AN IN-DEPTH ANALYSIS OF BEER FLAVOR AND ITS IMPACT ON CONSUMER REFRESHING PERCEPTION

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ABSTRACT

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Beer is the most consumed alcoholic beverage worldwide and a known source of refreshment. Refreshment is defined as an emotional response to a food consumption experience; nevertheless, there is a very limited understanding of beer refreshment and its impact factors. The objective of this study was to determine how sensory attributes, specifically flavor and alcohol-related, impacted refreshment post-beer consumption using a survey, consumer study, and volatile analysis. A preliminary beer survey (n = 1,050*)* indicated that consumers believed beer was highly refreshing (≥8 out of 10). Flavor and temperature were major impactful factors for refreshment, along with a "clean and crisp" flavor profile. According to survey results, a consumer study was conducted to test how flavor types and alcohol levels impacted beer refreshment. Participants (n = 322) rated 13 beer samples (three flavor types: citrus, cucumber, or lime x 4 alcohol levels: 0%, 2.5%, 5.0%, and 7.5% abv., plus the control: non-alcoholic beer base- Heineken 0.0) for liking and intensity of refreshment as well as six sensory attributes. The consumer study elucidated that alcohol levels and flavor types significantly affected consumer's perceived refreshment based on ANOVA, MANOVA, and partial least squares (PLS), $p < 0.01$. A majority of consumers preferred a citrus-flavored beer and an alcohol level of 2.5% abv. Volatile profiles of three pilsners (Carlsberg, Heineken 0.0, and Michelob

Ultra) were analyzed using solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS). Volatile analysis indicated specific esters (ethyl acetate, ethyl butyrate, ethyl hexanoate), terpenes (D-limonene and linalool), and aldehydes (acetaldehyde, isovaleric aldehyde, hexanal, nonanal) were common volatiles in three beers, which might associate with acceptance and preference by consumers. The significance derived from this thesis research revealed that flavor type and alcohol level significantly impact perceived refreshment. The addition of citrus flavor to the beer base with a 2.5% abv. created an ideal experience of sensory attributes and the highest overall acceptance. In the development of new and existing beers, a balance of flavor, alcohol, and volatiles are imperative for the refreshing perception.

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CHAPTER I

INTRODUCTION

The first known production of beer was found in in ancient Persia (modern day Iran and Iraq) as well as Egypt over 5,000 years ago (Hornsey, 2003; Nelson, 2005). These countries had access to barley and wheat, which are still currently the most preferred grains to use by brewers. Independent discoveries of fermentable grain, fruit, and honey alcoholic beverages were formed throughout Neolithic history. However, besides wine, nothing was as popular and experienced a global expansion craze as beer did. The history of how beer was discovered is still shrouded in mystery due to being consumed before experiences and recipes were written down (Hornsey, 2003). The common agreeance is the fermentable liquid was first consumed after being derived as a bread-making by-product. However, some scholars believe beer was naturally formed after cereals broke off from the plants, soaked by rain, heated by the sun, fermented, and consumed by adventurous and thirsty people (Meussdoerffer, 2009). The fundamental principles of brewing beer are still the same today as they were thousands of years ago—malting cereal grain to provide a source of enzymes, breaking down and germinating starch from cereal by high heat and water, utilizing lactic acid formed by fermentation to acidify mash to restrict microbial growth, and the production of alcohol and carbonation by yeast and fermentable sugars (Hornsey, 2003)

When Alexander the Great conquered Egypt in 331 BC, beer traveled to Greece and Italy. From Eastern Europe, the cultivation of grain and air-drying soaked grains techniques traveled to the Celts and Germans (Meussdoerffer, 2009). Christian monasteries began brewing beer with their newfound knowledge. The monasteries then spread to central Europe in early Middle Europe, Western Europe, and eventually the Americas, brewing and drinking beer every step of the way (Meussdoerffer, 2009).

Beer is the most consumed alcoholic beverage worldwide (Nelson, 2005). With global precedence, what makes beer enjoyable and easily consumable are ongoing investigative processes. Both flavor and aroma are important sensory characteristics for overall beer liking and preference (da Costa Jardim et al., 2018; Salanta et al., 2017). With more than 800 flavor and aroma compounds, beer has a very complex sensory profile (Brányjk, Vicente, Dostalele & Teixeira, 2008; Dong, Li, Yin, & Zhong, 2014; Holt, Miks, de Carvalho, Foulquie-Moreno, 2019; Humia et al., 2019). How beer's sensory profile is related to refreshment requires more in-depth research.

The term of refreshment, refreshing, refreshed, or occasionally freshness is considered an emotional response to a food consumption experience, instead of a food sensory attribute. The act of drinking a beverage and perceiving refreshing qualities is a very complex psychosocial system derived from a multitude of sensory contributions including visual, olfactory, gustatory, tactile, trigeminal, and auditory systems (Guinard, Souchard, Picor, Rogeaux, & Sieffermann, 1998; Labbe et al., 2009a; Saint-Eve et al., 2010). Refreshment is a subjective perception dependent on many different intrinsic and extrinsic factors. Common factors correlated with refreshment include temperature, carbonation, and pH (Green, 1992; Peyrot des Gachons et al., 2016). The beer variety (ale vs. lager), environment (drinking alone or socially), and socio-demographics (specifically age and profession) also contribute to what beer consumers choose as refreshing

(Aquilani, Laureti, Poponi, & Secondi, 2015; Yang, Allenby, & Fennell, 2002). Sensory analysis techniques have been utilized to relate consumer preferences with beer attributes and intensities. For instance, total carbonation, bitterness, duration after taste, and aroma of beer all increase consumer satisfaction (Hong, Choi, & Lee, 2017). Many factors have been related to refreshment. However, the relationship between specific beer flavor and ideal alcohol by volume (abv.) to refreshment has rarely been investigated.

This research aimed to fill the knowledge gap by investigating how sensory attributes, specifically beer flavor types and alcohol levels, impact the refreshment perception derived from beer consumption, both independently and collaboratively.

Hypothesis

The refreshing perception of beer consumption amongst participants will be affected by both flavor types and alcohol levels significantly. The ideal refreshing beer will be citric in taste and encompass a low alcohol percentage.

Specific Aims

- To validate that beer is considered refreshing by consumers and investigate what flavor profiles, beer varieties, and overall attributes consumers find refreshing using a preliminary survey.
- To examine if flavor and/or alcohol percentage affect refreshment using a consumer study.
- To correlate specific volatiles with perceived refreshment and acceptance using solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) volatile analysis.

CHAPTER II

LITERATURE REVIEW

Beer Production

Beer is the third most consumed beverage worldwide, right after water and tea, and the first most consumed alcoholic drink (Meussdoerffer, 2009; Nelson, 2005). The classic beer recipe utilizes malted cereals (such as barley, wheat, or rye), hops, yeast, and water as raw materials. The beer then ferments for approximately 2 weeks in which yeasts convert malted grain sugar (maltose) into ethyl alcohol (ethanol) and carbon dioxide to provide an alcoholic and bubbly beverage (Nelson, 2005). Diverse varieties of hops with respective yeast strains may be added to the beer base recipe to change aroma, flavor, and mouthfeel to what the brewery/brewmaster desires.

Both breweries and microbreweries around the world have had exponential growth in the past 20 years. According to the Brewers Association, there are 8,386 total U.S. breweries (regional craft: 240, microbreweries: 2,058, taprooms: 2,966, brewpubs: 3,011, and large/non-craft: 111; Brewers Association, 2020a). Other top breweries per capita include the UK with over 2,000 breweries and 218 breweries on the islands of New Zealand. New Zealand has the most breweries per capita- 4.56 breweries per 100,000 people. The UK has 3.04 breweries per 100,000 people and the US has 1.96 breweries per 100,000 people (Brewers Association, 2020b). Beer is consumed all over the world and in some countries, it is cheaper than bottled water (e.g., Czech Republic; Martin, 2014). In places where water is either readily available or unavailable, beer is a refreshing beverage of choice. In the US alone, 202.2 million barrels of beers were produced and sold leading to sales of $119 billion nationwide and $520 billion globally in 2018 (Marston's House, 2019). As a result, the industry of beer and breweries has skyrocketed while trying to meet the growing demand.

Key factors that influence how consumers pick a product commonly include store location and convivence, price of product and price of a competitor, product quality, packaging, brand familiarity and knowledge, taste, brand name, emotional status, personality traits, and peer pressure (Cerjack, Haas, & Damir, 2010; Vashishth & Tripathi, 2016). A consensus amongst consumers is that brand name, reputation, and loyalty, along with sensory characteristics, are the most important attributes that influence consumer purchase decisions (Aquilani et al., 2015; Chakraborty & Suresh, 2018; Yang et al. 2002). Professional status can impact beer selection. More successful socio-demographics will pay more for craft beer (Lerro, Marotta, & Nazzaro, 2020). Craft breweries are usually on a smaller scale than commercial and pay more detail to include a desirable beer aroma, foam, carbonation, and overall quality. Age can impact beer selection as well. Younger income adults, possibly students, will choose a cheaper beer compared to an older generation who may prefer more expensive craft beer (Thompson, 2018). Drinking alone versus drinking in a group also impacts beer selection. When drinking alone, the consumer is more likely to choose a craft beer. However, when drinking in a group, consumers are more likely to choose a beer based on a lower price and product availability (Kim & Chintagunta, 2012).

Sensory Characteristics of Beer

The sensory characteristics of beer include aroma, flavor, and mouthfeel components. Common attributes used to describe beer is floral, hoppy, nutty, worty, burnt, stale, sour, sweet, bitter, astringent, and thirst-quenching (Lermusieau, Bulens, & Collin, 2001; Meilgaard & Muller, 1987; Rudnitskaya et al., 2009). The overall sensory characteristics of a beer are derived from the various ingredients used such as malted cereals hops, and yeast. These ingredients and the type of fermentation (top, bottom, or spontaneous) greatly influence the overall aroma and flavor of the brewed beer (Nachay, 2018).

A quantitative descriptive analysis (QDA) is a common sensory evaluation tool that utilizes a highly trained table of 8-12 panelists. The panelists normally are trained for multiple sessions for beer attribute standards including flavor attribute intensity (e.g., malty, hoppy, floral, fruity, spicy, honey, roasted, sweet, bitter, salty, sour, alcohol), visual (e.g., foam persistency and turbidity), texture, and mouthfeel (e.g., fullness, carbonation, and astringency; François et al., 2006; Guinard et al., 1998; Medoro et al., 2016). Interval line scales are commonly used (1-9) without subjective descriptors including like, dislike, bad, and good. The main goal of a QDA is to train panelists on attribute standards and then have the panelists be able to rate unknown beer samples in the future for those attributes. The time-intensity method (T.I.) is used for lingering effects after beer consumption, mainly astringency and after-taste (François et al., 2006). Specifically, temporal dominance of sensations (TDS) is a dynamic method that analyzes attribute interactions over time to provide qualitative changes and is used in beer product analysis (Vázquez-Araújo, Parker, & Woods, 2013). These methods can be used to hone in on how consumers perceive a beer's sensory attributes.

A consumer study is an effective tool to understand what attributes of a beer are preferred and which are not. A consumer study can be used to assess attributes such as packaging bias for how a beer taste (Barnett, Velasco, & Spence, 2016). For example, 151 participants tasted a local IPA (Edinburgh, Scotland, UK) in a plastic cup. Participants were either handed a bottle or can of the beer they had just tasted and told that this was its packaging. They were asked to rate taste, quality, freshness, the likelihood of purchase, and cost. The bottled IPA was rated higher for taste and quality; however, there were no significant differences for freshness, purchase likelihood, and price between the bottled and can beer. To summarize, beer matrix packaging can influence perceived taste, acceptance, and refreshment amongst consumers (Barnett et al., 2016).

A beer consumer study including 240 college students found that consumers can distinguish beers by specific brands by both aroma and taste attributes (Mauser, 1979). Overall, the flavor is the most important sensory characteristic followed by aroma for overall beer liking and preference (da Costa Jardim et al., 2018; Salanta et al., 2017). The base beer flavor is a result of hops, malted cereal, yeast, additives like botanicals and fruits, the type of water, sugar, and the alcohol produced (Nachay, 2018). With each of these variables, comes a change in the overall beer flavor. The overall beer flavor includes aroma, alcohol, acidity, and bitterness. For further investigation into beer flavor, the method in which the beer has been fermented, and the ingredients used are the most influencing factors.

Past sensorial consumer experiences heavily impact and affect present beer choice (Sester, Dacremont, Deroy & Valentin, 2013). Fourteen commercial beers were analyzed for both packaging (common brand names vs. lesser-known brands and glass bottle vs. can) and taste (beer variety, degree of alcohol, and familiarity level). Participants were first asked to write down what thought came to their mind for all the beers based on packaging alone, then panelists were asked to describe the blinded and randomized beer samples (no hedonic scales given-all free response). According to the consumers, past experiences with a beer, mental representations, and attitude on taste and packaging, all contributed to if they chose to drink that beer again. Most consumers base a new beer based on their first experience so if the flavor expectation like bitterness, texture expectation like carbonation, and physiological expectation like being thirst-quenching, are not met, the beer will be rejected (Sester et al., 2013). Brand familiarity also significantly impacts consumer attitudes and preferences. The more familiar the consumer is with the product, the more acceptance was derived (Aquilani et al., 2015; Giacalone, Bredie, & Frøst, 2013; Giacalone et al., 2015; Yang et al., 2002).

Three Types of Beer Varieties and Their Corresponding Flavors

Top, bottom, and spontaneously fermented beers are composed of different characteristics such as foamability, aromas, and flavors (Gonzalez-Viejo, Fuentes, Torrico, Godbole, & Dunshea, 2019). The main differences in beer aromas and flavors are due to the two main beer variety types: ales and lagers. Ales are top-fermented meaning that the yeast is directly applied on top of the wort (the ground malt and grains that have been heated between 15-25 ℃ (Vidgren, Multanen, Ruohonen, Londesborough, 2010). Ales are the original type of beer fermentation method and utilize the yeast *saccharomyces cerevisiae* (readily available in nature and has many diverse yeast strains). Ale aromas included apple, pineapple, rose, and honey volatiles, with the addition of grape and banana. The flavors of ales are darker, fruity, and full of esters. Ales also include phenols such as tannins to provide bitterness to balance the fruity flavors (Bokulich & Bamforth, 2013).

Lagers are bottom-fermented beers and are what consumers describe as "clean and crisp" meaning light and carbonated (Bokulich & Bamforth, 2013). Lagers utilize *saccharomyces pastorianus.* The lager yeast strain is much more complex than ale and has evolved by the hybridization of *saccharomyces cerevisiae* and *saccharomyces bayanus* (Turakainen & Korhola, 1994). The yeast gathers at the bottom of the wort and is much more effective at a lower temperature (6-14 ℃) compared to ales (Vidgren et al., 2010). Due to the yeast preferring a lower temperature, the bottom-fermentation process is longer than the top-fermentation process and allows more time for carbonation. Like ales, lagers beers also contain fruity and floral volatiles such as apple, pineapple, rose, and honey (Gonzalez-Viejo et al., 2019). However, lagers are composed of less pronounced flavors compared to ales. Pilsners are the most common type of lager. There are three different styles of pilsners: Czech, German, and American. According to a consumer study that tested three lagers and three ales against each other for preference, the American pilsner was rated the highest due to the low intensity of hoppy flavor, phenols, and bitterness (da Costa Jardim et al., 2018).

Spontaneously fermented beer uses "wild" yeast strains such as *kloeckera* and *saccharomyces*, bacteria of the genus *pediococcus*, and *brettanomyces* yeasts. Spontaneously fermented beers are more acidic than ales and lagers and are also tangy (high concentration of acetic and lactic acid, ethyl acetate, and ethyl lactate; Spaepen, Oevelen, & Verachtert, 1978). Common spontaneous fermented beer aromas include pineapple, apple, grape, cherry, and raspberry (Gonzalez-Viejo et al., 2019). When comparing all fermentation types, top and spontaneous fermentation have more volatiles compared to bottom fermentation (Gonzalez-Viejo et al., 2019). The most common beer variety made by spontaneous fermentation is Lambic. Lambic beers originate in Belgium and are fermented through exposure to wild yeasts and bacteria residing within timber vessels (Spitaels et al., 2014).

Alcohol by volume (abv.) is a direct result of fermentation and differs based on beer variety (Missbach et al., 2017). Usually, ales have a higher abv. with intense sensory attributes such as hoppy and bitter. The highest abv. beers include Indian pale ales (single and double IPAs), Belgian Triple/Quadruple, Eisbock, and Imperial Stouts. With lower abv. beers, the flavor is much less bold due to the lack of complete fermentation and therefore lower levels of alcohol, carbonation, mouthfeel, and acidity. Alcohol content does impact beer flavor resulting in the requirement of nonalcoholic beer production to include additives to mimic natural beer flavor (Bokulich & Bamforth, 2013). A consumer survey was taken by university students (n = 1,057) which assessed alcohol consumption, product perception, and preference. Beer and wine (69.1%) were the most commonly consumed alcoholic beverages amongst consumers. Frequent emotions consumers experienced with alcohol consumption was relaxation and socialization (38.2%) followed by overall flavor and aroma enjoyment (32.1%), and euphoria (16%). Alcoholic beverages are consumed for these relaxing, refreshing, euphoric, thirst-quenching, stress-relieving, confidence-boosting, and appetite-inducing reasons (Ogbonna, 2009).

Beer Volatiles

With more than 800 flavor and aroma compounds, beer has a very complex sensory profile (Brányjk et al., 2008; Dong et al., 2014; Holt et al., 2019; Humia et al, 2019). The main compounds that are commonly investigated are diacetyl, ethyl caprylate, propanol, acetaldehyde, ethyl acetate, the alcohol ester ratio, and specific gravity (Brányjk et al., 2008; Dong et al., 2014). The important acetate and ethyl esters in beer are produced by yeast growth, lipid metabolism, and are a byproduct of fermentation (Kobayashi, Nagahisa, Shimizu, & Shioya, 2006; Olaniran, Hiralal, Mokoena, & Pillay, 2017). Other flavor components in beer include hops, ethanol, and low pH. Hops are the source of bitterness, floral, and fruity aroma notes, and act as a preservative. A weak acid is derived from ethanol production from the fermentation of yeast and malt. This acidy gives beer its character tang and bite.

There are specific compounds that all beer contains including ethyl caproate, ethyl octanoate, ethyl decanoate, ethyl laurate, and phenylethyl alcohol. However, there are differences such as top-fermented beers have a high percentage of volatiles, bottom-fermented in the middle, and spontaneous fermentation contained the least percentage of volatiles. When panelists were asked to rate which beer aromas they preferred, they chose ethyl caproate (apple/green banana/pineapple aroma), 4-ethylguaiacol (smoky/bacon/spicy aroma), and trans-β-ionone (violet/raspberry aroma; Gonzalez-Viejo et al., 2019).

The most common volatile extraction and identification technique is SPME-GC-MS. The headspace of beer can be analyzed and identified for specific organic volatile compounds. Beer fermented from the top, bottom, and spontaneously all encompass different aroma profiles according to SPME-GC-MS (Gonzalez-Viejo et al., 2019). Previous researchears have investigated the extraction efficiency, capacity, and variation of beer volatiles, with the use of four different methods: headspace solid-phase microextraction (HS-SPME), stir bar sorptive extraction (SBSE), headspace sorptive extraction (HSSE), and solvent-assisted flavor evaporation (SAFE) with dichloromethane (Richter, Eyres, Silcock, & Bremer, 2017). The study concluded that the SBSE method was the best for extracting acids and the HSSE method was the most efficient for esters and aldehydes. HS-SPME resulted in 40% fewer volatiles than the other methods and SAFE was not successful for hop-derived volatile extraction (better for alcohols and acids). HS-SPME and HSSE are both solventless techniques that extract volatiles and captures them for analysis. HS-SPME uses fused-silica coated fiber and HSSE uses polydimethylsiloxane (PDMS) coated film on a stir bar (Cavalli, Fernandez, Lizzani-Cuvelier, & Loiseau, 2003). Overall, HSSE was the best-found method for beer volatile extraction efficiency and capacity (Cavalli et al., 2003; Richter et al., 2017).

Alcoholic and nonalcoholic beers can be compared using SPME-GC-MS. The alcoholic beer's aroma profile consists of fermentation by-products including esters (e.g., isoamyl acetate, ethyl hexanoate, and octanoate), alcohols (e.g., isoamyl alcohol, 1-octanol, isobutanol), and fatty acids (e.g., hexanoic and octanoic acid). The nonalcoholic beer aroma profile consisted more of roasted and toasted aromas including pyrazines and furans (Riu-Aumatell, Miró, Serra-Cayuela, Buxaderas, & López-Tamames, 2014). Flavors and aromas are attributed to volatiles and non-volatiles, based on alcohol content, hops, and yeast, and together work harmoniously to contribute to refreshment.

Refreshing Perception and Impact Factors of a Beverage

The refreshing effect is a popular topic for beverages. Defining what is refreshing to a consumer is crucial in order to understand how to influence and enhance the perception of refreshment. Refreshment is defined as a way to restore strength and animation, revive, arouse, stimulate, and contain thirst-quenching properties (Labbe et al., 2009a). Refreshment is a multi-dimensional concept that is based on consumer opinion. Refreshing and thirst-quenching terminology go hand-in-hand to depict acceptability (McEwan & Colwill, 1996). To further investigate what beverage descriptors are associated with refreshing and thirst-quenching, a summary of seven attributes were derived and examined with the use of a focus group (McEwan & Colwill, 1996). The attributes were acidity, astringency, carbonation, fruity, the strength of flavor, sweetness, and thickness. After trained panelists tasted eight different beverages, acidity was the attribute most associated with thirst-quenching/refreshing/drinkability. Sweetness and thickness were the attributes associated with the least association with thirst-quenching and acceptance (McEwan & Colwill, 1996).

Impactful factors contributing to liking, preference, and refreshment of soft and alcoholic drinks come from a combination of low temperature, carbonation, acidity, and bitterness (Green, 1992; Missbach et al., 2017; Peyrot des Gachons et al., 2016; Satoh-Kuriwada, Shoji, Miyake, Watanabe, & Sasano, 2018). The main factor behind why beverages of cold temperature (40-50°F or 4-10°C) are perceived to be refreshing is due to oral cooling (Peyrot des Gachons et al., 2016). A possible correlation is a fact that people can inhale larger amounts and hold their breath for a longer duration when inhaling cool air than warm. People can also consume more cold water than warm. In previous literature, participants were able to drink a larger volume of cold water, water containing menthol, and cold-carbonated water than room temperature water suggesting that these oro-sensory traits strongly enhance the thirst-quenching properties of a beverage. Participants estimated intake for the cold and carbonated water was (22%) higher than the actual amount suggesting these traits make the brain believe the body is ingesting more fluids than it actually is (Peyrot des Gachons et al., 2016).

