perception (Figure S6). This shows that the residual extract had a suppressive effect on the resulting bitterness. The mean of the residual extract over the different products was ~4.7 w/w % (Figure S5). The residual extract is largely a function of the beer style in alcoholic beer and is composed of the fermentable (e.g., if remaining) and unfermentable carbohydrates, as well as protein and minerals.[43] In general, more bitter beer styles (e.g., IPAs and pale ales) tend to have higher residual extracts to make them more palatable. The beers consumers were most dissatisfied with (i.e., IPA3 and P1) had high BUs and low residual extracts (Figure 5B). IPA1 highlights the suppressive impact of Er on bitterness and increasing palatability because it had a similar BU to IPA3 but ~3× more residual extract to provide a bit more sweetness for a more balanced taste profile. The specific carbohydrate profiles of the different NABs were not measured, and individual carbohydrates can have a varying impact on sweetness. However, maltose is likely the main carbohydrate driving sweetness in NABs.[3,15] Therefore, different fermentation substrates, mashing profiles, yeast strains (i.e., the production of organic acids or assimilation of different carbohydrates) and/or varying fermentation attenuation levels could have a direct influence on the perceived sweetness of NABs.

An important factor to consider when increasing the residual extract is that this also increases the caloric content of the product. For example, P1 and IPA3 are estimated to be ~15 and 25 kcal/12 oz serving respectively, while IPA1 is ~76 kcal/12 oz serving. For perspective, Bud Light is marketed to have 110 kcal/12 oz serving. The products consumers were most satisfied with, R1 and HW2, also had very different residual extracts and calorie contents. The radler, R1, was very sweet and had a high residual extract, ~9 w/w %, resulting in ~127 kcal/12 oz serving, whereas the hop water, HW2, had a medium/low sweetness and had an Er of ~0.27 w/w %, resulting in 0 kcal/12 oz. HW2 contained some form of

alternative sweetener because it had some perceivable sweetness and such a low residual extract. Given that health and wellbeing are some main factors influencing consumers to choose to drink NABs, this is evidence that the use of alternative natural sweeteners (i.e., erythritol, stevia, xylitol, monk fruit, etc.) could also be a unique way to balance bitterness while minimizing the calorie content.[3] Also, while current regulations do not characterize hop water as NAB, there is evidence dating back to the late 19th century that breweries were manufacturing hop bitters and considered these products as alternatives to alcohol.[4]

4. CONCLUSIONS/INDUSTRIAL CONSIDERATIONS

There is clear evidence that sweet NABs [e.g., radlers (R1), hop water (HW1), and IPA (IPA1)] with citrusy, tropical, stone fruit aromas driven by volatiles such benzaldehyde, linalool, nerol, citronellol, myrcene, geraniol, and 2-methyl-buytl isobutyrate that are assumed to be extracted from botanicals (i.e., citrus juice or dry hopping) were the most highly preferred by American consumers. The survey data collected from consumers also supports this because the top three styles consumers indicated they would be most interested in trying were fruit beers, IPAs, and pale ales (Figure S4). Given that most brewers already have the equipment to add botanicals to NABs, these additions could be a relatively low-cost solution for brewers to improve and develop NABs with desirable flavors. However, care should be taken to microbially stabilize (e.g., pasteurize) these products as botanical additions have the potential to result in microbial contamination or lead to refermentations which could result in ABV being out of specification or spoilage. In addition, consumers were not satisfied with P1 and IPA3 because they were too bitter because of their low residual extracts and high isohumulone concentrations and characterized by herbal, black tea, grassy aromas driven by hexanal, pentanal, and dimethyl sulfide. Therefore, the selection of the appropriate amount and/or the right varieties of fruits or hops to promote the extraction of desired volatile and nonvolatile profiles during botanical extractions are then important considerations to the development of NAB with preferred aroma and taste profiles.

Consumers were satisfied with lager and wheat styles, which were sweeter and had fruity (i.e., banana and apple) and honey aromas. Apple character was positively correlated with 3-methylbutanal and 2-furfural, and banana character was positively correlated with phenylethyl acetate, 3-methyl-1-butanol, 2-methyl-1-butanol, isoamyl acetate, isobutanol, ethyl acetate, acetaldehyde, and 3-methylbutanal. Honey-like aroma was positively correlated with 2-furfural, methional, trans-2-nonenal, and acetaldehyde. As mentioned previously in Section 3.3, studies have shown that NABs produced using non-Saccharomyces yeasts can have aroma profiles with these characteristics/volatiles. Therefore, this study confirms that the use of non-Saccharomyces yeasts is also a promising technique to develop desirable NABs. Also notable is that, for lager- and wheat-type styles, consumers were the least satisfied with L16, L6, and W4, which were characterized by malty, skunk, and stale aromas, as well as thin and bitter taste profiles. The volatiles most positively correlated with malt flavor were ethyl nicotinate, 3-methylbutanal, phenyl ethanal, and trans-2-nonenal. Not mentioned is that skunk (also known as "lightstruck") aroma is typically related to the formation of 3-methylbut-2-ene-1-thiol (3-MBT) from either the direct or indirect irradiation of isohumulones.[44] Although 3-MBT was

not measured in this study, strategies to reduce the formation of 3-MBT have been developed, such as the implementation of reduced hop acids or light-filtering packaging in brown bottles or cans.[33]

Ultimately, these results provide direction to brewers and/or beverage producers on which techniques they should explore to develop desirable NAB offerings and suggest targets for chemical qualities, which are indicators of specific aroma or taste qualities that are either desirable or undesirable to American consumers.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c03168.

Recipes and descriptions for aroma, taste, and mouthfeel standards used in the experiments, descriptive analysis results, CATA results, concentrations of hop acids, select terpenes, aldehydes, alcohols, dimethyl sulfide, and esters in the NABs, principal component analysis biplots and external preference mapping of only the aromas and taste/ mouthfeels for the NABs, consumer demographic and survey information, least square means and Fischer LSD groupings for the liking scores of the consumer and trained panels, Pearson correlation data, box plots and scattergrams of select nonvolatile analyses, and multiple linear regression analysis of sensory bitternes (PDF)

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Notes

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