Carbonation is critical for a beer to have thirst-quenching and refreshing properties. People tend to crave cold and carbonated beverages such as mineral water, seltzer water, sodas, and beer when they are thirsty. When carbonated water is ingested at room temperature, the temperature of the water is perceived to be cooler than it is. The bubbly effects of carbonation are described as mentally awakening and invigorating. However, published research explains that carbonation and strong flavor profiles like malty, hoppy, and bitterness are negatively associated with liking and preference (Green, 1992; Peyrot des Gachons et al., 2016). The mouthfeel of a beer is a highly important aspect of sensory analysis. The nine concepts commonly focused on during beer assessment include the carbonation sting, carbonation bubble size, foam volume, total carbonation, density, viscosity, oily mouthfeel, astringency, and stickiness (Langstaff et al., 1991). Bottom-fermented beers such as lagers and pilsners have more carbonation and are perceived as more refreshing than top-fermented beers like ales and stouts. Research has proven that refreshment is impacted by carbonation thus perfecting the carbonation ratio in beer is imperative (Green, 1992; Peyrot des Gachons et al., 2016).

Acid can also play a part in refreshment (Peyrot des Gachons et al., 2016). Carbonation is an organic carbonic acid that can increase refreshment, especially when combined with cold temperatures. The five basic senses: sweet, salty, sour, bitter, and umami were investigated to understand which sense(s) contribute to labial minor salivary gland (LMSG) secretion and blood flow. All senses except for bitterness increased blood flow to the near LMSG. Umami and sour samples caused a greater secretion than the others. Acidic samples wet the mouth more than the other taste and give an impression of hydration (Satoh-Kuriwada et al., 2018). The citric acid in lime, lemon, orange, and grapefruit has also been shown to increase refreshment (Peyrot des Gachons et al., 2016). The recommended amount of citric acid is 50-250 parts per million (ppm) in beer and is the natural and direct result of yeast metabolism and fermentation (Green, 1992). Incomplete fermentation of beer can occur in low and nonalcoholic beers. In this case, citric acid can be added to increase acidity levels to a desirable range. Acid can also increase the preservation of the beer and slow microbial growth. Besides being a factor of refreshment, acidity in beer improves the overall quality of the beverage.

For thirst-quenching qualities, beer needs to have bitterness and carbonation (Missbach et al., 2017). Hops are a source of bitter flavor in beer (Almaguer, Schönberger, Gastl, Arendt, & Becker, 2014; Taniguchi, Yamada, Taniguchi, Matsukura, & Shindo, 2015). The bitter taste and hoppy floral aroma reside in the resin derived from hops (*Humulus lupulus L*; Olaniran et al., 2017). Along with these components contributing to flavor and aroma through essential oils, they also provide microbiological stability in the prevention of spoilage and help stabilize beer foam and cling (Almaguer et al, 2014). Since the origination of brewing beer, bitter herbs and hops have been used to balance sweet malt creating a desirable beverage (Mosher, 2009). A beer's bitterness is commonly assessed by international bitter units (IBUs). The higher the IBUs, the more bitter the beer will taste. Common beer variety IBU range of a lager is (5-15 IBU), pilsner (20-40 IBU), IPA (35-65 IBU), amber lager (18-30), Lambic (11-23 IBU), and porter and stout (20-40 IBU). To investigate the stages of bitterness during three stages of beer production, 60 beer samples were tested during wort (boiling), after fermentation, and once bottled (10 measurements for each stage). During the brewing process, bitterness gradually decreased as expected to a preferred bitterness level (losses in IBUs were between 4.7-41.54%). The role of bitterness in refreshment is more secondary in that bitterness alone would not lead to refreshment (Mosher, 2009). However, when used as a balancing agent, bitterness is imperative.

Refreshment Derived from Beer Consumption

Beer refreshment is important for both consumers and the beer industry for the reason that refreshment can make a beer stand out in a huge market, provide a pleasurable and enjoyable experience, and encourage the consumer to want to keep drinking more due to its thirst-quenching, oral cooling, and highly satisfying attributes. The degree of beer refreshment is related to the participant's perception of stimulation, invigoration, and hydration post beer consumption. The importance of analyzing beer refreshment is knowing exactly what consumers are tasting and experiencing when they consume a beer and describe refreshment. Eighteen different beers were analyzed by 12 panelists in a highly trained QDA setting to discover which sensory characteristics are significant determinants of thirst-quenching, refreshing, and drinkability (Guinard et al., 1998). Positive attributes that increased refreshing perception were carbonation and bubble density. Attributes that affected the determinants negatively included foam, overall aroma and flavor intensity, color, viscosity, malty, hoppy, burnt, acidic, metallic, astringent, and after taste. The study concluded that carbonation impacts refreshment greater than other sensory attributes including aroma and flavor (Guinard et al., 1998).

The expression “refreshing/refreshment” has been used in the beer industry to describe beers, especially those of a lemon/citrus profile and cold temperature (Petrak, 2012). However, three factors contribute to fluid maintenance and thirst-quench-ability: the sensory attributes of the beer itself, the internal environment of the consumer (water balance), and the external environment (social circumstances and temperature; Guinard et al., 1998; Ramsay & Booth, 2012). The common consumer desires a certain variety of beer depending on their flavor profile preference (ales, lagers, sours, wheats, IPAs, bocks, brown ales, stouts, etc.) and alcohol percentage preferences (average between 4-7% abv.). However, for some consumers, seasons can impact preference (spring and summer: light, crisp, fruity, wheat, pale and hoppy beers are the most commonly consumed varieties, fall: pumpkin, maple, amber/reds, Belgians, and Oktoberfest varieties are popular, and in the winter: stouts/porters, imperial, and bourbon barrel-aged varieties with flavors of chocolate, and coffee are the most desired; Miller, 2016; Rulkova, 2019).

There is a large research gap for the refreshing attribute of beer. Sensory attributes such as temperature and carbonation have been studied for their refreshing qualities. However, there is little research previously conducted to understand what specific attributes in beer (such as flavor and alcohol content) impact refreshing perception. This study was done to fill the knowledge gap as to what attributes directly impact consumer's perception of the refreshing qualities of beer.

Literature Summary

Beer has been around since between 3,500-2,900 B.C. (Nelson, 2005). In the centuries that have passed, consumers have redefined how to make beer more enjoyable and refreshing. Through tweaking the recipe with varieties of hops, cereals, and yeasts used, beer has evolved for optimal flavor, drinkability, and refreshment. Utilizing sensory analysis techniques such as QDAs, time-intensity methods, and consumer studies, beer drinkers have correlated acceptance and liking with multiple factors such as temperature, carbonation, acidity, bitterness, aroma, flavor, drinking environment, socio-demographics, and alcohol effects on the body. With SPME-GC-MS analysis, volatiles can be identified to certain aromas and flavors and can be related to consumers' preferences. Beer’s refreshment is imperative to its success. However, the in-depth mechanisms behind how flavor and alcohol impact perceived refreshment has not yet been well studied. Identifying what volatiles, flavors, and alcohol levels are correlated with optimal refreshment perception still needs further investigation.

CHAPTER III

METHODOLOGY

This study was composed of three main methods: an online preliminary survey, a consumer study, and volatile analysis. Human subjects were used and provided written and informed consent. All methods and procedures have been reviewed and granted approval by the Texas Woman's University Institutional Review Board (IRB) on July 18, 2019.

Online Survey

Survey Experimental Design

The main method of obtaining data was through the Texas Woman's University (TWU) email list. The list included the Dallas, Denton, and Houston, Texas campuses composing of approximately 20,000 students and faculty. The survey was launched through the Legacy Hall (a Plano, Texas food hall) employee email list to approximately 200+ people to obtain a diverse range of survey participants as well. The survey was also presented on a social media platform (Facebook; CA, USA).

The survey was made and completed on Google Forms (Google; CA, USA). Eleven questions about beer refreshment were asked, along with five questions about participant demographics shown in Appendix C. For all questions about beer refreshment, participants were allowed to choose as many answers that were applied to prevent the bias of choosing a sole answer. Incentives for participants were 10, $10 Amazon gift cards. Participants were asked if they would like to be included in the Amazon raffle and prompted to enter both their name and corresponding email addresses.

Winners for the gift cards were completely drawn at random A total of 1,050 participants completed this survey (*N* = 1,050).

Statistical Analysis

All data were collected and processed directly on Google Forms. Values are shown as a direct value and/or as a percentage. All figures were automatically generated through Google Forms.

Consumer Study

Beer Sample Preparation and Formulation

Non-alcoholic Heineken 0.0 (made by Heineken N.V. Netherlands, distributed by Heineken & Molson Coors Brewing Company [White Plains, New York]) was used as the base to spike different concentrations of alcohol and different types of flavor. This bland beer was also served as a "control" during consumer testing. The design of the consumer study is depicted in Figure 1.

Alcohol by volume (abv.) was controlled by adding ethyl alcohol (Sigma-Aldrich, 100% purity, food-grade) to the base (non-alcoholic Heineken 0.0). Each flavor type has four different abv. amounts (0%, 2.5%, 5%, and 7.5%) along with one control (no added flavor or alcohol-just beer base) to create a total of 13 unique samples. However, each control will be tested individually to achieve a total of 15 tested samples (see Figure 1).

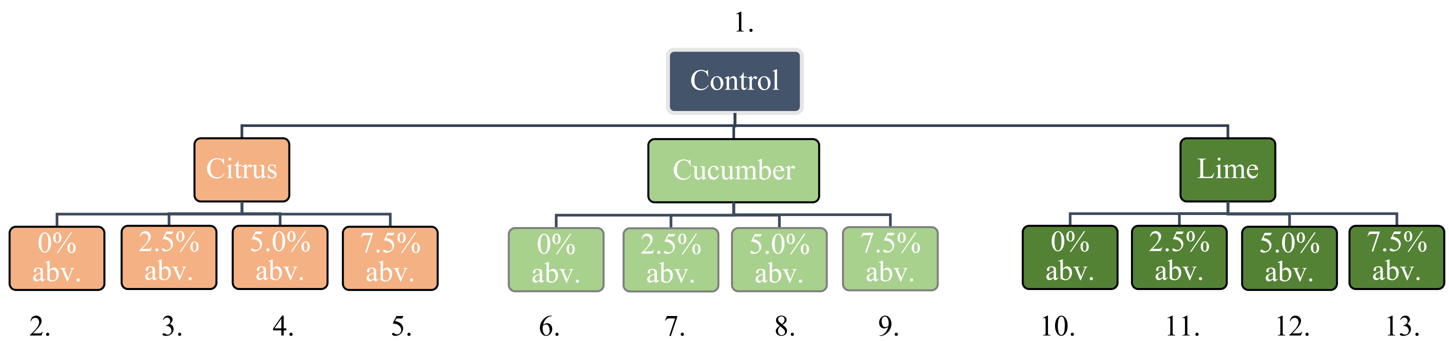


Figure 1. Consumer Study Design. Four alcohol levels (0% abv., 2.5% abv., 5.0% abv., and 7.5% abv.) were included in three, unique beer-flavored tests within the overall consumer study to compose of thirteen distinctive samples. The control (no added flavor or alcohol) was the same in each flavor test.

The beer samples spiked with different levels of alcohol were flavored using flavorings from commercial resource. The citrus-flavored beer samples contained 100ppm of the citrus flavor (Firmenich 516819T,10μL of citrus flavor per 100mL beer base), the cucumber-flavored beer samples contained 20ppm cucumber aldehyde (Sigma (*E, Z)*-2,6-nonadienal, 2μL cucumber aldehyde per 100mL beer base), and the lime-flavored beer samples contained a mixture of a concentrated lemon flavor (Firmenich 560059CW lemon flavor, 15μL per 100 mL beer base) plus fresh lime juice (75mL of fresh strained lime juice per 100mL of beer base). Lime was purchased from a local grocery store (Kroger, Denton, TX, USA). The dose of three flavors was pre-tested, optimized by trial and error, and selected according to the most accurate representation of authentic citrus, cucumber, and lime flavors as well as pleasant flavor intensity levels.

To control carbonation, commercial CO_2 cartridges (16g, 3/8-24 pitch threaded, Drink Tanks, Bend, Oregon, USA) were used to attach to a growler dispenser tap (Spotted Dog, Amazon, Fayetteville, Arkansas, USA). On the day of the consumer studies, beer samples were formulated and stored in 64-ounce growlers and outfitted with brand new CO_2 cartridges. The optimal PSI (pounds of force per square inch area) for each sample was between 1-3 PSI. Pressure in the growler was regulated by having a visible pressure gauge and a pressure relief valve on top of the dispenser tap.

Due to the consumer study taking place in the US, the optimal serving temperature for each sample was between 6-9 ℃ (42-48 °F; Mosher, 2009), although countries all over the world have a difference in the optimal serving temperature of a lager and most countries serve lagers warmer than the United States (7-10 ℃). The beer samples' temperatures were controlled by placing growlers inside a small refrigerator (6 ℃) for a minimum of 90 min before the start of each test. There was a noted temperature fluctuation of the refrigerator between 6-10 ℃ (42-50 °F) due to the opening/closing of the door. To maintain the desired serving temperature for the beer samples, the opening of the refrigerator doors was kept to a minimum.

Consumer Study Design

The study was separated into three tests based on flavor: Test One- citrus flavored (4 citrus samples with 4 varying abv. and a control), Test Two- cucumber flavored (4 cucumber samples with 4 varying abv. and a control), and Test Three- lime-flavored (4 lime samples with 4 varying abv. and a control; see Figure 1). The control was the same in all three tests, which was non-alcoholic Heineken 0.0, without spiking with alcohol and flavors. The beer sample container was clear, 4-ounce glasses with transparent plastic caps, which sealed the glassware. All samples had the same color to prevent optical bias. However, the aromas of the samples were different and unique to that of the tested flavor. Samples were presented to participants at 1-2 PSI and at a temperature range of 6-9 ℃ (42-48 °F).

Participants were allowed to partake in one beer-flavored consumer study at a time, either composing exclusively of citrus, cucumber, or lime-flavored samples. Participants could come back in a few hours or the next weekend to take a different beer-flavored consumer study. However, most participants only completed one flavor test. Data from the same participants were not taken collaboratively and, therefore, were statistically analyzed independently.

Each consumer study participant was given a tray including the five randomized beer samples (corresponding to the tested flavor, 60 mL for each), palate cleaners including a cup of water and unsalted saltine crackers (Keebler-Zesta unsalted, saltine crackers), an iPad on which participants would rate the samples (Compusense), and a napkin. The beer samples contained a randomized 3-digit code that would match the test on the iPad (Compusense software cloud link) to ensure study participants were rating the appropriate sample. Only the test administrator was aware of which codes were responsible for which sample. The samples were presented randomly for each participant; sample orders were randomized via Compusense. An example of the test ballot can be seen in Appendix D.

Consumer Tests of Formulated Beers

All methods and procedures have been reviewed and granted approval by the TWU IRB. The beer refreshment survey used human subjects provided with written and informed consent. The consumer tests were performed over two weekends. One flavor test was administered at a time to prevent bias and confusion. Each flavor test was taken by a minimum of 100 participants (citrus [n = 114], cucumber [n = 105], lime [n = 103]). The location of the test was at Legacy Hall in Plano, Texas. This venue was chosen due to its vast range in demographics, accessibility to large crowds of beer consumers, and allowance of customers to participate in this consumer study. In addition, this venue encompassed a typical bar-like environment where beer is consumed in a social setting. During study recruitment, the ideal participant was one who consumes beer one to five times a week.

Consumers were blind to all tested samples. Each flavor test was taken on an iPad using Compusense software (Compusense, Guelph, ON, Canada). Each test was composed of same 15 questions for each corresponding tested flavor. Questions of the consumer study included seven hedonic questions: the likings of refreshing, overall beer flavor, tested flavor, alcohol, carbonation, acidity, and bitterness. The liking questions were based on a 1-9 hedonic scale. For all purposes throughout this study, *liking* and *hedonic* will be used interchangeably. The consumer study also included six just-about-right (JAR) questions that corresponded to the attribute intensity on either a 1-9 JAR scale (only for refreshing intensity) or a 1-7 JAR scale (for all other attribute intensities). One CATA (check-all-that-apply) question was applied. The participants were asked to try the sample and then check-all-that-apply to the sample with specific descriptors like clean, green, lemon, lime, citrus, crisp, cucumber, astringent, candy, and other. The CATA question was included in the study to observe what descriptors can describe highly rated refreshing beer samples.

On the Compusense test, directions were given to consumers to take palate cleanser breaks. Between each tested beer sample, consumers were met with a mandatory 15-second break in which they were instructed to cleanse their palate by eating a saltine cracker and drinking filtrated water. At the end of each test, participants were prompted to enter demographics, including age (≥ 21), gender, beer consumption frequency, education, and employment status. The consumer study was taken on an iPad using Compusense to collect data. Participants were given a unique login and password in order to collect every participant's data independently and with their corresponding demographic data.

Statistical Analysis

Analysis of variance (ANOVA), multivariate analysis of variance (MANOVA), and Pearson correlation analysis were performed using SPSS (IBM Corp. 2017. IBM SPSS Statistics for Mac, Version 25.0. Armonk, NY, USA). JAR penalty analysis, principal component analysis (PCA), partial least squares (PLS), and an agglomerative hierarchical clustering (AHC) were all analyzed using XLSTAT (XLSTAT V. 2020.1. Data Analysis and Statistics Software for Microsoft Excel 2020. Addinsoft, New York, NY, USA). Although the three controls were the same for all tests, their data were analyzed independently using ANOVA, MANOVA, PCA, and PLS-R. The differences of each attribute between beer samples were determined by Tukey's posthoc test ($p \leq 0.05$).

Volatile Analysis

Beer Volatile SPME-GC-MS Method Optimization

To understand if beer volatile quality and quantity analyses are influenced by ethanol, a series of gradual dilutions were performed on beer samples. The abv. of Michelob Ultra is 4.2%. The beer was then diluted to 2.1% abv., 1% abv., and 0.5% abv. with deionized water. Each dilution set was performed in triplicate. The end result was 12 samples. Three milliliters of each diluted sample was placed into a 20 mL autosampler glass vial with 1 g of sodium chloride (100% purity, Food Grade) to preserve samples and to improve the extraction efficiency of volatile compounds in the headspace due to the salting-out effect. The vials were then sealed with an aluminum crimp cap.

To investigate the impact of gas (CO2) on beer volatile extraction, beer samples were degassed. Fifty milliliters of each beer sample was poured into individual beakers and degassed in an ultrasonic bath for 10 min at 20 °C (temperature was controlled throughout the 10 min and monitored via thermometer) to remove CO2. Carbonation was judged by the beer in the beaker being completely flat, meaning no visible gas bubbles indicating carbonation. Next, 3 mL of each degassed sample was poured into a 20 mL autosampler glass vial and 1 g of sodium chloride (Food Grade) was added. The vials were sealed with an aluminum crimp cap. All samples were prepared in triplicate.

Beer Sample Selection

Three lagers were compared: Heineken 0.0, Michelob Ultra, and Carlsberg. These specific lagers were chosen based on the preliminary consumer survey, the beer variety rated the lightest, crispest, and most refreshing. All three samples were also tested for pH, titratable acidity (9TA; Metrohm 905 Titrando- citric acid setting), and °Brix (measuring sugar content) using a refractometer (Atago 3810). For all pH and TA calculations, 10mL of the beer sample was added to 50mL deionized water in a 250mL beaker. For °Brix, no dilution was performed, only direct application of beer to refractometer. TA, pH, and °Brix were performed in triplicate for all three samples and an average was taken.

SPME-GC-MS Volatile Identification

Volatiles of three selected beers were compared to understand what specific compounds are in common amongst the lager beer variety that contributes to refreshment. The SPME fiber was conditioned at 200℃ for 20 min while the beer sample in the vial was shaken and heated. The SPME fiber is released from the conditioning port and inserted (split-less) into the headspace for 3 min preconditioning time, the incubation temperature was 40℃ with an incubation time of 15 min, agitator speed of 250rpm, sample vial depth of 22 mm, sample extract time of 20 min, and a sample desorption time of 2 min.

The SPME fiber is then inserted into GC-MS (Line 1). A DB-wax packed with polyethylene glycol capillary column (30 m × 0.25 mm, and 0.25 μm film thickness) was used with helium carrier gas with a column-head pressure of 51 kPa and flow rate at 1.0

mL/min. The total and purge flow was 3mL/min. The column temperature started at 40 °C and held for 3 min, increased to 120°C at a rate of 5 °C min^{-1}, increased to 250 °C at a rate of 10 °C min^{-1}, and then held for 5 min. For the MS, the ion source was 200°C, interface of 230°C, a lower vacuum of 5.2e +000 Pa, and a higher vacuum of 1.2e-004 Pa. The total analysis time was 47 min. Blank runs (empty vials) were used in between each unique beer sample to ensure no carryover of analytes from previous injections. Post-run analysis using Shimadzu GC Solutions was used for volatile identification. Volatiles were identified by comparing their mass spectra with NIST and Wiley libraries for all three replications.

Statistical Analysis

Data collection from SPME-GC-MS triplicates was utilized to perform ANOVA and *t*-test statistical analysis using SPSS. Tukey's HSD test was used to identify significant differences between samples. A α-error of less than 0.05 was deemed statistically significant.

CHAPTER IV

RESULTS

Online Survey

Potential Reasons Why Consumers Drink Beer

Factors and reasons as to why consumers drink beer were answered in using an online survey (see Figures 2 and 3). Participants were asked what factors were considered when choosing which beer to drink. Out of 1,050 responses, 965 participants (91.9%) answered that flavor was a factor when choosing which beer to drink (see Figure 2). This indicates that the flavor is a major concern when consumers are choosing a beer. The next top responses include the brand of beer (n = 526, 50.1%), the beers locality (n = 415, 39.5%), alcohol percentage (n = 427, 40.7%), and a specific beer variety (n = 406, 41%). In addition, participants were asked to indicate the main reasons for drinking beer (see Figure 3). Beer taste was a key factor that consumers looked for. Over 77% of participants said they drink beer for the taste (n = 810, 77.1%). The next most commonly chosen reasons for why people drink beer include feeling refreshed after consumption (n = 583, 55.5%), beer alcohol effects on the body (n = 447, 42.6%), and 350 participants stated they have a preference for beer over liquor (33.3%). One hundred and sixty-nine participants stated they drink beer due to social pressure (16.1%). Overall, beer flavor/taste was the most important factor, followed by the factor of "feeling refreshed after beer consumption," for people drinking beer. The concept of beer refreshment was further investigated then.

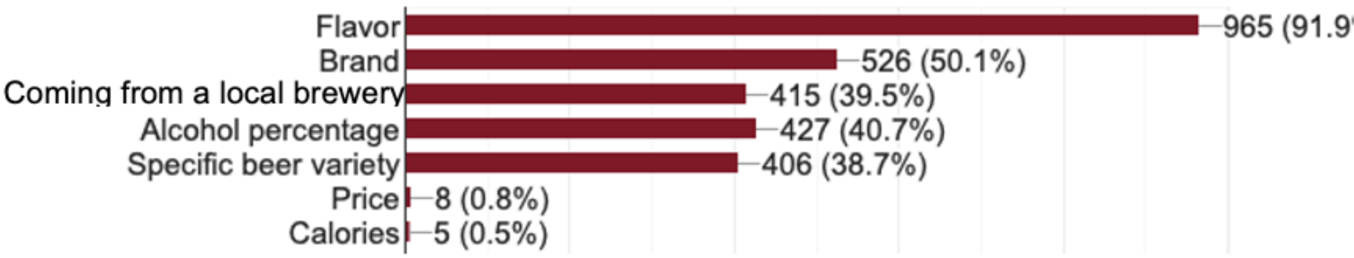


Figure 2. Important Factors When Choosing a Beer/Brand.

Figure 3. Main Reasons Why Participants Drink Beer.

Consumers Preconceived Opinions on Beer Refreshment

To first examine if consumers find beer refreshing, participants were asked to rate how refreshing they thought beer was on a scale from 1 (*very low or no refreshment*) to 10 (*very refreshing*). Consumers, as a consensus, claim beer refreshment rate was high (see Figure 4). A majority of participants, 961 out of 1,050 (91.5%), rated above the middle point (above 5, out of 10). This included 165 participants rating refreshment a 10 (15.7%), 127 participants rated it a 9 (12.1%), 296 participants rated an 8 (28%), 205 participants rated it a 7 (19.5%), 105 participants rated it a 6 (10%), and 63 participants rated it a 5 (6%). Much smaller percentages of participants rated beer's refreshment below 5 (middle point).

Participants claimed the major feeling associated with refreshment was a lightened mood (n = 915, 87.1%; see Figure 5). The next most chosen feelings from refreshment included quenched thirst (n = 514, 49%), feeling invigorated (n = 271, 25.8%), and re-energized (n = 134, 12.8%). One other refreshing feeling that many people experienced was relaxation (n = 15, 1.4%). This indicates that beer refreshment perception may be a complexed feeling.

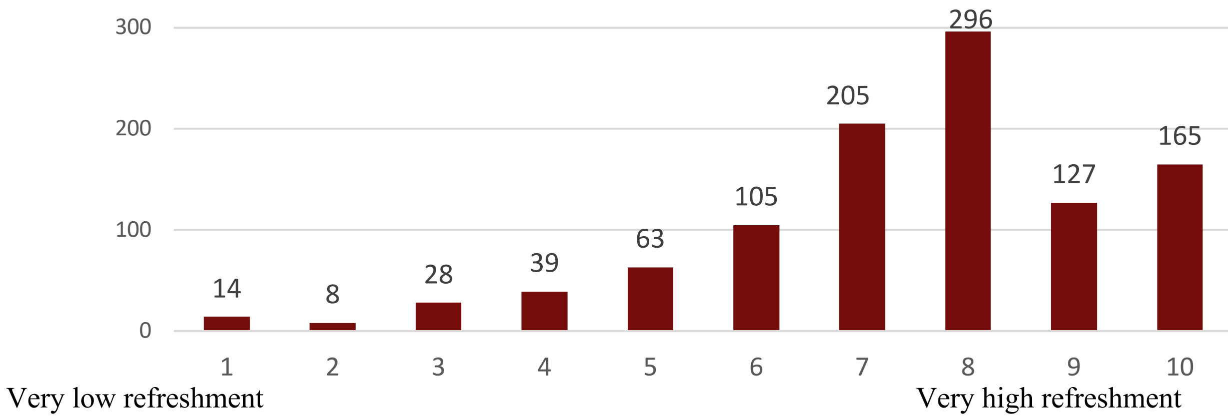


Figure 4. Perceived Refreshment on a 1-10 Hedonic Scale. Values shown above the bar are the number of participants who chose the respective hedonic value.

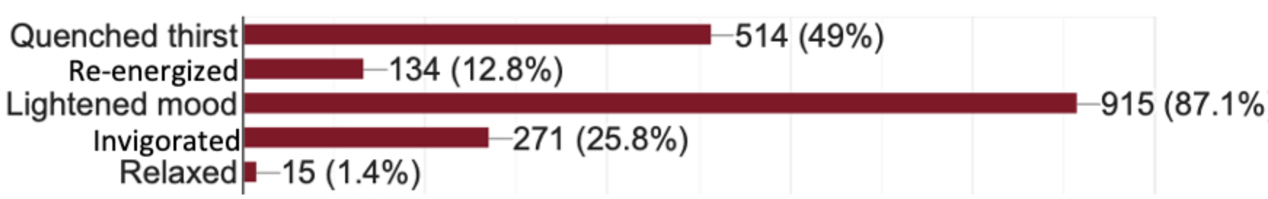


Figure 5. Feelings from Refreshment.

Product Factors Related to Perceived Refreshment Derived from Beer Consumption

Sensory factors of beer that impact refreshment are explained in Figure 6. Out of 1,050 responses, a majority of 1,002 participants (95.4%) answered that a cold/chilled temperature impacts beer refreshment perception. The next most chosen factor was flavor. Flavor was chosen by 930 participants (88.6%) as an impactful refreshment factor. Less commonly chosen were the matrix the beer is presented in (aluminum can, bottle, draft, etc.) with 464 participant responses (44.2%), carbonation (*n* = 441, 42%), and the variety of beer (*n* = 345, 32.9%). The least chosen beer factor that impacts beer refreshment was alcohol effects on the body (*n* = 315, 30 %).

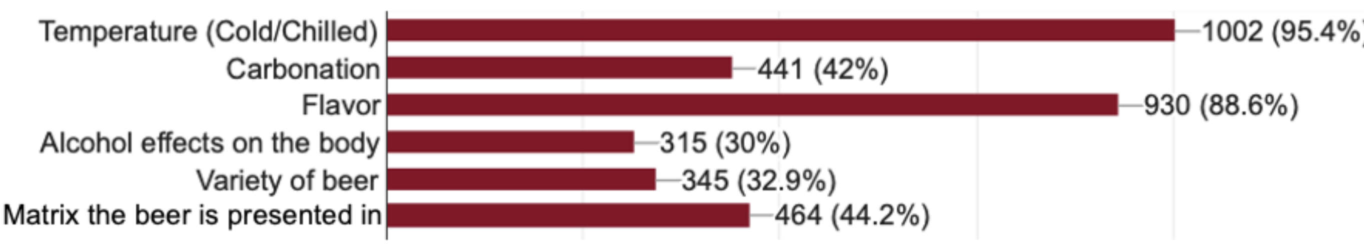


Figure 6. Impactful Sensory Factors on the Perception of Beer Refreshment.

Beer flavor was very important to consumers as explained in the previous question. When asked what flavors are preferred, the most commonly chosen beer flavor was lime (*n* = 543, 51.7%) as depicted in Figure 7. The next majority beer flavors chosen by respondents include lemon (*n* = 452, 43%), orange (*n* = 425, 40.5%), amber (amber-colored American style light lager; *n* = 380, 36.2%) and grapefruit (*n* = 375, 35.7%). Other commonly chosen beer flavors include hoppy (*n* = 276, 26.3%), honey (*n* = 266, 25.3%), strawberry (*n* = 270, 25.7%), raspberry (*n* = 224, 21.3%), blackberry (*n* = 106, 15%), pumpkin/cloves/cinnamon (n = 158, 15%), blueberry (n = 144, 13.7%), bitter (n = 118, 11.2%), and chocolate (n = 92, 8.8%).

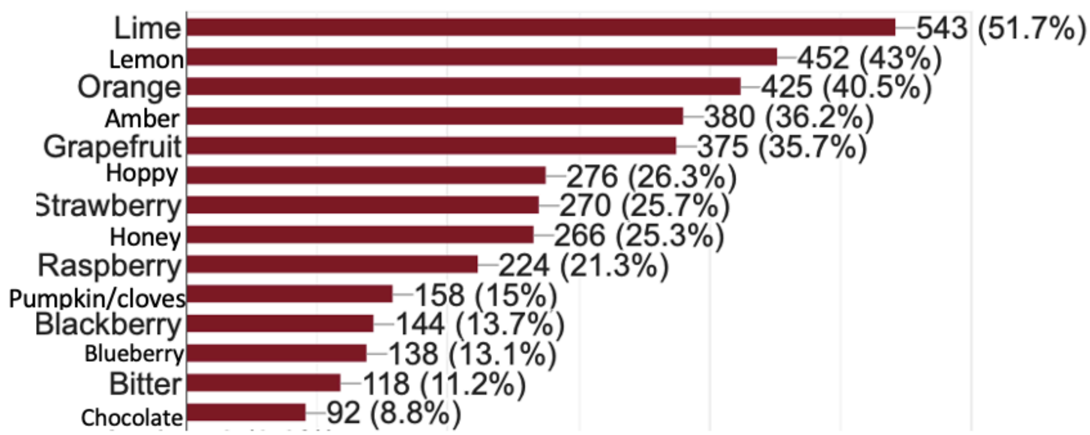


Figure 7. Refreshing Beer Flavors.

Consumer opinions on refreshing beer flavor profile were analyzed in Figure 8. When asked what flavor profile was the most refreshing, 917 participants (87.3%) chose a crisp/clean flavor profile. The next commonly chosen flavor profile was fruity (n = 555, 52.9%), sour/tart (n = 271, 25.8%), hoppy/bitter (n = 244, 23.2%), malty/sweet (n = 213, 20.3%), deep/chocolate/coffee (n = 115, 11%), and spice (n = 101, 9.6%). Twenty-seven participants said they "are not sure" what beer flavor profile they find refreshing.

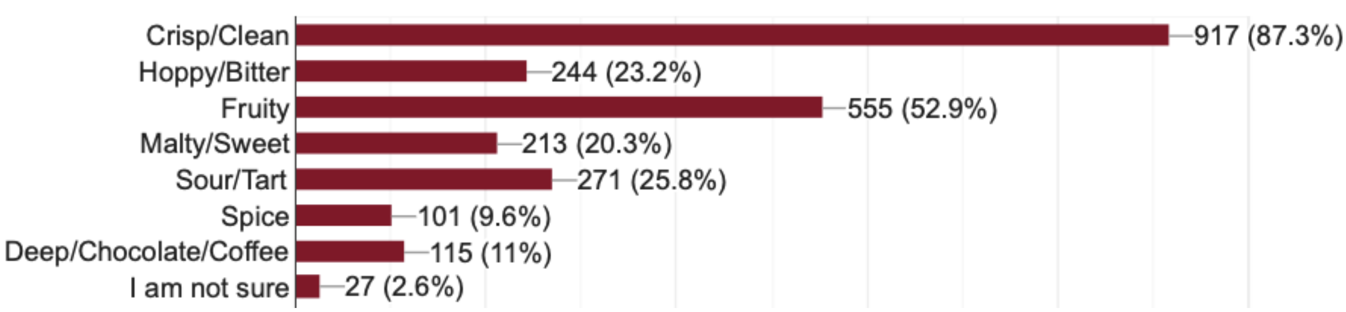


Figure 8. Refreshing Beer Flavor Profiles.

To not overwhelm participants with the number of beer varieties, the vast amount of varieties was broken down into two questions (see Figures 9 and 10). Both questions had answer choices that included light, medium, and dark beer varieties to avoid bias. The most common type of refreshing beer type was the Hefeweizen variety (*n* = 532, 50.7%). Followed by Blonde Ale (*n* = 496, 47%), American Lager (*n* = 461, 43.9%), American Amber Lager (*n* = 402, 38.3), American Pale Wheat (*n* = 386, 36.8%), and the Vienna (amber) Lager (*n* = 365, 34.8%). Less commonly chosen beer varieties include the Belgian Witbier (*n* = 288, 27.4%), American Pale Ale (*n* = 202, 19.2%), American IPA (*n* = 191, 18.2%) and the German Pilsner (*n* = 180, 17.1%).

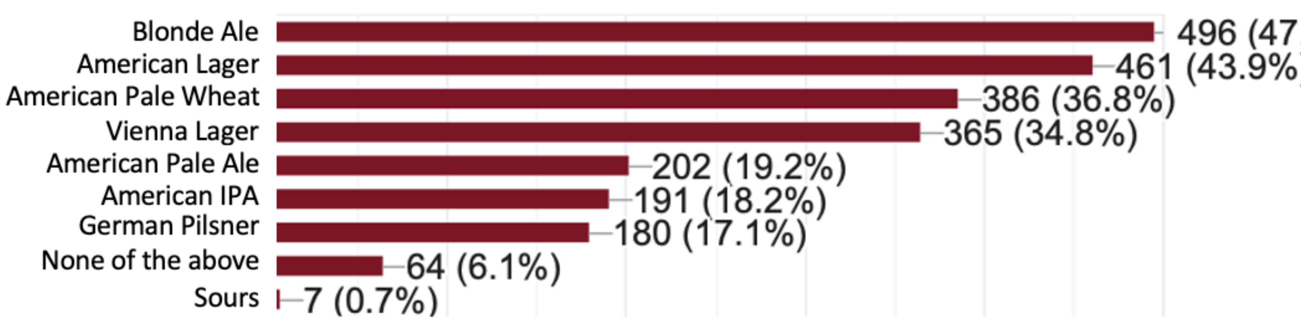


Figure 9. Refreshing Beer Varieties.

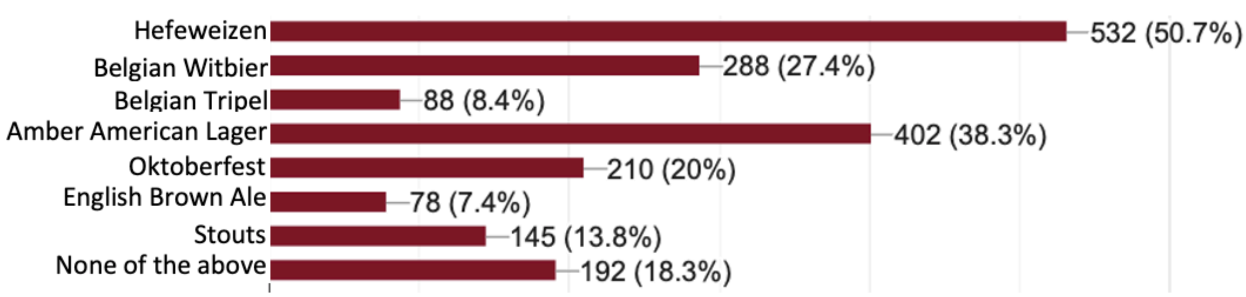


Figure 10. Refreshing Beer Varieties (continued).

External Factors Related to Perceived Refreshment Derived from Beer Consumption

External factors related to perceived beer refreshment including seasons and beer-food interactions were analyzed in Figure 11. According to the survey participants, 42.3% find summer beers (citrus-forward and fruit-forward beers) the most refreshing (444 participants). More than a third of participants do not think a beer variety is more or less refreshing depending on the present season (*n* = 356, 33.9%). A smaller portion of participants chose the spring variety of beers (pilsners, sours, and wheat) to be the most refreshing (*n* = 117, 11.1%). Followed by the fall variety of beers (IPA, pale ales, ambers, spice-forward beers) with 100 participants (9.5%) and winter beers (bocks, stouts, and porters) with 32 participants (3%).

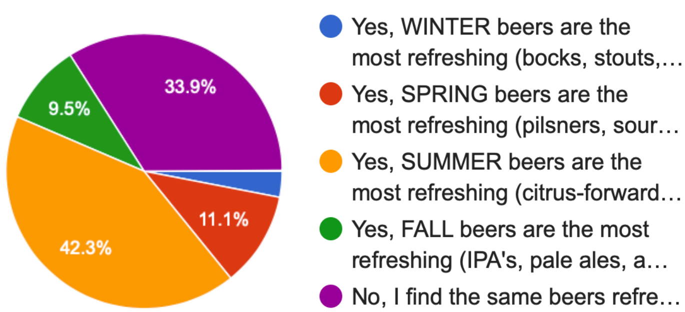


Figure 11. Do Seasons Influence Beer Refreshment?

Does pairing food with beer impact the perception of refreshment? As seen in Figure 12, out of 1,050 survey participants, 69.5% believe pairing food with beer impacts the perception of refreshment (*n* = 730).

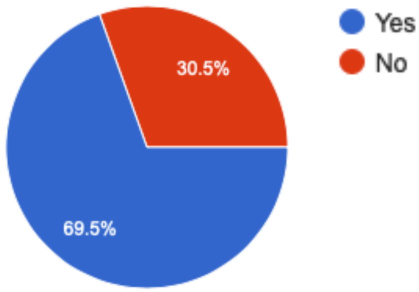


Figure 12. Does Food Influence on Refreshment?

Demographics

To better understand the population that was taking the beer refreshment survey, demographic questions were asked. As shown in Figure 13, 782 out of 1,050 participants were female (74.5%), 262 were male (25%). Participants could also state “gender fluid” or “prefer not to say.” Participant age ranges were examined in Figure 14. The most common age range for the survey participants was 21-25 years old (n = 437, 41.6%) followed by 26-35 years old (n = 342, 32.6%). Beer consumption frequency was an important factor for the survey (see Figure 15). The majority of the survey participants said they consume beer 1-3 times per week (n = 594, 56.6%). Less than a quarter of participants (18.2%) only consume beer once every 2 weeks (n = 191), 9.7% of participants consume beer a few times a year (n = 102), 7.5% consume beer 5 or more times per week (n = 79), and 7.5% consume once a month (n = 79).

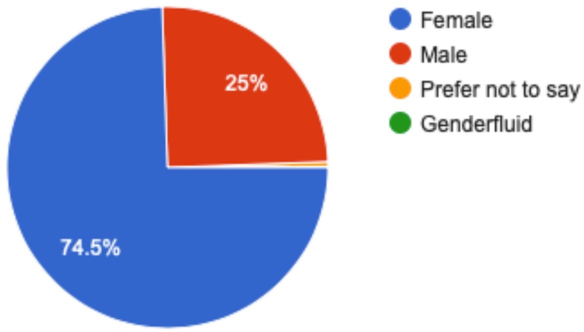


Figure 13. Survey Demographics- Gender.

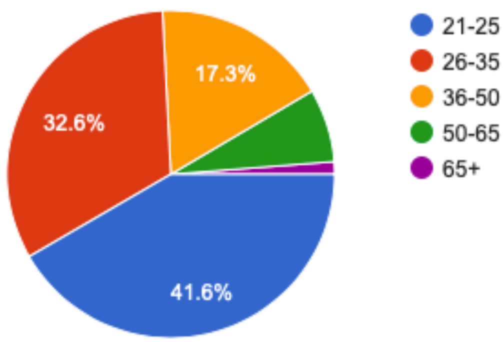


Figure 14. Survey Demographics- Age.

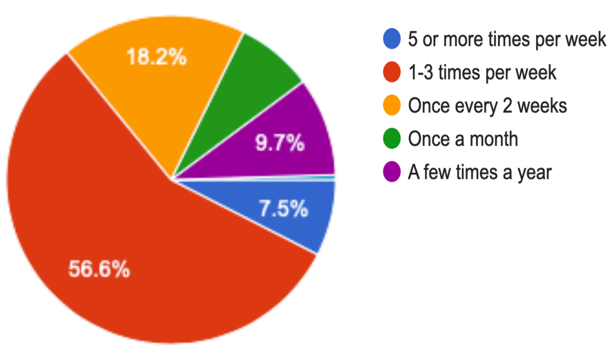


Figure 15. Beer Consumption Frequency of Participants.

Survey participant education levels indicated that over 42% of the survey's participants have completed and obtained a bachelor's degree (*n* = 449; see Figure 16). A lesser portion of participants have a master's degree (*n* = 184, 17.5%,), completed some college (*n* = 179, 17%), an associate's degree (*n* = 141, 13.4%), doctoral degree (*n* = 73, 7%), or a high school diploma (*n* = 14, 1.3%). For participant employment status almost half (49%) of survey participants were employed full-time (*n* = 515), 24.4% (*n* = 256) were students, 19% were employed part-time (*n* = 199), 2.8% are self-employed (*n* = 29), 1.8% were unemployed (*n* = 19), 0.9% were retired (*n* = 9), and 2.1% were a miscellaneous combination of part/time employee and student (see Figure 17).

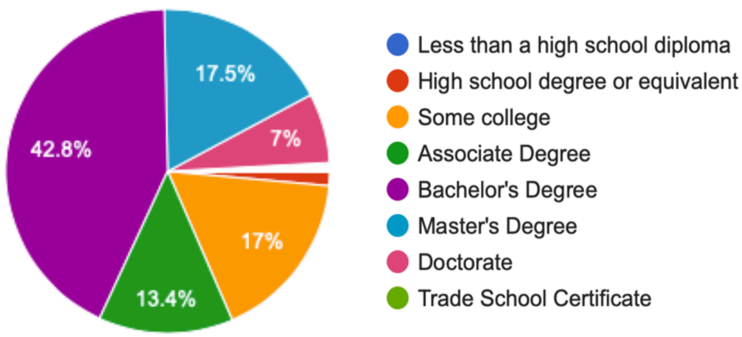


Figure 16. Highest Level of Education of Survey Participants.

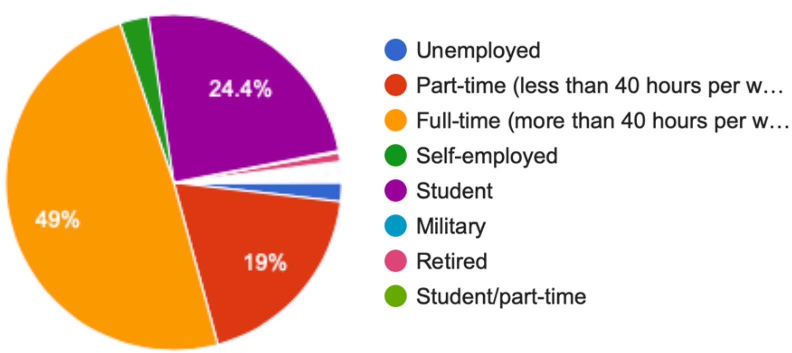


Figure 17. Employment Status of Survey Participants.

Consumer Study

Consumer Attitudes toward Formulated Beers and Impact of Flavor and Alcohol

To investigate consumer attitudes on the attributes of 12 formulated beer samples, a one-way ANOVA and MANOVA were performed (see Table 1). Nine beers (0%-5.0% abv) (excluding controls) received hedonic scores above 5 (neither *like* nor *dislike*) for all attributes. The 2.5% abv. samples possessed the most preferred alcohol level amongst consumers and the citrus was the most preferred overall and tested flavor. For the favored sample, the citrus 2.5% abv. sample was rated highest for the liking of overall beer flavor, tested flavor, alcohol, carbonation, and acidity. Lime 0% abv. was rated highest for the bitterness liking by 0.02 over the citrus 2.5% abv. sample. Three beers with 7.5% abv. had attributes scored below 5. The lowest- rated sample was the citrus 7.5% abv. This sample had the lowest hedonic scores for overall beer flavor and alcohol liking and was significantly different (p < 0 .05) than the other four samples included in its respective flavor group according to ANOVA. These results implied participants strongly disliked the higher alcohol level samples compared to the favored lower alcohol samples.

Table 1.

Mean Scores of Seven Liking Attributes of Formulated Beers.

Sample Name		Beer Flavor (L)	Tested Flavor (L)	Alcohol (L)	Carbonation (L)	Acidity (L)	Bitterness (L)	Refreshing (L)	Refreshing (I)
Bbase(Citrus)		6.33^{b}	5.14^{a}	5.93^{b}	5.89^{b}	5.46^{b}	5.36^{b}	6.54^{bc}	6.24^{bc}
BCitrus0abv		6.83^{bc}	6.96^{bc}	6.55^{c}	5.81^{b}	6.07^{c}	5.49^{bc}	6.72^{c}	6.49^{cd}
BCitrus2.5abv		7.13^{c}	7.35^{c}	7.24^{d}	6.51^{c}	6.41^{c}	5.96^{c}	7.11^{c}	6.93^{d}
BCitrus5.0abv		6.32^{b}	6.75^{b}	6.11^{bc}	5.68^{b}	5.94^{bc}	5.40^{b}	6.14^{b}	5.82^{b}
BCitrus7.5abv		3.54^{a}	5.13^{a}	2.99^{a}	4.31^{a}	4.61^{a}	4.22^{a}	3.04^{a}	3.24^{a}
Bbase(Cucumber)		5.73^{b}	5.02^{a}	5.41^{b}	5.21^{b}	5.46^{b}	5.39^{b}	5.76^{b}	5.69^{b}
BCucumber0abv		6.29^{bc}	6.24^{b}	6.21^{cd}	5.95^{c}	6.12^{c}	5.87^{b}	6.32^{b}	6.20^{bc}
BCucumber2.5abv		6.49^{c}	6.50^{b}	6.56^{d}	6.10^{c}	6.29^{c}	5.90^{b}	6.47^{b}	6.48^{c}
BCucumber5.0abv		5.76^{bc}	5.87^{b}	5.65^{bc}	5.25^{b}	5.39^{b}	5.30^{b}	5.75^{b}	5.67^{b}
BCucumber7.5abv		3.65^{a}	4.38^{a}	3.56^{a}	3.81^{a}	4.24^{a}	4.10^{a}	3.61^{a}	3.51^{a}
Bbase(Lime)		5.40^{b}	4.01^{a}	4.95^{b}	5.24^{b}	5.19^{a}	5.28^{b}	5.43^{b}	5.49^{b}
BLime0abv		6.22^{c}	6.47^{c}	6.40^{d}	6.06^{c}	6.31^{bc}	5.98^{c}	6.24^{c}	6.30^{c}
BLime2.5abv		6.50^{c}	6.62^{c}	6.61^{d}	6.24^{c}	6.40^{c}	5.96^{c}	6.43^{c}	6.43^{c}
BLime5.0abv		5.89^{bc}	6.15^{c}	5.76^{c}	5.80^{c}	5.82^{b}	5.46^{bc}	5.91^{bc}	5.89^{bc}
BLime7.5abv		4.10^{a}	5.08^{b}	4.06^{a}	4.56^{a}	4.90^{a}	4.52^{a}	4.02^{a}	3.91^{a}
Flavor type	No added flavor	5.84^{ab}	4.74^{a}	5.45^{a}	5.46^{ab}	5.38^{a}	5.93^{b}	5.34^{a}	5.82^{b}
	Citrus	5.96^{b}	6.55^{d}	5.72^{a}	5.57^{b}	5.76^{bc}	5.75^{ab}	5.27^{a}	5.62^{ab}
	Cucumber	5.55^{a}	5.75^{b}	5.5^{a}	5.28^{a}	5.51^{ab}	5.54^{a}	5.29^{a}	5.46^{a}
	Lime	5.68^{ab}	6.08^{c}	5.71^{a}	5.67^{b}	5.86^{c}	5.65^{ab}	5.48^{a}	5.63^{ab}
Alcohol level	0 abv w/h flavor	5.84^{b}	4.74^{a}	5.45^{b}	5.46^{b}	5.38^{b}	5.93^{b}	5.34^{b}	5.82^{b}
	0 abv	6.46^{c}	6.57^{bc}	6.39^{d}	5.93^{c}	6.16^{d}	6.44^{c}	5.77^{c}	6.34^{c}
	2.5 abv	6.72^{c}	6.84^{c}	6.82^{e}	6.29^{d}	6.37^{d}	6.68^{c}	5.94^{c}	6.62^{c}
	5.0 abv	6.00^{b}	6.27^{b}	5.85^{c}	5.57^{b}	5.72^{c}	5.94^{b}	5.39^{b}	5.80^{b}
	7.5 abv	3.75^{a}	4.88^{a}	3.52^{a}	4.23^{a}	4.58^{a}	3.54^{a}	4.28^{a}	3.54^{a}
Flavor type (F)		11.62***	28.65***	4.75**	8.83***	3.81*	1.61	6.18**	2.66
Alcohol level (A)		148.47***	117.45***	201.42***	80.36***	76.45***	59.89***	167.58***	161.80***
(F) x (A)		3.09**	4.31***	6.87***	2.56**	2.04*	1.08	6.06***	2.91**

Note. F-values and sources of variation with their interactions from tested flavor and alcohol levels are analyzed by MANOVA and ANOVA analysis of each attribute across formulated beers with same type of flavor. Different letters within a column indicates significant differences according to Tukey 's HSD test *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.(L): liking; (I): intensity.

A MANOVA was performed to investigate the impact factors: flavor types, alcohol level, and flavor types x alcohol level on the acceptance scores of attributes (see Table 1). When these factors were compared independently, alcohol level played a larger factor by significantly ($p < 0.001$) impacting all sensory attributes while flavor type significantly ($p < 0.05$) impacted all attributes with the exception of bitterness.

To evaluate the impact of alcohol levels on hedonic scores, the controls were compared against the tested flavor's abv. samples: 0% abv., 2.5% abv., 5% abv., and 7.5% abv (see Table 1). The highest-rated alcohol level was 2.5% abv. for overall beer flavor, tested flavor, alcohol, carbonation, acidity, and bitterness, while the 7.5% abv. samples were rated the lowest for each attribute and were significantly different ($p < 0.05$) than all other samples. The 2.5% abv. samples were not significantly different ($p < 0.05$) than the 0% abv. samples for the hedonic scores of beer flavor, tested flavor, acidity, and bitterness showing that low-alcohol samples shared common sensory characteristics.

To evaluate the impact of flavor type on beer sensory attributes, the control was compared against the attribute hedonic scores for citrus, cucumber, and lime-flavored beers (see Table 1). The citrus-flavored beer was rated the highest acceptability for overall beer flavor, tested flavor, and alcohol, while the lime-flavored beer received the highest hedonic scores for carbonation and acidity. Cucumber-flavored samples received the lowest hedonic scores for beer flavor, carbonation, and bitterness, whereas the control was the favorite for bitterness and the least favorite for tested flavor, alcohol, and acidity hedonic scores.

How flavor types and alcohol levels impacted attribute hedonic scores was further investigated, as shown in Table 1. The two factors impacted sensory attribute ratings independently as well as conjointly. All attributes (overall beer flavor, tested flavor, alcohol, carbonation, acidity, and bitterness) were impacted significantly ($p < 0.05$) by alcohol level alone. For the factor of flavor type alone, all attributes were also impacted significantly ($p < 0.05$) with the exception of bitterness. Flavor types and alcohol levels, collaboratively, also impacted the hedonic scores significantly. Similar to flavor type alone, alcohol levels and flavor types were co-dependent on each other and impacted all sensory attributes significantly ($p < 0.05$) with the exception of bitterness. As a result, bitterness may be affected more by alcohol level rather than flavor type. High alcohol levels presented in a beer may evoke a higher intensity of bitterness and consequently, influence the overall flavor.

Impact of Attribute Intensities on Hedonic Scores

All 15 samples were analyzed together for seven JAR attribute intensities: overall beer flavor, tested flavor, alcohol, carbonation, acidity, and bitterness (see Figure 18). The majority of consumers (>50%) rated the intensities for beer flavor, tested flavor, alcohol, acidity, and bitterness JAR, implying that the intensities of these sensory attributes for the formulated beers were acceptable. However, overall carbonation had the least amount of JAR intensities (42%) and was primarily of too little in intensity (54%).

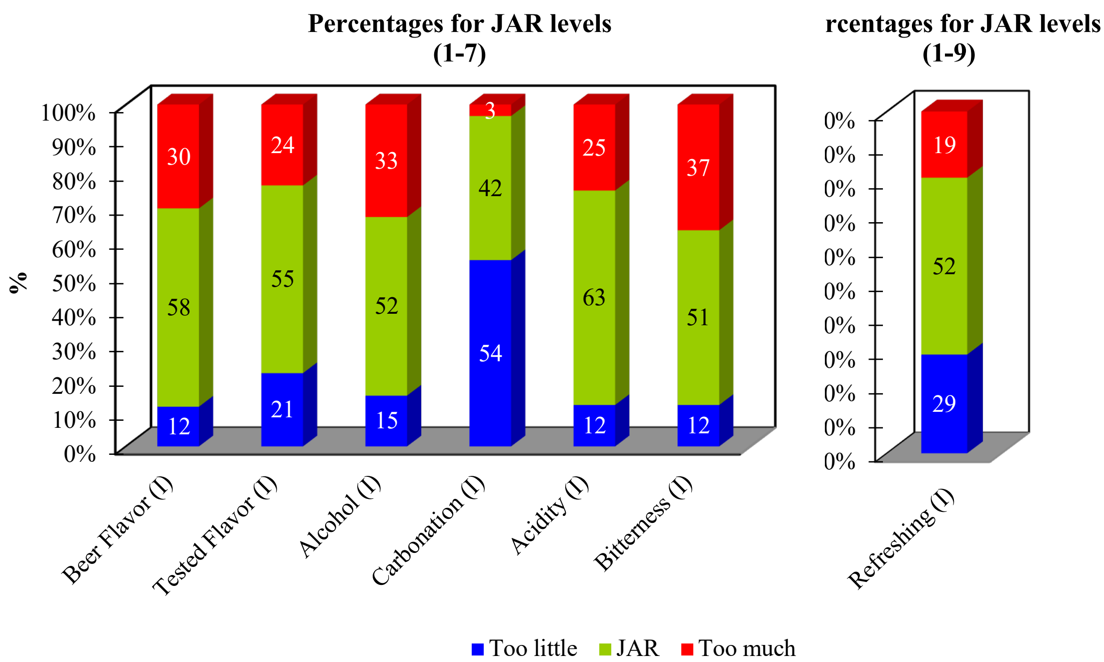


Figure 18. Percentages for JAR Levels for Sensory Attributes and Refreshment. Too little of the attribute (I) intensity is shown as a blue percentage, too much attribute intensity is shown as a red percentage, and a just-about-right intensity is shown as the green percentage. All samples (n = 15) were tested simultaneously.

The JAR mean drops depicted in Figure 19 investigated if an attribute with not enough or too much intensity impacted the hedonic score of the respective attribute significantly. According to the mean drops, the beer samples in which the overall beer flavor, alcohol level, acidity, and bitterness attributes were too intense, the hedonic score was significantly impacted ($p < 0.05$) and highly penalized (meaning the hedonic score was lowered by consumers). For the tested flavor, having either too little or too much flavor was highly penalized by consumers. Lastly, for carbonation, when a sample did not contain enough carbonation, it was high penalized.

JAR Mean Drops

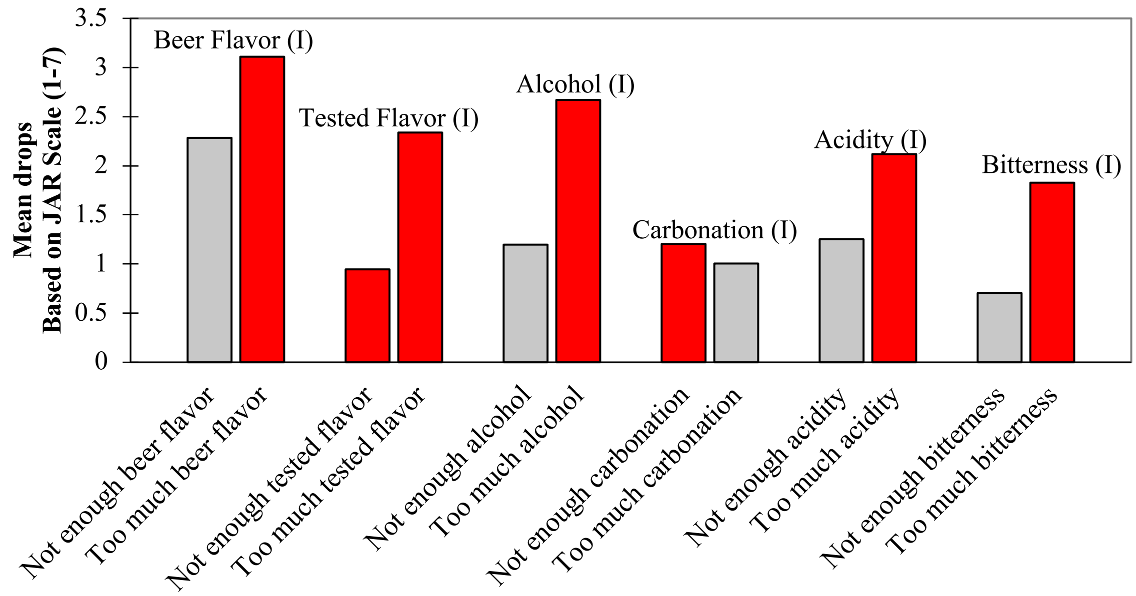


Figure 19. JAR Mean Drops for Sensory Attributes. The JAR mean drops are derived from the difference from the respective attribute liking score minus the "too much" or "too little" levels for (I) intensity. Significance is depicted by a red bar ($p < 0.05$). A red bar means the beer sample score was significantly impacted by the attribute having too much or too little intensity. A gray bar depicts that there were not enough cases of the respective intensity being chosen for a significant test to be computed.

PCA of Formulated beers

To explain the variances of attributes among all 15 samples, a PCA biplot is illustrated in Figure 20. The first principal component (PC1) accounted for 75.52% of the variance, while the second principal component (PC2) accounted for 18.81%. PC1 and PC2 together explained 94.33% of the total variance. PC1 was the major PC to separate samples according to their sensory attributes. Six formulated beers with alcohol volume either at 0% or 2.5% and three different types of flavors (citrus, lime, and cucumber) were separated at the positive side of PC1 along with high intensities in refreshing and carbonation as well as received high hedonic scores for refreshing, overall beer flavor, tested flavor, alcohol, carbonation, acidity, and bitterness. Oppositely, three formulated beers with alcohol levels at 7.5% with three flavors (citrus, lime, or cucumber) were separated at the negative side of PC1. Those three beers had high intensities in alcohol, beer flavor, bitterness, and acidity.

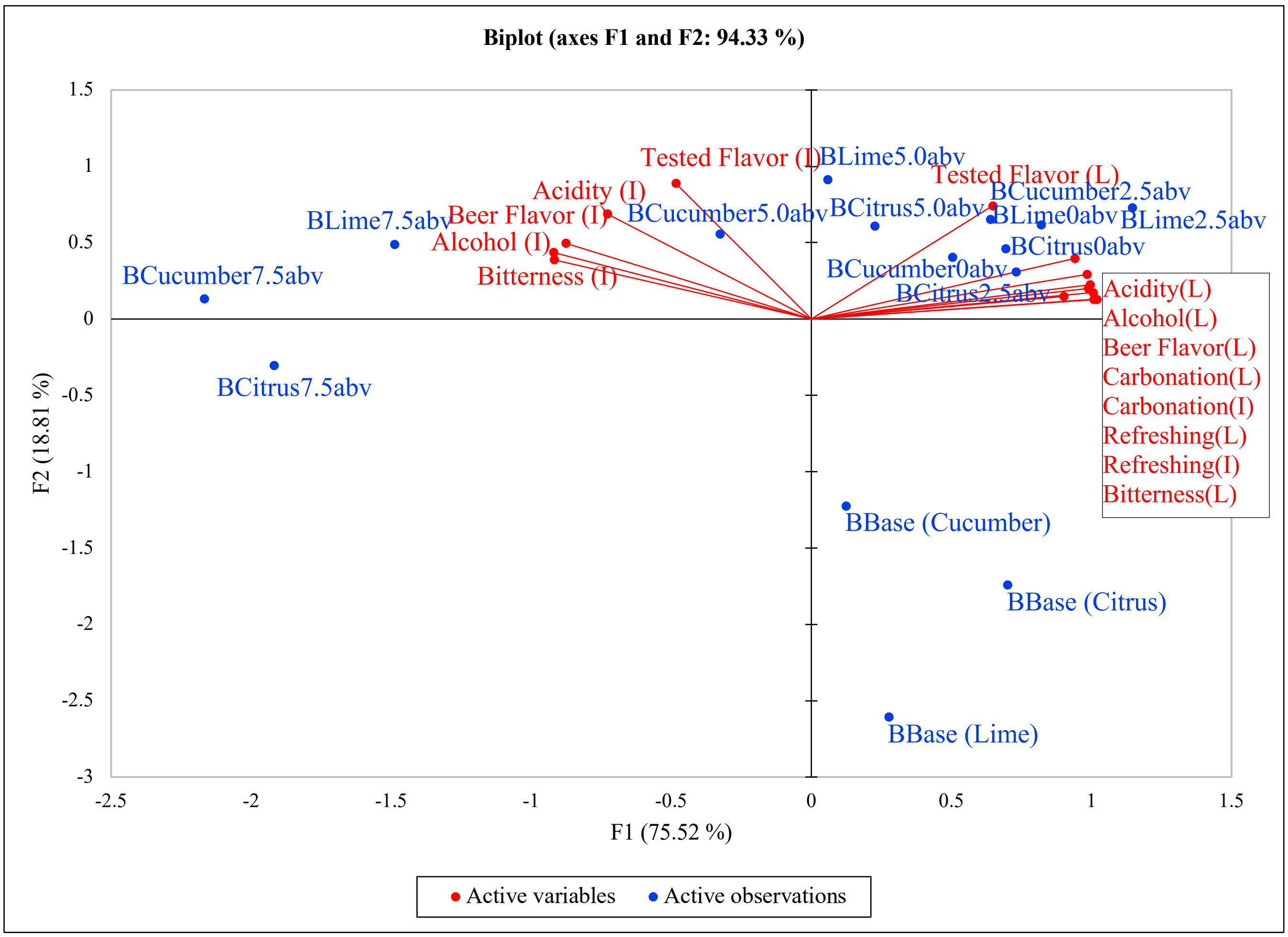
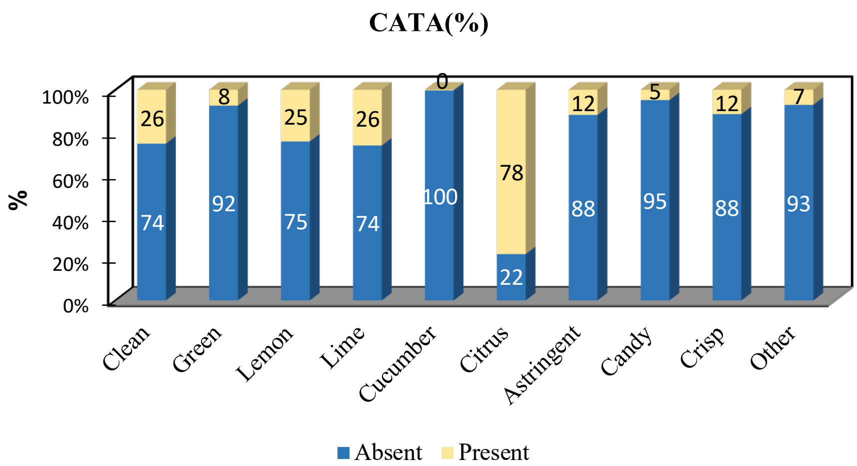


Figure 20. PCA Biplot for Formulated Beers and Sensory Attributes. PCA biplot (F1 versus F2) showing the sensory attributes as active variables (red) and the 15 beer samples as active observations (blue). PC1 (F1) contains 75.52% of variability on the x-axis and PC2 (F2) contains 18.81% of variance on the y-axis. PC1 and PC2 collaboratively make up 94.33% of total variance in the consumer study. The main sources of variation are composed of the flavor controls (Bbase) and 7.5% abv samples. (L): liking, (I): intensity

Six beer samples were separated in PC2 (see Figure 20). Three 5.0% abv. samples with three different flavors (citrus, lime, and cucumber) were separated on the positive side of PC2 and possessed high intensities in tested flavor. On the contrast, three beer base controls (same sample) were separated on the negative side of PC2. The control and 5.0% abv samples, along with the tested flavor variable provided variance amongst the consumers while the majority of consumers rated the other attributes and samples more in sync.

CATA and PCA Biplot

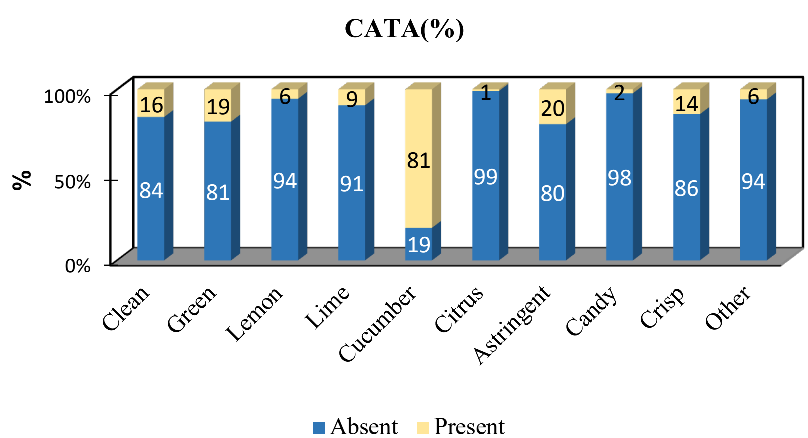
A CATA question with 9 descriptors and an "other" option was asked to participants for each beer sample: clean, green, lemon, lime, cucumber, citrus, astringent, candy, and crisp (see Figure 21). The most frequented checked descriptors for the citrus beer samples were citrus (78%), clean (26%), lime (26%), and lemon (25%). A chi-square test for independence for the citrus beer samples showed significance ($p < 0.001$). The Cochran's Q test compared products independently for each attribute and described seven out of 10 descriptors as significant ($p < 0.05$): clean, green, citrus, astringent, crisp, lime, and candy. These descriptors depicted the flavor profile of the citrus-flavored samples according to participants



Attributes	p-values
Clean	**< 0.0001**
Green	**< 0.0001**
Lemon	0.124
Lime	**0.014**
Cucumber	0.092
Citrus	**< 0.0001**
Astringent	**< 0.0001**
Candy	**0.003**
Crisp	**< 0.0001**
Other	0.291

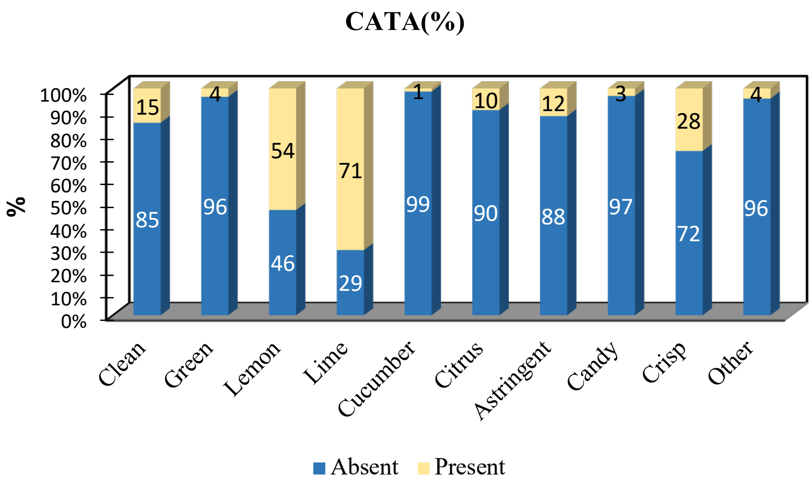
Figure 21. CATA Bar Chart for Tested Flavors.

A. *Citrus CATA% Bar Chart*. According to Cochran's Q test for significance, 7/10 attributes were rated significantly different ($p < 0.01$) amongst consumers: clean, green, lime, citrus, astringent, candy, and crisp.



Attributes	p-values
Clean	**< 0.0001**
Green	0.690
Lemon	**0.041**
Lime	**0.000**
Cucumber	**< 0.0001**
Citrus	0.736
Astringent	**< 0.0001**
Candy	0.382
Crisp	**< 0.0001**
Other	**< 0.0001**

B. *Cucumber CATA% Bar Chart*. According to Cochran's Q test for significance, 7/10 attributes were rated significantly different ($p < 0.05$) amongst consumers: clean, lemon, lime, cucumber, astringent, crisp, and other.



Attributes	p-values
Clean	**< 0.0001**
Green	**0.008**
Lemon	**< 0.0001**
Lime	**< 0.0001**
Cucumber	0.446
Citrus	**0.011**
Astringent	**< 0.0001**
Candy	0.429
Crisp	**< 0.0001**
Other	**< 0.0001**

C. *Lime CATA% Bar Chart*. According to Cochran's Q test for significance, 8/10 attributes were rated significantly different ($p < 0.05$) amongst consumers: clean, green, lemon, lime, citrus, astringent, crisp, and other

The most frequently checked descriptors for the cucumber samples were cucumber (81%), astringent (20%), green (19%), and clean (16%). According to Cochran's Q test for significance, seven descriptors were deemed significant and described the flavor profile of the cucumber samples: clean, lemon, lime, cucumber, astringent, crisp, and other. Although green was a commonly chosen descriptor, its *p*-value determined by Cochran's Q test value was 0.690 and not deemed significantly different between the cucumber samples.

The most frequently checked descriptor for the test lime-flavored beer were lime (71%), lemon (54%), and crisp (28%). Eight descriptors were deemed significant ($p < 0.05$) according to Cochran's Q test: clean, green, lemon, lime, citrus, astringent, crisp, and other were what a majority of the consumers tasted when describing the lime-flavor beer.

All 15 samples were analyzed using a CATA PCA biplot in Figure 22. Two major principal components accounted for 70.5% of variance- PC1 (41.21%) and PC2 (29.29%). Four formulated lime beer samples (0%, 2.5%, 5.0%, and 7.5% abv.) with descriptors including clean, crisp, lime, lemon, and candy were separated on the positive side of PC1. On the negative side of PC1 was the sensory descriptor of cucumber as well as the tested cucumber-flavored beer samples (0%, 2.5%, 5.0%, and 7.5% abv.). PC2 was composed of green, astringent, and other descriptors on the positive side along with the controls of each flavor test. The citrus sensory descriptor was on the negative side of PC2 along with the 0%, 2.5%, 5%. and 7.5% abv. citrus-flavored beer samples.

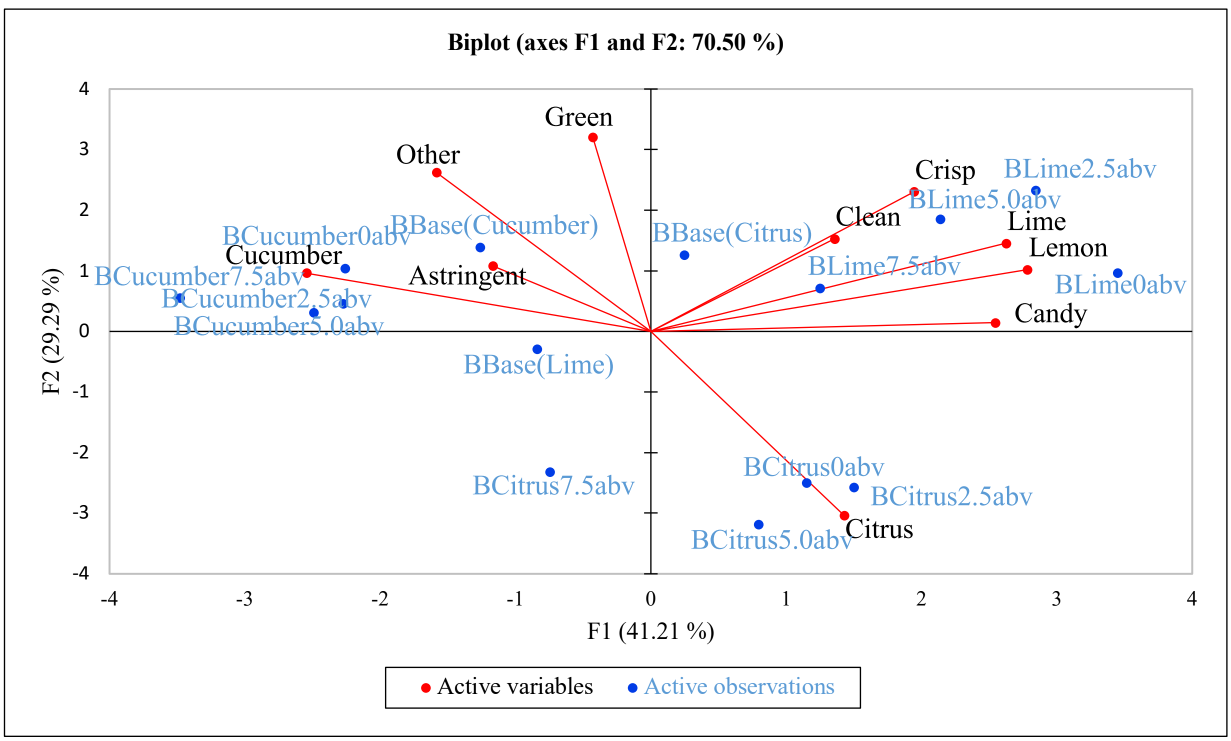


Figure 22. PCA Biplot for CATA Questions for Formulated Beer Samples. PCA biplot (F1 versus F2) showing the 15 beer samples as active observations in blue and sample descriptor in black. PC1 (F1) contains 41.21% of variability on the x-axis and PC2 (F2) contains 29.29% of variance on the y-axis. PC1 and PC2 collaboratively make up 70.50% of total variance in the consumer study.

Beer Refreshing and Impact of Flavor Types and Alcohol Levels

In this study, both refreshing liking and intensity of the formulated beers were rated on a 1-9 hedonic scale (see Table 1). The lowest score for refreshing liking (3.04) and intensity (3.24) both derived from the same sample, citrus 7.5% abv., while the highest refreshing liking (7.11) and intensity scores (6.93) belonged to the citrus 2.5% abv. sample. There were significant differences in refreshing scores between the 2.5% abv. sample with the 5.0% and 7.5% abv. samples. The latter two had substantially lower ratings on refreshing liking, 6.14 and 3.04 respectively. For refreshing intensity, there were significant differences between the citrus 2.5% abv. sample with other samples-control, 5.0%, and 7.5% abv. sample. Overall, the lower abv. samples (0%, and 2.5% abv.) were rated the highest for both refreshing liking and intensity while the higher abv. samples (5.0% and 7.5% abv.) were rated the lowest.

The beer base (control) was compared against citrus, cucumber, and lime-flavored samples to understand the impact of flavor on refreshment derived from beer consumption (see Table 1). The control had the highest scores for both refreshing liking and intensity although not significantly different ($p < 0.05$) compared to the citrus and cucumber-flavored beers. The citrus-flavored samples were rated with the highest refreshing liking and intensity scores, while the cucumber-flavored samples were rated the lowest. The cucumber-flavored samples were significantly different ($p < 0.05$) than the control for tested flavor and bitterness liking as well as refreshing intensity. The MANOVA results showed no significance in refreshing liking according to flavor type. However, there was for refreshing intensity; the cucumber-flavored beers were rated lower than all other flavors and significantly different ($p < 0.05$) from the control according to Tukey's post hoc test.

To evaluate the impact of alcohol levels on refreshment, the controls were compared against the tested flavor abv. samples: 0% abv., 2.5% abv., 5% abv., and 7.5% abv. The highest-rated samples in each respective flavor were the 2.5% abv. samples for refreshing (hedonic and intensity) while the 7.5% abv. samples were rated the lowest according to ANOVA (see Table 1). The 2.5% abv. samples were not significantly different ($p < 0.05$) from the 0% abv. and control samples for both citrus and cucumber-flavored beers but were for the lime-flavored beers in regard to refreshing hedonic scores. When all flavor's abv. levels were averaged, the highest hedonic and intensity for refreshment belonged to the 2.5% abv. level although not significantly different ($p < 0.05$) from the 0% abv. level. However, the 2.5% level was significantly different ($p < 0.05$) from the control, 5.0%, and 7.5% alcohol levels. According to MANOVA, alcohol level showed extreme significance ($p < 0.001$) for both hedonic and intensity score for refreshment. This demonstrated that the alcohol level was very influential in the overall refreshing perception and that a low intensity of alcohol was preferred amongst consumers.

The two separate factors of flavor type and alcohol level collectively impacted perceived refreshment as well (see Table 1). The factors of flavor and alcohol influenced both refreshing hedonic and intensity scores significantly ($p < 0.01$). Flavor and alcohol, both conjointly and independently, considerably impact perceived refreshment post beer consumption.

Relationships Between Sensory Attributes and Refreshment

A Pearson correlation association test was conducted to understand the relationships between perceived refreshment (liking and intensity) and all attributes (liking and intensity), as shown in Table 2. For refreshment (liking and intensity), the attributes with the highest correlation were beer flavor ($r = 0.947$) and alcohol acceptance ($r = 0.765$). Beer flavor and alcohol liking were highly correlated with each other as well ($r = 0.778$). The tested flavor acceptability was more correlated with alcohol ($r = 0.721$) than overall beer flavor ($r = 0.698$) and refreshing ($r = 0.666$) hedonic scores. The four attributes: refreshing, overall beer flavor, tested flavor, and alcohol were all highly correlated with each other more than the rest of the attributes (carbonation, acidity, and bitterness).

Table 2.

Pearson Correlation Between Refreshment with Sensory Attribute Likings and Intensities.

		Refreshing (L)	Beer Flavor (L)	Tested Flavor (L)	Alcohol (L)	Carbonation (L)	Acidity (L)	Bitterness (L)	Refreshing (I)	Beer Flavor (I)	Tested Flavor (I)	Alcohol (I)	Carbonation (I)	Acidity (I)	Bitterness (I)
Refreshing (L)	Pearson Correlation	1.000	0.947	0.666	0.765	0.611	0.641	0.579	0.955	-0.447	-0.245	-0.431	0.329	-0.238	-0.321
	Sig. (2-tailed)		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0
Beer Flavor (L)	Pearson Correlation	0.947	1.000	0.698	0.778	0.625	0.663	0.591	0.924	-0.435	-0.223	-0.407	0.324	-0.226	-0.305
	Sig. (2-tailed)	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Tested Flavor (L)	Pearson Correlation	0.666	0.698	1.000	0.721	0.584	0.666	0.546	0.652	-0.223	-0.050	-0.224	0.250	-0.121	-0.199
	Sig. (2-tailed)	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.047	0.000	0.000	0.000	0.000
Alcohol (L)	Pearson Correlation	0.765	0.778	0.721	1.000	0.712	0.716	0.660	0.759	-0.405	-0.173	-0.415	0.391	-0.225	-0.316
	Sig. (2-tailed)	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Carbonation (L)	Pearson Correlation	0.611	0.625	0.584	0.712	1.000	0.670	0.595	0.613	-0.284	-0.225	-0.336	0.587	-0.206	-0.331
	Sig. (2-tailed)	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Acidity (L)	Pearson Correlation	0.641	0.663	0.666	0.716	0.670	1.000	0.680	0.641	-0.291	-0.146	-0.287	0.374	-0.224	-0.276
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Bitterness (L)	Pearson Correlation	0.579	0.591	0.546	0.660	0.595	0.680	1.000	0.581	-0.315	-0.178	-0.336	0.359	-0.200	-0.356
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000
Refreshing (I)	Pearson Correlation	0.955	0.924	0.652	0.759	0.613	0.641	0.581	1.000	-0.446	-0.239	-0.433	0.337	-0.250	-0.340
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000
Beer Flavor (I)	Pearson Correlation	-0.447	-0.435	-0.223	-0.405	-0.284	-0.291	-0.315	-0.446	1.000	0.528	0.603	-0.168	0.447	0.439
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000

Tested Flavor (I)	Pearson Correlation	**-0.245**	**-0.223**	**-0.050**	**-0.173**	**-0.225**	**-0.146**	**-0.178**	**-0.239**	**0.528**	1.000	**0.573**	**-0.104**	**0.520**	**0.403**
	Sig. (2-tailed)	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000
Alcohol (I)	Pearson Correlation	**-0.431**	**-0.407**	**-0.224**	**-0.415**	**-0.336**	**-0.287**	**-0.336**	**-0.433**	**0.603**	**0.573**	1.000	**-0.180**	**0.540**	**0.540**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000
Carbonation (I)	Pearson Correlation	**0.329**	**0.324**	**0.250**	**0.391**	**0.587**	**0.374**	**0.359**	**0.337**	**-0.168**	**-0.104**	**-0.180**	1.000	**-0.065**	**-0.204**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.009	0.000
Acidity (I)	Pearson Correlation	**-0.238**	**-0.226**	**-0.121**	**-0.225**	**-0.206**	**-0.224**	**-0.200**	**-0.250**	**0.447**	**0.520**	**0.540**	**-0.065**	1.000	**0.519**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009		0.000
Bitterness (I)	Pearson Correlation	**-0.321**	**-0.305**	**-0.199**	**-0.316**	**-0.331**	**-0.276**	**-0.356**	**-0.340**	**0.439**	**0.403**	**0.540**	**-0.204**	**0.519**	1.000
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Note. Pearson product-moment correlation coefficient (r) and two-tailed significance (p) are shown for each attribute for both liking (L) and intensity (I). Values in bold depict significance ($p < 0.05$).

Two partial least square regression (PLS-R) analyses were performed. One investigated refreshing liking related with all other sensory attributes, and the other investigated the overall beer flavor acceptance related with the other sensory attributes. If an attribute's variable importance for the projection (VIP, term from XLSTAT software) was less than 0.8, it was disqualified from the analysis due to a small contribution to the regression. Refreshment liking were closely related (VIPs $\geq$ 0.8) with refreshment intensity, as well as overall beer flavor, alcohol, and carbonation (see Figure 23). Although tested flavor had a high VIP, the attribute was depicted separate and independent from other attributes including refreshment. To summarize, refreshment liking were the most related overall beer flavor, alcohol, and carbonation liking.

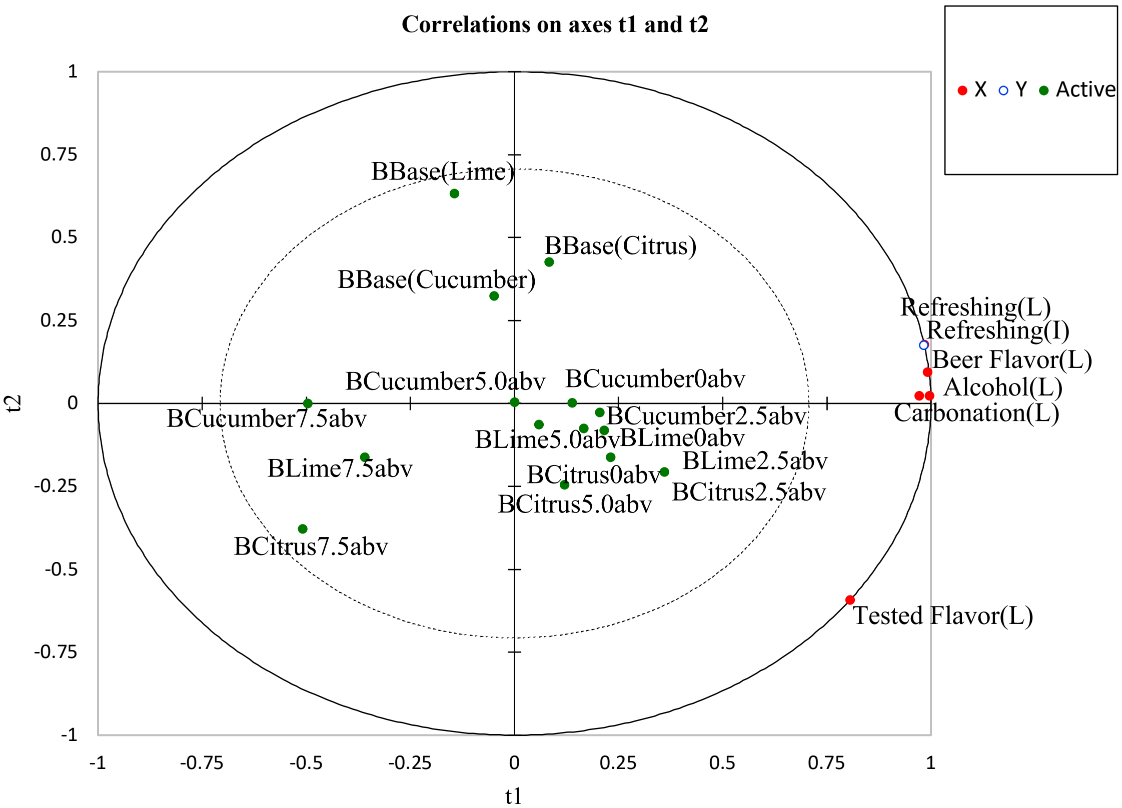


Figure 23. PLS-R of Refreshing(L) Projected with Formulated Beer Samples and Sensory Attributes. PLS-R plot depicting VIPs ≥ than 0.8 for refreshing liking (y-dependent variable) were (x-independent variables) refreshing intensity and the likings of alcohol, beer flavor, tested flavor and carbonation. (L): liking, (I): intensity

The overall beer flavor liking (y-dependent variable) was compared against the overall beer flavor intensities and 12 other attributes-(liking and intensity) for refreshing, tested flavor, alcohol, carbonation, acidity, and bitterness (x-explanatory variables) by PLS-R (see Figure 24). Refreshment (hedonic and intensity) as well as the liking of alcohol, tested flavor, and carbonation were the most related (VIPs ≥ 0.8) to overall beer flavor. Tested flavor liking was also depicted independent from the other attributes, similar to the PLS-R of refreshment.

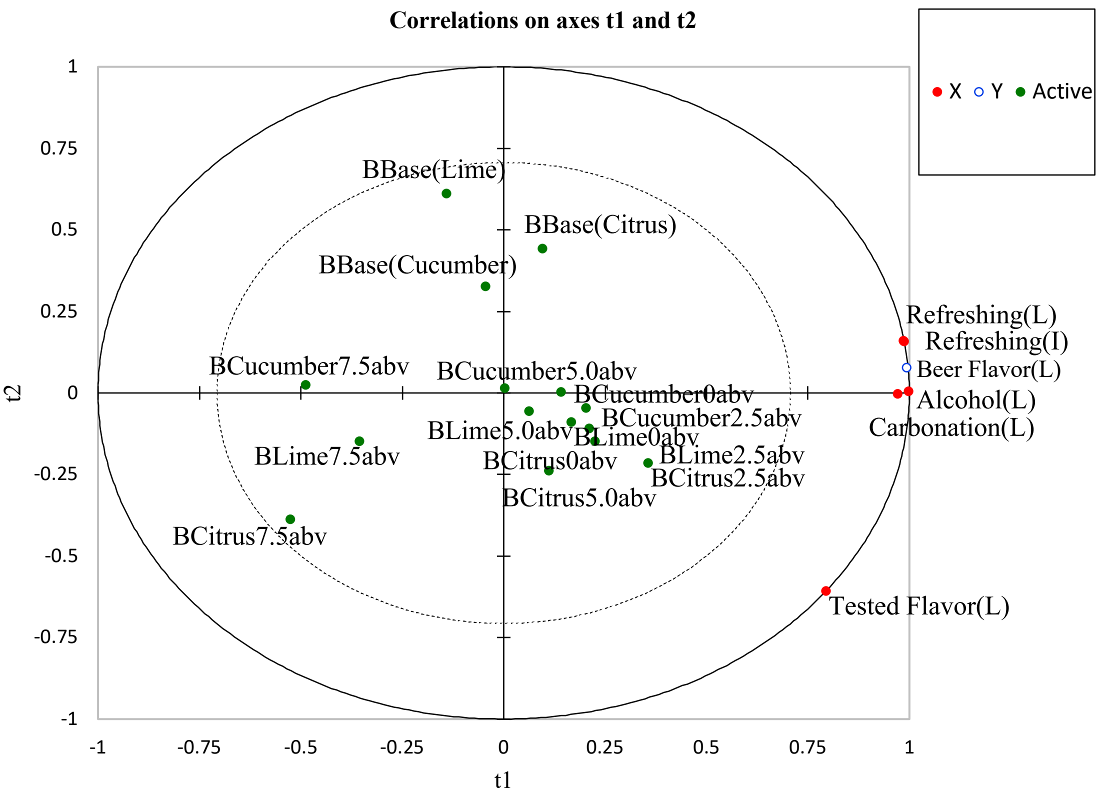


Figure 24. PLS-R of Beer Flavor(L) Projected with Formulated Beer Samples and Sensory Attributes. PLS-R plot depicting VIPs ≥ than 0.8 for beer flavor liking (y-dependent variable) were (x-independent variables) refreshing (liking and intensity) and the likings of alcohol, tested flavor, and carbonation. (L): liking, (I): intensity

In summary, both refreshment and overall beer flavor liking were closely related to the consumer liking of alcohol, carbonation, and tested flavor. Tested flavor established variance and represented the diversity amongst consumers in the beer market as to what flavors are considered refreshing.

Consumer Preference Segments

To relate consumer demographics to sensory attribute preferences in samples, an agglomerative hierarchical clustering (AHC) was conducted. Demographics for the unique beer flavor studies are depicted in Table 3. The average consumer for the citrus test was male, between 36-50 years old, consumed beer 5 or more times per week, obtained a bachelor's degree, and employed full-time. For the cucumber test, the average consumer was male, between 21-25 years old, consumed beer 1-3 times per week, obtained a bachelor's degree, and employed full-time. For the lime test, the average consumer was male, between 50-65 years old, consumed beer 1-3 times per week, obtained a bachelor's degree, and employed full-time. Based on how similarly the beer samples were rated, the consumers were separated into three unique clusters by AHC depicted in Figure 25.

Table 3.

Beer Consumer Study Demographics.

Demographic data	Citrus		Cucumber		Lime	
Age	*n*	%	*n*	%	*n*	%
21-25	32	28.1	31	29.5	22	21.4
26-35	26	22.8	25	23.8	35	34
36-50	41	36	20	19	16	15.5
50-65	12	10.5	18	17.1	27	26.2
65+	3	2.6	11	10.5	3	2.9
Gender						
Female	43	37.7	31	29.5	30	29.1
Male	70	61.4	70	66.7	71	68.9
Prefer not to say	9	0	3	2.9	2	1.9
Gender fluid	1	0.9	1	1	0	0
Beer Consumption						
5 or more times per week	51	44.7	40	38.1	28	27.2
1-3 times per week	28	24.6	45	42.9	63	61.2
Once every 2 weeks	29	25.4	13	12.4	10	9.7
A few times a year	5	4.4	4	3.8	1	1
Never	1	0.9	3	2.9	1	1
Highest Level of Education						
Less than high school	2	1.8	5	4.8	2	1.9
High School degree	18	15.8	30	28.6	31	30.1
Trade School degree	5	4.4	7	6.7	11	10.7
Associate Degree	19	16.7	19	18.1	12	11.7
Bachelor's Degree	57	50	36	34.3	39	37.9
Master's Degree	12	10.5	5	4.8	6	5.8
Doctorate Degree	1	0.9	3	2.9	2	1.9
Employment Status						
Unemployed	3	2.6	6	5.7	3	2.9
Part-time (less than 40 hours)	6	5.3	4	3.8	10	9.7

Full-time (40 or more hours per week)	72	63.2	59	56.2	56	54.4
Self-employed	4	3.5	8	7.6	7	6.8
Student	8	7	7	6.7	2	1.9
Student and employed	9	7.9	1	1	4	3.9
Military	4	3.5	2	1.9	4	3.9
Retired	8	7	18	17.1	17	16.5

Note. The beer consumer study table describes the combined demographics for citrus (n = 114), cucumber (n = 105), and lime (n = 103) consumer tests.

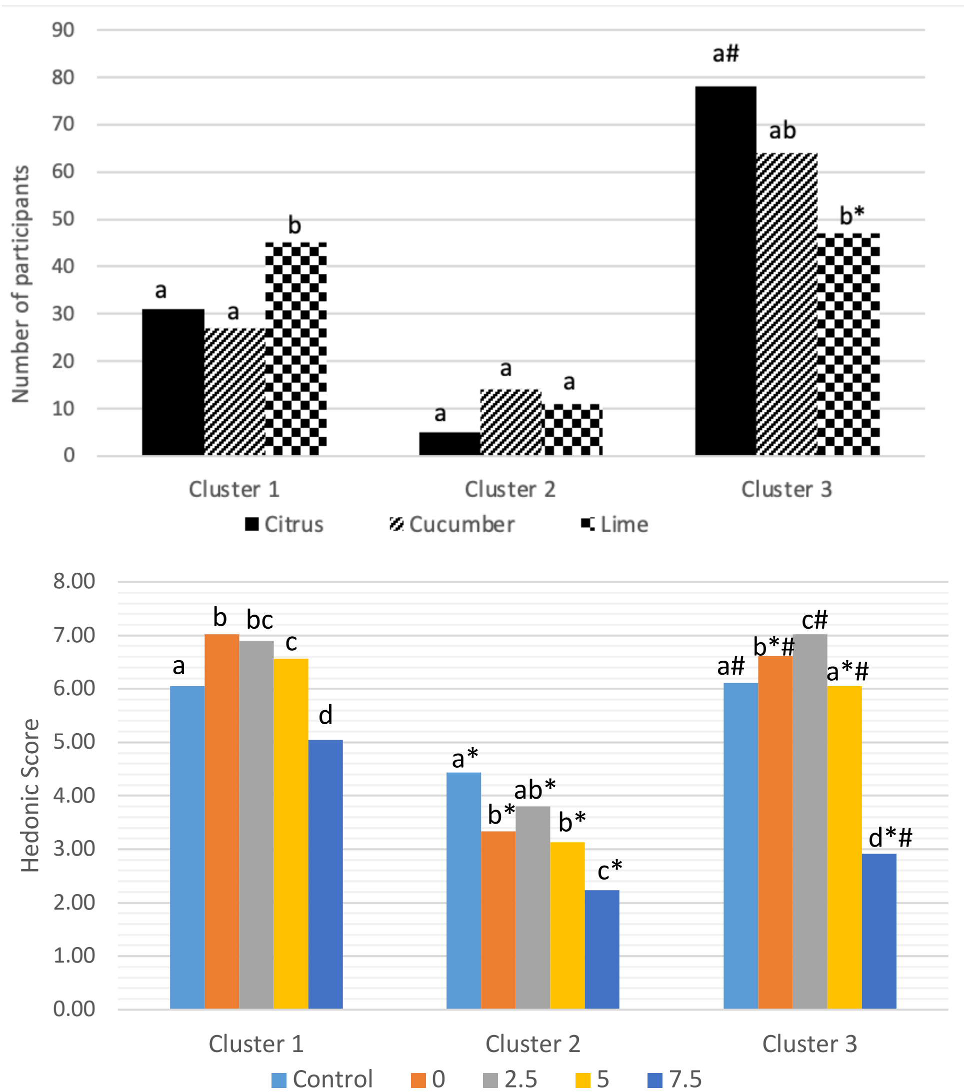


Figure 25. AHC Bar Charts Based on Demographic Data. Three clusters are depicted for flavor and alcohol preferences. Cluster 1 (*n* = 103), Cluster 2 (*n* = 30), and Cluster 3 (*n* = 189) all show different preferred flavors amongst clusters. Cluster 3 is the majority and prefers citrus and 2.5% abv. while cluster one prefers lime and 0% abv. and the minority Cluster 2 prefers cucumber and the control (0% abv.).

Different letter indicates significant difference between the three flavors within each cluster. *, significant from Cluster 1; #, significant from Cluster 2. All values shown as abv. levels.

Cluster 3 (*n* = 189) was the majority cluster, followed by Cluster 1 (*n* = 103), and Cluster 2 (*n* = 30). The participants in Cluster 3 were primarily male, aged 21-25, consumer beer 5+ times per week, with a collegiate degree, and employed full-time. The citrus flavor was the highest-rated flavor for hedonic attribute scores in this cluster trailed by the cucumber and lime flavors. Cluster 3 preferred the 2.5% abv. level the most, followed by 0% abv., and the control.

Cluster 1 was the second biggest cluster and was composed primarily of males, between 26-35 years of age, consumed beer 1-3 times per week, had a collegiate degree, and employed full-time. Cluster 1 scored the lime flavor the highest shadowing the citrus and cucumber-flavored samples. The 0% abv. sample received the highest hedonic scores, followed by the 2.5% abv., 5.0% abv., control, and 7.5% abv. samples.

The minority Cluster 2 was composed primarily of females, between 50-65 years of age, consumed beer 1-3 times per week, had a collegiate degree, and employed full-time. This cluster preferred the cucumber flavor over both the lime and citrus-flavored samples. Cluster 2 rated the control the highest in alcohol preference followed by the 2.5% abv., 0% abv., 5% abv. and 7.5% abv. samples.

Volatile Analysis

Volatile Isolation Method Validation

To determine how carbonation influences volatile analysis in quality and quantity, samples containing either a degassed beer sample or a gassed sample were analyzed by SPME-GC-MS. A chromatograph comparing the degassed versus gassed samples is presented in Figure 26. The compounds shown the most significant differences between the degassed and gassed samples included acetaldehyde, ethanol, 1-butanol, isoamyl acetate, isoamyl alcohol, ethyl hexanoate, hexyl acetate, ethyl heptanoate, ethyl octanoate, 1-octanol, phenethyl acetate, 1-dodecanol, octanoic acid, nonanoic acid, n-decanoic acid, and phenol, 4-(1,1-dimethylpropyl). These compounds in the gassed sample have larger peak areas, peak area percentages, and are more reliable in replications than the degassed beer samples. It should be noted that beer samples should not be degassed in an ultrasonic bath prior to SPME-GC-MS analysis due to the significant volatile loss and decrease in reproducibility.

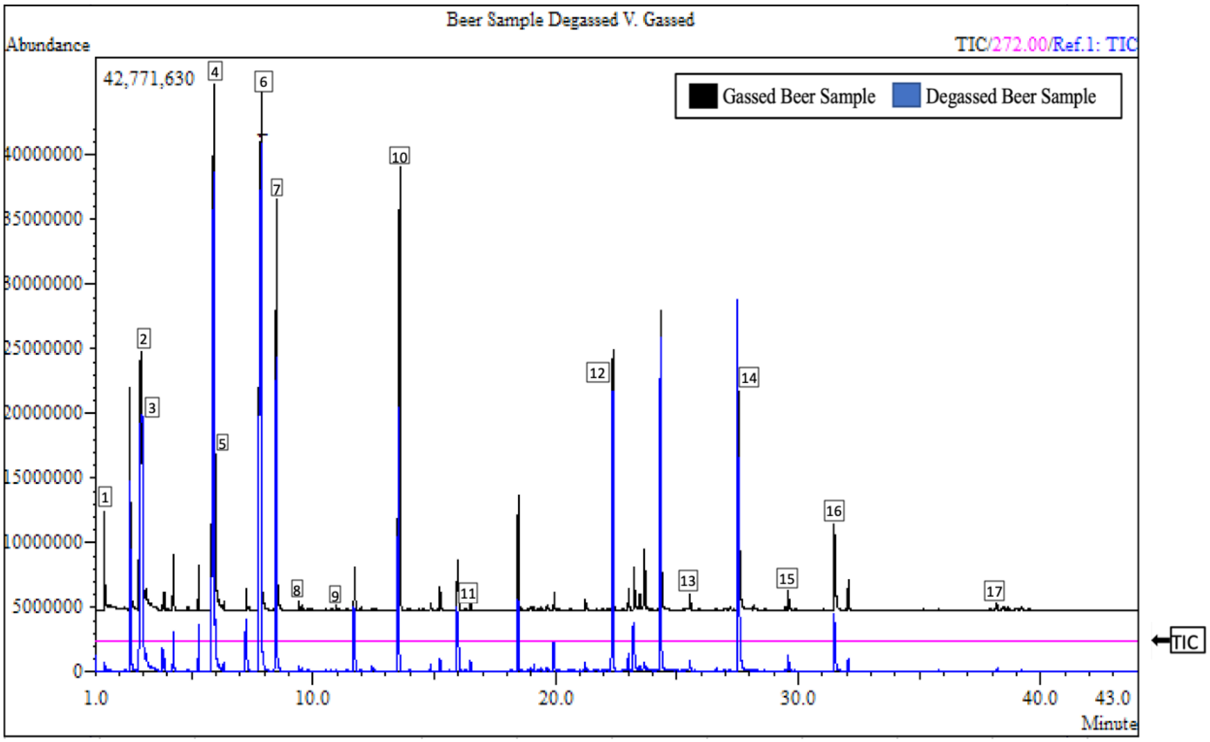


Figure 26. Gassed v. Degassed Beer Samples Chromatograph.

Key: **1.)** Acetaldehyde **2.)** Ethanol **3.)** Ethanol **4.)** 1-Butanol, 3-methyl-, acetate **5.)** 1-Butanol **6.)** 1-Butanol, 3-methyl- **7.)** Hexanoic acid, ethyl ester **8.)** Acetic acid, hexyl ester **9.)** Heptanoic acid, ethyl ester **10.)** Octanoate <ethyl-> **11.)** 1-Octanol **12.)** Acetic acid, 2-phenylethyl ester **13.)** 1-Dodecanol **14.)** Octanoic acid **15.)** Nonanoic acid **166.)** n-Decanoic acid **17.)** Phenol, 4-(1,1-dimethylpropyl)-

To determine how ethanol influences volatiles quality and quantity, a series of gradual dilutions were performed. A chromatograph comparing the gradual dilution is presented in Figure 27. The compound list (see Figure 2) shows the greatest differences within the gradual dilutions. The 0.5% abv. and 1% abv. had very small peaks and low reproducibility, while the 4.2% abv. samples had high peaks with better reproducibility. The 2.1% abv. had smaller peaks than the 4.2% abv. samples, greater than the 0.5% abv. and 1% abv., and the best reproducibility. Considering all dilutions, the 2.1% abv. beer was the leading sample with the second-largest peaks, greatest reproducibility, and overall favorable quality.

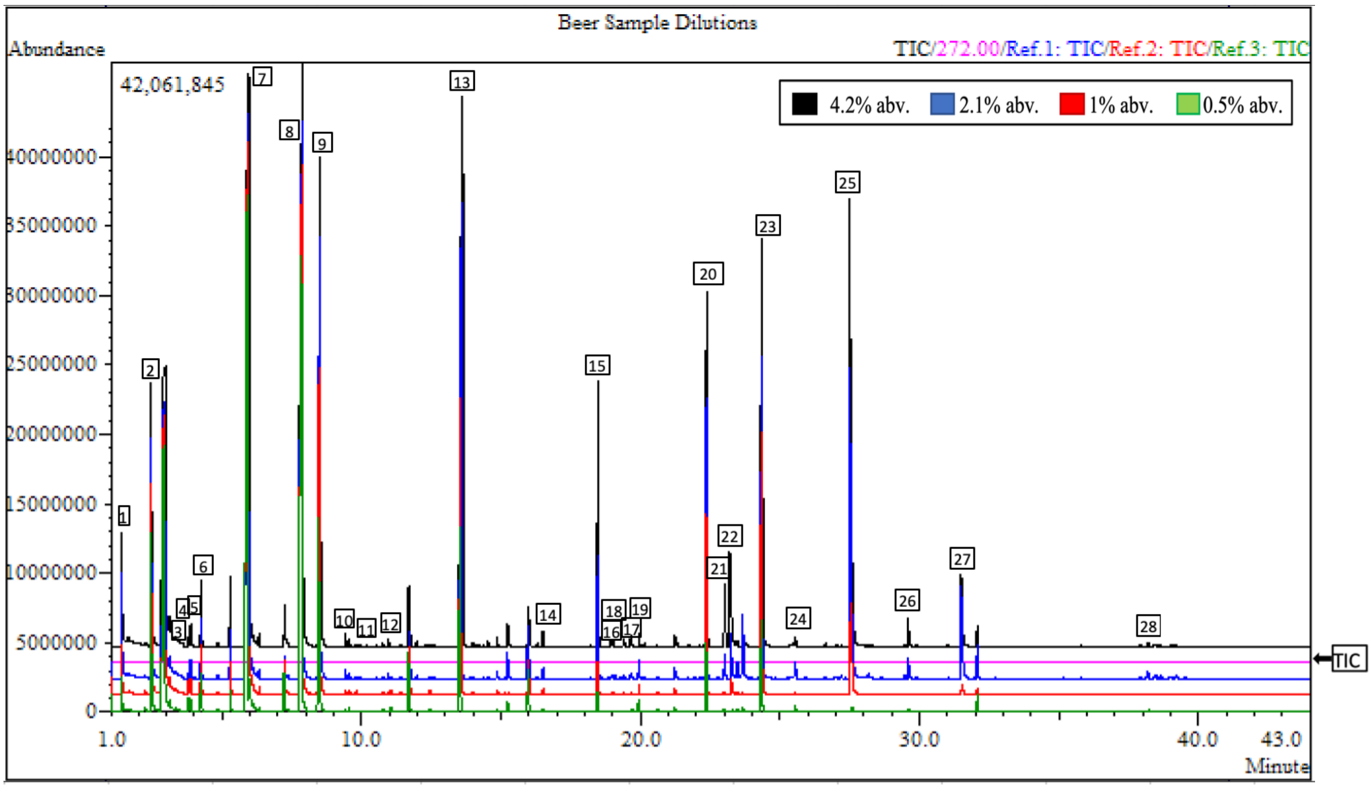


Figure 27. Gradual Alcohol Dilutions Chromatograph.

Key: **1.)** Acetaldehyde **2.)** Ethyl Acetate **3.)** Propanoic acid, 2-methyl-, ethyl ester **4.)** n-Propyl acetate **5.)** Isobutyl acetate **6.)** Butyrate <ethyl-> **7.)** 1-Butanol, 3-methyl-, acetate **8.)** 1-Butanol, 3-methyl- **9.)** Hexanoic acid, ethyl ester **10.)** Acetic acid, hexyl ester **11.)** 3-Heptanol, 3,5-dimethyl- **12.)** Heptanoic acid, ethyl ester **13.)** Octanoate <ethyl-> **14.)** 1-Octanol **15.)** Decanoic acid, ethyl ester **16.)** Octanoic acid, 3-methylbutyl ester **17.)** Benzoic acid, ethyl ester **18.)** Pentanoic acid, 3-methyl- **19.)** Ethyl 9-decenoate **20.)** Acetic acid, 2-phenylethyl ester **21.)** Dodecanoate <ethyl-> **22.)** Hexanoic acid **23.)** Phenylethyl Alcohol **24.)** 1-Dodecanol **25.)** Octanoic acid **26.)** Nonanoic acid **27.)** n-Decanoic acid **28.)** Phenol, 4-(1,1-dimethylpropyl)-

When comparing both carbonation and ethanol, the premier beer sample analyzed was the 2.1% abv. gassed sample. With a moderate alcohol content and full carbonation, this sample depicted repeatable results with stable isolated compounds detected via SPME-GC-MS.

Characteristics of Selected Three Beers

Table 4.

Physical and Sensory Qualities of Branded Pilsners.

Brand	°Brix	IBU	pH	Abv.	Aroma	Flavor
Michelob Ultra	2.4%	10 IBU	7.3	4.2% abv.	Yeast, sour, grain	Light intensity with notes yeast, sourness, and grain
Carlsberg	4.8%	22 IBU	6.3	5% abv.	Notes of hops, skunk, and yeast	Hoppy, herbal, bitter, sweet, spice, grass, and pine
Heineken 0.0	5.4%	23 IBU	6.4	< 0.05% abv.	Hoppy, sweet, notes of unfermented wort	Mild skunky, grainy, and hoppy

Characteristics of the three selected beers are depicted in Table 4. Michelob Ultra (St. Louis, Missouri, USA) was a light lager with a 4.2% abv. and 10 IBUs. Aroma and flavor notes of this beer were very light in intensity, mostly yeasty, slightly sour, with a touch of grainy notes. The average pH for Michelob Ultra was 7.3, a TA of 8.64 (2.06 mL), and a °Brix of 2.4%.

Carlsberg Pilsner (Copenhagen, Denmark) was a medium intensity lager with a 5% abv. and 22 IBUs. Flavor notes of Carlsberg were hoppy in both taste and aroma, a slight skunky and yeast-like aroma with a balance of bitter, sweet, grassy, and piney flavors. The mouthfeel was smooth and full. The average pH for Carlsberg was 6.3, a TA of 8.57 (3.25 mL), with a °Brix of 4.8%.

Heineken 0.0 (non-alcoholic; Holland, Netherlands) was a light lager as well and the beer base of the beer consumer study. The Heineken 0.0 has an abv. of < 0.05% and 23 IBUs. The Heineken 0.0 flavor notes were light in aroma and flavor intensity, mildly skunky, grainy, and hoppy with an aroma of hops, sweet, and unfermented wort (malted grain mash before fermentation). The average pH for Heineken 0.0 was 6.4, a TA of 8.55 (3.28 mL), and a °Brix of 5.4%.

The three beers chosen are regularly consumed around the world and represent what a typical lager is. Slight differences in aroma, flavor, pH, °Brix, and IBUs are what makes each pilsner unique. Michelob Ultra represents light flavor intensity with a slight twist of grainy notes, Carlsberg are for the hop-loving beer enthusiasts, and Heineken 0.0 showcases a beer with classic and tradition flavor that can be consumed by everyone.

SPME-GC-MS Volatile Identification of Selected Three Beers

The volatiles of the three beers are listed in Table 5. Lowercase letters show significant ($p < 0.05$) differences between beer samples obtained from ANOVA. The peak area percentage (PA%) are the values that depict the proportional amount of the PA of the volatile that is presented in the beer sample according to GC-MS. The total amount of these volatiles can be found in Table 6**.**

Table 5.

Volatile Compounds.

RT	Functional Group	CAS #	Beer Brand (Peak Area %)			
	Esters		**Heineken 0.0**	**Michelob Ultra**	**Carlsberg**	**p-value**
2.436	Ethyl Acetate	141-78-6	1.952 b	1.185 a	1.286 a	0.000
3.14	Ethyl propanoate	105-37-3	0.203 b	0.034 a	0.022 a	0.000
3.233	Ethyl 2-methylpropanoate	97-62-1	-	0.001	-	-
3.334	n-Propyl acetate	109-60-4	0.05 c	0.013 a	0.03 b	0.000
3.865	Isobutyl acetate	110-19-0	-	0.08	0.045	0.000
4.228	Ethyl butyrate	105-54-4	0.145 a	0.252 b	0.247 b	0.000
4.488	Ethyl 2-methlybutanoate	7452-79-1	-	0.002	0.003	N/A
4.76	Ethyl isovalerate	108-64-5	-	0.002	0.005	0.001
4.84	Acetic acid, butyl ester	123-86-4	-	0.013	0.045	0.000
5.933	1-Butanol, 3-methyl-, acetate	123-92-2	13.504 b	6.608 a	6.436 a	0.000
6.124	Ethyl pentanoate	539-82-2	-	0.01	0.04	0.408
6.75	2-Butenoic acid, ethyl ester, (E)-	623-70-1	-	0.001	-	-
6.985	Pentyl acetate	628-63-7	0.027 c	0.003 a	0.009 b	0.000
7.377	Isopentyl propanoate	105-68-0	0.019 a	0.03 a	0.009 a	0.015
7.515	Propanoic acid, 2-methyl-, 2-methylbutyl ester	2445-69-4	-	-	0.01	-
7.524	3-Methyl-3-buten-1-ol, acetate	7/2/05	-	0.002	-	-
8.373	Hexyl acetate	142-92-7	-	0.002	0.002	N/A
8.501	Ethyl hexanoate	123-66-0	2.366 a	2.986 b	2.224 a	0.000
9.234	Butyrate <isopentyl->	106-27-4	0.012 b	0.003 a	0.002 a	0.000
9.418	Hexyl acetate	142-92-7	0.007 a	0.043 b	0.109 c	0.000
9.524	3-methylbutyl 2-methlybutanoate	27625-35-0	0.006	-	-	-
9.798	Heptanoic acid, ethyl ester	106-30-9	-	0.015	-	-
9.923	Hex-4-enoic acid, ethyl ester	500027-11-2	-	0.009	0.024	0.002
9.976	3-methylbutyl 3-methlybutanoate	659-70-1	0.032	-	-	-
10.152	Ethyl 3-hexenoic acid	2396-83-0	-	0.009	0.02	0.000
10.54	Ethyl 2-methyl pent-3-enoate	609-26-7	-	0.01	0.047	0.000

10.955	Ethyl heptanoate	106-30-9	-	0.027	0.031	0.070
11.176	Ethyl lactate	97-64-3	-	0.007	-	-
11.217	Ethyl 2-Hexenoic acid	1552-67-6	-	-	0.003	-
11.968	Heptyl acetic acid	112-06-1	-	0.016	0.034	0.001
12.098	(E)- Ethyl 4-heptenoic acid	54340-70-4	-	-	0.003	-
12.226	Hexyl 2-ethyl acetate	103-09-3	-	0.001	-	-
12.992	Acetic acid, cis-4-methylcyclohexyl ester	22597-23-5	-	-	0.005	-
13.584	Ethyl octanoate	106-32-1	0.258 a	3.66 b	4.09 b	0.000
14.098	Isopentyl hexanoate	2198-61-0	0.004	0.005	-	0.024
14.774	Ethyl oct-7-enoate	35194-38-8	-	0.001	0.001	0.116
16.163	Pentanoic acid, 2-hydroxy-4-methyl-, ethyl ester	10348-47-7	-	-	0.003	-
16.744	Menthyl valerate	64129-94-8	0.003	-	-	-
16.792	3-Nonenoic acid, ethyl ester	123-29-5	-	0.002	-	-
18.451	Decanoic acid, ethyl ester	110-38-3	0.027 a	0.612 b	0.524 b	0.000
18.915	Octanoic acid, 3-methylbutyl ester	2035-99-6	-	0.012	-	-
19.011	Ethyl benzoate	93-89-0	-	0.028	-	-
19.648	Ethyl 9-decenoate	67233-91-4	-	0.025	0.149	0.007
20.643	1,3-Propanediol, diacetate	628-66-0	0.005	-	-	-
20.779	Undecanoic acid, ethyl ester	627-90-7	-	0.005	0.002	0.205
21.711	Benzeneacetic acid, ethyl ester	101-97-3	-	0.008	0.01	0.497
22.117	Methyl 4-hydroxybutanoate	1487-49-6	0.004 a	0.008 a	0.004 a	0.085
22.252	Ethyl nicotinate	614-18-6	-	-	0.007	-
22.35	2-Phenylethyl acetate	103-45-7	2.028 a	1.63 a	1.312 a	0.085
23.016	Dodecanoic acid, ethyl ester	106-33-2	0.011 a	0.131 b	0.031 a	0.000
23.8	Benzenepropanoic acid, ethyl ester	2021-28-5	-	0.016	0.007	0.235
24.105	Diethyl adipate	141-28-6	-	-	0.003	-
25.408	.beta.-Phenylethyl butyrate	103-52-6	-	0.01	0.003	0.011
26.409	Methyl tetradecanoate	124-10-7	-	0.002	-	-
26.984	Isopropyl myristate	110-27-0	-	0.026	-	-
28.637	Ethyl cinnamate	103-36-6	-	0.005	0.004	0.349
30.117	Salicylate <hexyl->	6259-76-3	-	0.004	0.002	0.067
31.023	Hexadecanoic acid, ethyl ester	628-97-7	0.003	-	0.003	0.008
34.236	Homosalate	118-56-9	0.001 a	0.009 b	0.001 a	0.002
37.13	Benzyl Benzoate	120-51-4	-	0.003	-	-
	Alcohols					

2.84	Ethanol	64-17-5	0.134 a	2.821 b	4.66 c	0.000
5.284	2-Methyl propanol	78-83-1	-	0.171	0.119	0.003
5.727	3- Methyl pentanol	77-74-7	-	0.001	0.001	0.374
6.332	1-Butanol	71-36-3	0.003 a	0.029 a	0.113 b	0.000
7.872	3-Methyl butanol	123-51-3	6.83 b	5.838 a	5.715 a	0.008
8.81	1-Pentanol	71-41-0	0.022 b	0.002 a	0.011 a,b	0.012
8.887	1,3-Dimethylcyclopentanol	19550-46-0	-	0.001	0.003	0.016
10.421	4-methyl- 1-Pentanol	626-89-1	0.005 c	0.004 b	0.003 a	0.000
10.74	3-methyl-1-Pentanol	589-35-5	0.001 a	0.015 b	0.047 c	0.000
10.769	3,5-dimethyl-3-Heptanol	19549-74-7	-	0.013	0.02	0.011
11.043	3,6-dimethyl-3-Heptanol	1573-28-0	-	0.015	0.022	0.002
11.395	1-Hexanol	111-27-3	0.007 a	0.012 a	0.029 b	0.000
12.166	3-Hexen-1-ol, (Z)-	928-96-1	0.001	-	-	-
12.89	3-methyl-1-Hexanol	13231-81-7	-	0.002	0.001	0.116
13.107	2-Octanol	123-96-6	-	-	0.004	-
13.892	1-Octen-3-ol	3391-86-4	-	-	0.007	-
13.978	1-Heptanol	111-70-6	0.002 a	0.013 b	0.012 b	0.001
14.857	2-ethyl-1-Hexanol	104-76-7	0.034 b	0.046 b	0.011 a	0.001
15.597	2-Nonanol	628-99-9	-	-	0.009	-
16.499	1-Octanol	111-87-5	0.006 a	0.062 b	0.102 c	0.000
16.918	2,3-Butanediol	513-85-9	0.012 a	0.004 a	0.006 a	0.369
17.467	1-Hexanol, 5-methyl-2-(1-methylethyl)-	2051-33-4	0.011	-	-	-
18.942	2-Furanmethanol	98-00-0	0.07	-	0.109	0.027
19.549	2-propyl-1-Heptanol	10042-59-8	-	0.004	-	-
20.293	2-Undecanol	1653-30-1	-	-	0.004	-
21.211	1-Decanol	112-30-1	0.009 a	0.059 b	0.057 b	0.004
24.34	Phenylethyl Alcohol	60-12-8	2.894 a	2.341 a	2.144 a	0.098
25.546	1-Dodecanol	112-53-8	0.032 a	0.128 b	0.027 a	0.001
26.123	3-Tetradecanol	1653-32-3	-	0.004	-	-
	Terpenes					
6.718	.beta.-Myrcene	123-35-3	-	-	0.005	-
7.44	D-Limonene	5989-27-5	0.016 a	0.003 b	0.005 b	0.000
12.786	Linalool	78-69-3	-	0.003	0.004	0.374
16.183	Menthol	1490-04-6	0.003	-	-	-
16.301	Linalool	78-70-6	0.03 b	0.011 a	0.113 c	0.000
16.822	Caryophyllane <4,8-beta-epoxy->	1139-30-6	-	-	0.011	-

17.07	Fenchol	1632-73-1	-	-	0.010	-
17.49	Terpinen-4-ol	562-74-3	-	-	0.003	-
17.735	Carvomenthol	499-69-4	0.007	-		-
17.816	Myrcenol	543-39-5	-	-	0.008	-
18.799	Verbanol	94480-83-8	-	-	0.001	-
19.699	.alpha.-Terpineol	98-55-5	0.005	-	0.038	0.000
21.111	Geranyl acetate	105-87-3	-	0.007	0.008	0.891
21.31	Citronellol	106-22-9	-	0.013	0.042	0.001
22.03	Nerol	106-25-2	-	-	0.002	-
26.993	(E)-Nerolidol	142-50-7	0.015	-	-	-
27.049	Caryolan-8-ol	178737-45-6	-	-	0.026	-
27.321	Epicubenol	19912-67-5	-	-	0.003	-
28.297	Cedrol	77-53-2	-	0.012	0.001	0.001
29.139	Humulol	28446-26-6	-	-	0.017	-
29.357	tau.-Cadinol	05937-11-1	-	-	0.004	-
30.233	Eudesmol<alpha->	473-15-4	-	-	0.003	-
30.444	Cadin-4-en-10-ol	481-34-5	-	-	0.005	-
	Aldehydes					
1.638	Acetaldehyde	75-07-0	0.204 b	0.037 a	0.011 a	0.000
2.15	2-Butenal	123-73-9	-	0.006	-	-
1.976	2-Methyl-propanal	78-84-2	0.152	-	0.002	0.011
2.69	Isovaleric aldehyde	590-86-3	0.007 c	0.001 a	0.002 b	0.000
5.00	Hexanal	66-25-1	0.009 b	0.001 a	0.003 a	0.000
9.797	Octanal	124-13-0	0.016	-	0.08	0.118
12.44	Nonanal	124-19-6	0.066 a	0.017 a	0.237 a	0.175
15.078	Decanal	112-31-2	0.015 a	0.002 a	0.044 a	0.29
15.595	Benzaldehyde	100-52-7	0.007	-	-	-
15.934	2-Nonenal	18829-56-6	0.001	-	0.003	0.346
20.063	Dodecanal	112-54-9	0.081 b	0.004 a	0.009 a	0.000
24.614	Tetradecanal	124-25-4	0.031	-	0.002	0.097
26.866	Cinnamaldehyde, (E) -	14371-10-9	-	0.003	-	-
32.74	Cinnamaldehyde <(2Z)-, hexyl-	101-86-0	0.002 a	0.01 a	0.006 a	0.051
	Carboxylic Acids					
14.34	Acetic acid	64-19-7	0.012 a	0.014 a	0.028 a	0.144
18.057	4-Hydroxy-butanoic acid	591-81-1	0.003 a	0.002 a	0.002 a	0.63
19.396	3-Methyl-butanoic acid	503-74-2	0.037 a	0.034 a	0.052 a	0.272
23.216	Hexanoic acid	142-62-1	0.204 a	0.35 a	0.218 a	0.092

25.372	2-ethyl-hexanoic acid	149-57-5	0.002	0.018	-	1.000
27.505	Octanoic acid	124-07-2	1.112 a	2.443 b	1.719 a,b	0.014
29.592	Nonanoic acid	112-05-0	0.017 a	0.229 b	0.053 a	0.004
31.512	n-Decanoic acid	334-48-5	0.133 a	0.698 b	0.259 a	0.005
32.62	9-Decenoic acid	14436-32-9	0.004 a	0.009 a	0.015 a	0.355
35.168	Dodecanoic acid	143-07-7	-	0.013	0.006	0.212
	Phenols					
29.89	4-Vinyl-guaiacol	7786-61-0	0.017 b	0.006 a	0.021 b	0.015
38.179	4-(1,1-Dimethylpropyl)phenol	80-46-6	0.025 a	0.064 a	0.022 a	0.047
38.431	4-(1,1,3,3-Tetramethylbutyl)phenol	140-66-9	0.01 a	0.021 b	0.002 a	0.003
38.487	2-Methyl-4-(1,1,3,3-tetramethylbutyl)phenol	2219-84-3	0.008	-	-	-
38.618	2-Methyl-4-(1,1,3,3-tetramethylbutyl)phenol	2219-84-3	0.007	-	-	-
38.685	3,5-Diisopropylphenol	26886-05-5	0.002a	0.005a	0.006a	0.022
38.915	4-(1,1,3,3-Tetramethylbutyl)phenol	140-66-9	-	0.006	-	-
39.212	4-(1,1-Dimethylpropyl)Phenol)	80-46-6	0.011 a	0.027 a	0.011 a	0.221
39.521	2-Methyl-4-(1,1,3,3-tetramethylbutyl)phenol	2219-84-3	0.003 a	0.006 a	0.005 a	0.64
	Ketones					
9.678	2-Octanone	111-13-7	0.003	-	-	-
12.309	2-Nonanone	821-55-6	0.001	-	-	-
17.469	Undecan-2-one	112-12-9	-	0.002	-	-
18.598	Acetophenone	98-86-2	0.003	-	-	-
21.081	β-Damascenone	23726-93-4	0.003	-	-	-
27.188	Tetramethyl acetyloctahydronaphthalenes	54464-57-2	0.004 a	0.026 b	0.007 a	0.014
32.221	Benzene, p-diacetyl-	1009-61-6	-	0.005	-	-
34.688	Benzophenone	119-61-9	-	0.001	0.001	N/A
	Furans					
1.906	Furan	110-00-9	0.075	-	-	-
3.074	Furan, 2,5-dimethyl-	625-86-5	0.001	-	-	-
8.355	Furan, 2-pentyl-	3777-69-3	0.002	-	-	-
14.231	Furfural	98-01-1	0.043 c	0.006 a	0.023 b	0.000
21.892	6,7-Dimethyl-3H-isobenzofuran-1-one	569-31-3	-	-	0.003	-
26.643	2(3H)-Furanone, dihydro-5-pentyl-	104-61-0	0.017 a	0.019 a	0.016 a	0.734

33.391	2,3-dihydro-benzofuran	496-16-2	0.001 a	0.006 b	0.002 a	0.000
	Sulfurs					
1.577	Methanethiol	74-93-1	0.06	-	-	-
4.427	S-Methyl thioacetate	1534-08-3	-	-	0.001	-
5.178	S-Ethyl ethanethioate	625-60-5	-	-	0.001	-
16.71	3-(Methylthio)propanoic acid ethyl ester	13327-56-5	-	-	0.002	-
18.187	3-(Methylthio)propyl acetate	16630-55-0	0.001 a	0.008 b	0.013 c	0.000
20.143	3-(methylthio)-propanol	505-10-2	0.031 a	0.016 a	0.039 a	0.132
	Pyrazines					
9.131	Pyrazine, methyl-	109-08-0	0.001	-	0.001	0.23
10.513	Pyrazine, 2,5-dimethyl-	123-32-0	0.002	-	-	-
10.662	Pyrazine, 2,6-dimethyl-	108-50-9	0.001	-	-	-
	Other					
20.73	Naphthalene	91-20-3	0.004 a	0.021 a	0.007 a	0.046

Note. N/A: *T*-test could not be performed because the standard deviation (SD) was zero

Table 6.

Volatile Quantification for Pilsners and Functional Groups.

	All Beers	Heineken 0.0	Michelob Ultra	Carlsberg
Esters	60	22	48	43
Alcohols	29	17	22	25
Terpenes	23	6	6	20
Aldehydes	14	12	9	10
Acids	10	9	10	9
Phenols	9	8	7	6
Ketones	8	5	4	2
Furans	7	6	3	4
Sulfurs	6	3	2	5
Pyrazines	3	3	0	1
Other	1	1	1	1
Total	170	92	112	126

Amongst the beers compared in Tables 5, 6, and 7, esters and alcohols were the two largest categories of functional groups which is to be expected. Esters composed 35.3% (60 volatiles) of the total volatiles. Between the beer samples, Michelob Ultra had the largest portion of esters with 42.9% of total volatiles (48 volatiles – 17.6 PA%), Carlsberg in the middle with 43 compounds making up 34.1% of total volatiles (16.9 PA%), and Heineken 0.0 with the fewest esters (22 volatiles - 23.9% of total volatiles) but the largest PA% was 20.7%. Statistically significant difference ($p < 0.05$) between each pilsner (a, b, c) was found specifically in n-propyl acetate (melons, pears, hard and soft fruits), pentyl acetate also known as amyl acetate (banana and apple aromas), and hexyl acetate (green, sweet banana, apple, pear). These volatiles showed significant differences in peak area percentages between each beer sample and provided each beer with their own unique aroma and flavor complex.

Table 7.

Functional Groups Peak Area Percentages (PA%).

	Heineken 0.0	Michelob Ultra	Carlsberg
Esters	20.67%	17.57%	16.85%
Alcohols	10.07%	11.59%	13.24%
Terpenes	0.08%	0.05%	0.31%
Aldehydes	0.59%	0.08%	0.40%
Acids	1.52%	3.81%	2.35%
Phenols	0.08%	0.14%	0.07%
Ketones	0.01%	0.03%	0.01%
Furans	0.14%	0.03%	0.04%
Sulfurs	0.09%	0.02%	0.06%
Pyrazines	0.00%	0.00%	0.00%
Other	0.00%	0.02%	0.01%

Alcohols composed 17.1% (29 volatiles) of total volatiles (see Table 5). The Carlsberg pilsner had 25 alcohol volatiles (19.8% of total volatiles - 13.2 PA%), Michelob Ultra had 22 alcohol volatiles (19.6% of total volatiles - 11.6 PA%), and Heineken 0.0 had the fewest alcohols (17 volatiles - 18.5% of total volatiles - 10.1 PA%). Like esters, Carlsberg and Michelob Ultra shared more volatiles in common while Heineken 0.0 had a lesser number of shared volatiles. All three pilsners showed significance ($p < 0.05$) in 11 alcoholic compounds. Statistical significance ($p < 0.05$) between each pilsner (a, b, c) was found in four compounds: ethanol, 4-methyl-1-pentanol (isohexanol- nutty, light fruity aroma), 3-methyl-1-pentanol (fruity-fermented-cognac-whiskey aroma), and 1-octanol (aromatic, fruity, waxy). These alcohol volatiles were found at significant differences amongst pilsners and can be distinguishable through the sensory experience.

The terpene category (see Table 6) was larger in the Carlsberg (20 volatiles - 15.9% of total volatiles - 0.3 PA%) pilsner than Heineken 0.0 (6 volatiles - 6.5% of total volatiles - 0.1 PA%) and Michelob Ultra (6 volatiles - 5.4% of total volatiles - 0.1 PA%), which contributes to the hoppy, herbal, and aromatic aroma and flavor of the beers. Two terpenes shared by all three pilsners showed a statistically significant *p*-value ($p < 0.05$): d-limonene (primarily lemon and citrus aromas) and linalool (sweet orange, lavender, floral, and strawberry). Significant differences ($p < 0.05$) between each pilsner (a, b, c) was found only in linalool; Michelob Ultra and Carlsberg were not statically significantly different from each other for d-limonene.

Heineken 0.0 had the highest volatile percentage for aldehydes (12 volatiles - 13% of total volatiles - 0.6 PA%) while Carlsberg (10 volatiles - 7.9% of total volatiles - 0.4 PA%) and Michelob Ultra (9 volatiles - 8% of total volatiles - 0.1 PA%) had the lowest volatile percentage (see Table 7). Four aldehydic volatiles shared amongst the three pilsners had a p-value < 0.05: acetaldehyde (green, acetic, ethereal aroma), isovaleric aldehyde (also known as 3-methylbutanal- fruity, nutty, cocoa, nutty, chocolate), hexanal (fresh cut grass, fruity, berry, tropical fruit), and dodecanal (soapy, citrus, fruit rinds, floral, green). Only one aldehyde had statistically significant differences ($p < 0.05$) between each pilsner (a, b, c) and that was isovaleric aldehyde. These aldehydes all contribute to individualized green and earthy aromas and flavors of the beers.

Heineken 0.0 had the most acids out of total volatiles percentage (9.8% - 9 volatiles), however, the lowest PA% (1.5 PA%; see Table 7). Michelob Ultra had the highest PA% (3.8 PA%) with 10 volatiles (8.9% of total volatiles). Carlsberg had 9 volatiles (7.1% of total volatiles) and a 2.4 PA%. There were three carboxylic acids that were shared amongst the three beers that had a statistic significance of $p < 0.05$: octanoic acid (sour, cheesy, fatty, acidic), nonanoic acid (waxy, fatty, cheesy), and n-decanoic acid (rancid, sour, fatty, soapy, waxy). There was not an instance where the three pilsners were significantly different from each other (a, b, c) for any acidic volatile.

Heineken 0.0 had the most phenols (8 volatiles - 8.7% of total volatiles; see Table 6). Michelob Ultra had 7 phenols (6.3% of total volatiles) and Carlsberg had 6 phenols (4.8% of total volatiles). However, all three pilsners had the same PA% for phenols - 0.1

PA%. There were four phenols that shown statistical significance ($p < 0.05$) that all three pilsners shared: 4-vinyl-guaiacol (woody, smoky, nutty), 4-(1,1-dimethyl propyl) phenol (no specific organoleptic properties), 4-(1,1,3,3-tetramethyl butyl) phenol (no specific organoleptic properties), and 3,5-diisopropyl phenol (no specific organoleptic properties). There was no phenol that showed significant difference amongst all three pilsners (a, b, c).

Smaller functional group categories included ketones, furans, sulfurs, and pyrazines (see Table 5). There was only one ketone that all three pilsners shared in common and also happens to be statically significant ($p < 0.05$): tetramethyl acetyloctahydronaphthalenes (also known as iso E super gamma, amber and woody aroma). There were two furans that showed statical significance ($p < 0.05$) amongst all three samples: furfural (baked goods, almond, caramel, sweet, woody aroma) and 2,3 dihydro-benzofuran (weak aromatic). Only one sulfur was in common amongst all beers and was statistically significant ($p < 0.05$): 3-(methylthio)propyl acetate (sulfur, vegetal, and cheesy aroma). There were no pyrazines that all pilsners shared and that was statically significant. Both furfural and 3-(methylthio)propyl acetate were significantly different amongst all three pilsners (a, b, c). The different compositions of peak area percentages for these functional groups give subtle and distinctive characteristics to the individual beer samples.

CHAPTER V

DISCUSSION

Online Survey

Survey Demographics

The overall demographics of the survey participants were depicted in Figures 12-16. Due to the large portion of participants belonging to TWU, the average demographic was female, aged 21-25 years old, with a bachelor's degree, either employed full-time (the survey was sent out over the summer) or a student (n = 256, 34.4%), and consumed beer 1-3 times per week. According to recent studies investigating the typical beer drinker, there seems to be a clear divide between craft and traditional macro-brewed beer drinkers. The craft beer drinker demographic is younger in age (19-40 years of age), highly educated, with a greater annual income than the traditional beer drinker (Lerro et al., 2020; Malone & Lusk, 2018). For both studies, there was a heterogeneity of consumers where the average beer consumer's demographic was equally a man or woman. However, in a separate poll, there were twice as many male beer drinkers than female (Nadeau & Coletto, 2013). The demographics for this consumer survey match closer to the craft beer drinker than the traditional beer drinker. There are huge opportunities for brewers to market to craft beer-drinking females using the results from the current survey.

Why Consumers Enjoy Beer

Acknowledging the findings of this survey, the two most important aspects as to why participants consume beer was due to enjoying the flavor and feeling refreshed. Flavor is both why consumers enjoy beer (n = 810, 77.1%) and why they chose one beer product over the other (n = 965, 91.9%). Flavor is a very significant and influential factor for people to prefer one beer over another (Aquilani et al., 2015; Chakraborty & Suresh, 2018; Guinard et al., 1998; Labbe et al., 2009a). After flavor, the next most commonly chosen factor was feeling refreshed after consumption (n = 583, 55.5%). Refreshed, refreshing, or refreshment is an emotional response to food consumption experience, instead of a sensory attribute of food. Refreshment is an important aspect of a product and gaining more attention recently, as we can see more newly released papers including this concept. Feeling refreshed after beer consumption has been linked to many different factors including flavor, the temperature of the beer, and the water aiding in hydration (Desbrow, Murray, & Leveritt, 2013; Guinard et al., 1998; Labbe, 2009a;). A balance of flavor attributes is imperative to refreshment as stated in previous literature (McEwan & Colwill, 1996). Flavor and refreshment are the main reasons why consumers drink beer and should be the forefront priorities for all brewers.

Consumer Opinion on Beer Refreshment

The majority of survey participants linked lightened mood to feelings of refreshment (n = 915, 87.1%). The feeling of a lightened, happy mood amongst consumers can be considered both mental and physical effects. Consuming beer has rewarding effects on the mind (Fairbairn et al., 2015). Once the first sip is consumed the mental feelings of a lightened and refreshed mood are already ensuing. Alcohol effects on the body can also seem to constitute a lighter mood. On average, 1-2 beers in 2 hours is enough to make a person feel euphoric and at ease (Baum-Baicker, 1985). The next most chosen feeling was thirst-quenching (n = 514, 49%). Thirst-quenching's association with refreshment has been supported by previous literature (Guinard et al., 1998; Labbe et al., 2009a; Meilgaard & Muller, 1987; Peyrot des Gachons et al., 2016). Refreshment is defined as a way to restore strength and animation, revive, arouse, stimulate, and contain thirst-quenching properties (Labbe et al., 2009a). It is imperative to relate refreshment with thirst-quenching in which both descriptors were chosen by the majority of survey participants when describing beer. Together, a lighter mood and to be without thirst are the direct effects of feeling refreshed.

Product Factors Related to Refreshment

Two internal sensory factors that impact refreshment derived from beer consumption stood out amongst the others. Both temperature (n = 1002, 95.4%) and flavor (n = 930, 88.6%) were chosen by a large majority of survey participants. The temperature has been related back to refreshment due to oral cooling causing a craving for more fluid to be ingested (Peyrot des Gachons et al., 2016). The flavor of the beer is more complex. Consumers tend to rate familiar beers more refreshing than new beers (Giacalone et al., 2015) relating that nostalgia and familiarity alone can be linked to refreshment. Nevertheless, few studies have investigated the impact of beer flavor on refreshing perception.

Many factors contribute to both beer aroma and flavor such as hops, malted cereal, yeast, additives like botanicals and fruits, the type of water, sugar, and the alcohol produced. With each of these variables, comes a change in the overall beer flavor. The top three refreshing beer flavors chosen in this survey were lime (n = 543, 51.7%), lemon (n = 452, 43%), and orange (n = 425, 40.5%). These citrus descriptors could be profiled in both ale and lager varieties resulting in refreshment in either. The main consensus was that consumers chose citrus flavors as the most refreshing amongst other common beer flavors including fruity (strawberry, raspberry, blueberry, blackberry), sweet (honey), bitter, hoppy, amber, spice (pumpkin/cloves/cinnamon), and chocolate flavors. The citrus aroma has been linked to increased physical activity, shortened response time, decreased negative emotions, feeling refreshed, and associated with both crisp and clean sensory descriptors (de Wijk & Zijlstra, 2012; Matsumoto, Asakura, & Hayashi, 2014; Private Label Buyer, 2006). The main contributor to citrus refreshment is the sour taste derived from citric acid. Beverages containing citric acid are known to trigger increased saliva excretion to wet the mouth and cause the perception that the beverage is more hydrating (Bozorgi, Holleufer, & Wendin, 2020; Froehlich, Pangborn, & Whitaker, 1987; Murugesh et al., 2015). Acidity has been shown to be positively associated and highly correlated with thirst-quenching properties (McEwan & Colwill, 1996).

The most refreshing beer flavor profiles mimic the previous results by participants choosing "crisp and clean" (*n* = 917, 87.3%) and "fruity" (*n* = 555, 52.9%) as the most preferred profiles. These two flavor profiles adequately describe the two most common beer varieties: ales and lagers. Ales are considered fruiter due to esters formed during a long, warm fermentation while lagers are lighter in ester intensity and more carbonated due to specific yeast strains and low fermentation temperatures (Barth, 2013). The clean descriptor is related to low intensity while the crisp descriptor is related to carbonation in beer (Willis, 2020). Participants linked both flavor profiles to refreshment elucidating that either ales or lagers can be found refreshing respective to what the consumer prefers.

Beer varieties can be broken down into specific subdivisions within the overall ale and lager guidelines or can be completely independent. The three most chosen refreshing beer varieties were hefeweizen: a citrus-forward wheat beer (*n* = 532, 50.7%), blonde ale: light in intensity, a balance of yeast and hops (*n* = 496, 47%), and American lager: more golden and full-bodied than a blonde ale yet still light in flavor and aroma intensity (*n* = 461, 43.9%). According to previous literature, the top five most desirable traits for a lager are refreshing, crisp, citrus, sweet, and light (Bettenhausen et al., 2020). Lagers traditionally have a minor malt complexity, a light to medium body, carbonated, and can be linked to grass, grain, and yeast aroma and flavor notes (Donadini & Fumi, 2010). However, due to the longer and warmer fermentation process of ales, more flavor-active esters are allowed to be produced to allow the ale's signature fruity and malty notes and full-bodied mouthfeel (Hiralal, Olaniran, & Pillay, 2014). Ales have their own niche preference amongst consumers. However, in literature, the lager beer variety is typically related to refreshment (Bettenhausen et al., 2020; Guinard et al., 1998).

External Factors Related to Refreshment

For the purpose of this survey, subdivisions of beer varieties were associated with seasons of the year. The most commonly chosen season participants found refreshing was the summer, citrus-forward beers (n = 444, 42.3%). Citrus has been linked to refreshment and beer preference in previous literature for internal factors such as acidity balancing out other attributes, proving crisp and clean characteristics to beverages, and aiding in a happier emotional state (Bettenhausen et al., 2020; de Wijk & Zijlstra, 2012; Matsumoto et al., 2014; Private Label Buyer, 2006). However, citrus is also related to being an external factor of refreshment due to synergy of citrus aroma and flavor cravings during the warm weather months (Dunn, 2013; Zarzo, 2012).

Food pairings are also shown to influence refreshment with a majority of survey participants (n = 730, 69.5%). The top two alcoholic beverages when dining out are beer and wine (Zan & Fan, 2010). Due to the complexity of both beer and wine, the duo has the ability to be paired with multiple types of cuisine; however, the traditional beer pairing has been with calorie-rich foods (Pettigrew & Charters, 2006). In a study researching beer and pizza pairings, findings concluded that consumers prefer stouts with spicy pizza while lagers were preferred with non-spicy pizza (Harrington, Miszczak, & Ottenbacher, 2008). The balance of flavors grows in complexity when a beer is added to the meal experience. There is now a demand for optimal food and beer pairings across the world and it is a consumer's market (Martinez, Hammond, Harrington, & Wiersma-Mosley, 2016).

Consumer Study

Consumer Attitude Toward Formulated Beers and Endogenous Attributes

The citrus 2.5% abv. samples were rated the most preferred for overall beer flavor, tested flavor, alcohol, and carbonation level in this study. The acidity favorite was rated similarly amongst consumers between the citrus 2.5% abv. and the lime 2.5% abv. samples with a difference of only 0.01. The bitterness attribute was rated highest between citrus 2.5% abv., lime 0% abv., and lime 2.5% abv. samples with a difference of 0.02. The main consensus was that a low abv. improved all attributes acceptance (Vasiljevic, Couturier, & Marteau, 2018). The citrus and lime-flavored beers were rated greater than the cucumber-flavored beers for the majority of attributes. These two flavors could contribute to the encompassing acidity that might balance out the alcohol and bitterness attributes, positively impacting and synergizing with the carbonation and aiding in refreshment (Bettenhausen et al., 2020; Labbe et al., 2009a; McEwan & Colwill, 1996). Carbonation has been linked to refreshment perception in beverages (Green, 1992; Guinard et al., 1998; Peyrot des Gachons et al., 2016) and was crucial in this consumer study. Amongst all flavored beers, carbonation hedonic scores decreased when the abv. increased. In order for a beer to be considered refreshing, the abv. had to be low ~ 2.5% abv., citric in flavor, and able to withhold its palatable carbonation.

For the impact of intensities on hedonic scores, all attributes were rated just-about-right with the exception of the carbonation intensity. The carbonation intensity was shown to be too little. Carbonation was readily lost in beer once exposed to air due to the buoyancy of the bubbles reaching the surface where they either burst or float (Zenit & Rodríguez-Rodríguez, 2018). Consumers' expectations of beer were both high in carbonation and expected to have a bite (Wise, Wolf, Thom, & Bryant, 2013). Although the carbonation intensity was described as too little, all other attribute intensities including overall beer flavor, tested flavor, alcohol, acidity, and bitterness were rated JAR. As a result, the refreshing intensity was also described as JAR. This elucidated that even though the carbonation was very important for thirst-quenching, the overall product could still be deemed refreshing if carbonation was not present, but the flavor and alcohol levels were JAR (Chauhan et al., 2014; Morata et al., 2020).

To support this finding, having not enough carbonation was shown to have a significant effect on hedonic ratings according to the JAR mean drops while having too much carbonation does not. Other attributes such as overall beer flavor, alcohol, acidity, and bitterness levels were the opposite and showed significance when the intensity was too high and no significance when the intensity was too low. Too high of an attribute intensity was off-putting for consumers and can change the perception of other attributes (Rankin & Marks, 1991, 1992). However, for tested flavor, having either too much or too little intensity was shown to significantly affect consumer hedonic scores. These attributes influenced the overall beer flavor intensity and confirmed that the balance of flavor should be considered during future beer development (Aquilani et al., 2015; Guinard et al., 1998).

To further understand the relationship and patterns between the unique samples and their attributes, a PCA was performed. Six low-alcohol-level beers (0% and 2.5% abv.- all tested flavors) were shown to be liked by consumers the most along with their respective attributes. The results mimicked those of previous literature elucidating how refreshment can be influenced by a balance of sensory attributes with low to moderate alcohol levels (Bettenhausen et al., 2020; Labbe et al., 2009a; McEwan & Colwill, 1996). Oppositely, beers with alcohol levels at 7.5% had high intensities in alcohol, beer flavor, bitterness, and acidity. This matches the current market trends in which consumers in recent years, prefer lower-strength and non-alcoholic beverages with a balance of bitterness and acidity intensity (Higgins, Bakke, & Hayes, 2020; Vasiljevic et al., 2018). Three 5.0% abv. samples along with the tested flavor intensity were separated on the positive side of PC2 while the three beer base controls were separated on the negative side of PC2. The tested flavor was shown to be a highly independent factor shown by the clear isolation of the controls from the tested flavored samples. Consumers have shown in previous sensory studies that flavor is the most important attribute to determine product quality (Barrett et al., 2010). Consumers do not differ in this study and show through hedonic scores that flavor absolutely matters by the contrast of controls and high abv. beer samples away from the preferred lower-abv. samples that were correlated with attribute hedonic scores and desirable intensities.

The two main descriptors applied to each respective beer sample were clean (no bold flavors, light overall flavor intensity) and crisp (slight carbonation/bite) according to the results from the CATA question. Crisp and clean descriptors have precedence in previous literature describing their positive impact on refreshment in beer consumption (Green, 1992; Labbe et al., 2009a; Peyrot des Gachons et al., 2016). According to the CATA PCA biplot, refreshing related descriptors such as crisp and clean were associated with the lime-flavored samples. Lime flavor has previous associations with refreshment (Manjunatha, Raju, & Bawa, 2012; Spence, 2015). The citrus samples had one main descriptor which was just citrus itself, and the cucumber samples were correlated with green, cucumber, and astringent descriptors. Green and cucumber descriptors have previously been associated with refreshment as well (El-Saadony et al., 2020; Wyers, 2016). However, the cucumber-flavored beer samples did not have the acidity to balance the other attributes like the citrus and lime samples in this study and were thus perceived more astringent (Cohen et al., 2014).

Beer Refreshment and Impact Factors

Investigative impact factors of beer refreshment for this study were focused on alcohol and flavor. These factors showed to have the closest association with and the greatest predictors of refreshment, according to both PLS-R and Pearson correlation. When refreshing liking and intensity scores of the tested flavors: citrus, cucumber, and lime, were compared against the controls, all three flavors showed preference up to the 5.0% abv. level. This elucidates that the addition of citrus, lime, or cucumber is preferred over the foundational beer flavor when the alcohol level is low and balanced. Citrus and lime flavors have precedence in literature by their association with refreshment in beer and have shown to specifically make the flavor of non-alcoholic beer more balanced, bold, and enjoyable (Lafontaine et al., 2020; Zellner & Durlach, 2003). Cucumber has been linked to refreshment in beverages (An, 2020; Kausar, Saeed, Ahmad, & Salam, 2012; Mukherjee et al., 2013; Tatlioglu, 1993). However, flavor type shows only significance in refreshment liking and not intensity. To summarize the impact of flavor type on refreshment, flavor can heavily influence refreshment liking ratings while the intensity of refreshment may remain consistent.

The alcohol level was significant ($p < 0.001$) and influential on refreshing perception shown by the results of MANOVA (see Table 1). The lower alcohol level samples (0% and 2.5% abv.) were rated with the highest refreshing liking and intensity scores. These low abv. samples were the most refreshing to consumers confirming the results found throughout this study. Consumers prefer the taste of low alcohol level beverages (Vasiljevic et al., 2018). The taste of ethanol has been generally aversive by consumers in previous studies due to its bitterness and irritation when ingested and flavor dominance (Missbach et al., 2017; Thibodeau & Pickering, 2017). Low alcohol content relates to a more enjoyable tasting beer leading to a higher perceived refreshment by consumers.

Both refreshing liking and intensity ratings were significantly impacted by the flavor and alcohol conjointly, according to the results of MANOVA. The balance of alcohol and flavor for optimal refreshment has been imperative throughout this study as well as in previous literature (Aquilani et al., 2015; Chakraborty & Suresh, 2018; Guinard et al., 1998; Labbe et al., 2009a; Ninjouji, Takekawa, Hoshi, & Tomoko, 2005). For optimal refreshment to be achieved, the favor and alcohol levels must be equilibrized. One cannot overpower the other and a synergy must be met.

In general, flavor (e.g., acidity and a low overall flavor intensity) and alcohol are intrinsic sensory factors that have impacted beer refreshment in previous literature (Aquilani et al., 2015; Labbe et al., 2009a; McEwan, & Colwill, 1996). These respective attributes significantly impacted perceived refreshment throughout this consumer study as well, shown by multiple statistical analyses including PLS-R, Pearson Correlation, ANOVA, MANOVA, and JAR scales. These attributes stimulate the olfactory and gustatory systems which in turn influences perceived refreshment and overall beer acceptance (Baum-Baicker, 1985; Guinard et al., 1998a). For future beer flavor development, how flavor types and alcohol levels independently and collaboratively impact beer's perceived refreshment should be intently focused on.

Consumer Study Demographics

The consumers were divided into three clusters by AHC based on demographics and how samples and attributes were scored. Cluster 3 was primarily composed of college-aged males currently in school and drink beer multiple times per week. This principal cluster preferred the citrus-flavored beer profile with a lower alcohol content (~2.5% abv.). Cluster 1 was composed of slightly older males with a collegiate degree, employed full-time, and enjoyed beer 1-3 times per week. This cluster of consumers has just graduated from college and is now entering the working force and drinking a bit less often than Cluster 3. Cluster 1 prefers the lime-flavored beer samples with a medium alcohol content (~ 2.5 - 5.0% abv.). Similarities in Cluster 1 and 3 demographics can be considered the average craft-beer consumer found throughout the literature (Lerro et al., 2020; Malone & Lusk, 2018). Both clusters preferred the citrus and lime-flavored beer samples, which have been linked to refreshment in previous literature (Lafontaine et al., 2020; Zellner & Durlach, 2003). Beverages containing citric acid are known to trigger increased saliva excretion to wet the mouth and make a beverage be perceived as more hydrating, refreshing, and thirst-quenching (Bozorgi et al., 2020; Froehlich et al., 1987; McEwan & Colwill, 1996; Murugesh, et al., 2015).

The smallest cluster, Cluster 2, describes the minority population of beer drinkers that were educated, well-established, full-time working females, who were slightly older in age than the other two larger clusters. This cluster preferred the cucumber-flavored beer samples with low alcohol content. Previous studies (Gemousakakis et al., 2011; Higgins et al., 2020) have recognized the difference in the bitterness receptor between men and women that could contribute to why the cucumber-flavored beer samples were preferred amongst the women and the citrus-flavored samples amongst the men.

The current U.S. consumer segment market for beer consumption is nearly equally split between men and women with the younger generation consuming more craft beer and the older generation choosing more commercial beers (Jaeger, Spinelli, Ares, & Monteleone, 2020; Malone & Lusk, 2018). Beer consumers on average are educated, both male and female, and enjoy 1-3 beers per week which coincides with the demographics of this consumer study as well (Malone & Lusk, 2018). Consumer study participants scored the lower abv. beer samples the highest, which is on-trend for the current beer market, as well as rated the citrus and lime-flavored samples greater than the control. Both citrus and lime-flavored beers are shown to be popular amongst consumers in the market and this study shows their preference over the taste of an average beer. Overall, the demographics from this consumer study represents the average beer consumer found throughout the U.S. marketplace.

Volatile Analysis

Beer Volatile Overview

In general, the majority of volatiles derivatized naturally from beer fermentation are esters, alcohols, acids, aldehydes, ketones, and sulfurs (Olaniran et al., 2017; Stewart, 2017). Previous studies also show other volatile identifications including terpenes and terpenoids as well as Maillard reaction products (i.e., furans, pyrans, and pyrroles) and phenols (Gu et al., 2015). Each one of these functional groups was confirmed in all three pilsners. These compounds are the result of barley and hop fermentation, yeast metabolism by-products, potentially contaminated microorganisms, and the result of flavor stability/degradation balance during storage (Kobayashi et al., 2007; Olaniran et al., 2017).

Michelob Ultra had the lowest °Brix (2.4%) and IBU (10 IBU units), the highest pH of 7.3, the lowest abv. of 4.2%, and the lightest in both aroma and flavor intensity amongst the three pilsners. This pilsner had the lowest number of terpenes (0.05%), aldehydes (0.08%), furans (0.03%), and sulfurs (0.02%) and the highest acids (3.81%), phenols (0.14%), and ketones (0.03%). Carlsberg was composed of a °Brix of 4.8% with 22 IBU units, 6.3 pH, and an abv. of 5%. The Carlsberg pilsner was the strongest in aroma and flavor intensity composing mostly of hops, herbal, pine and grass, yeast, spice, and slight sweetness with the lowest PA% of esters (16.85%), phenols (0.07%), and ketones (0.01%) and highest alcohols (13.24%) and terpenes (0.31%). Heineken 0.0 had the highest °Brix of 5.4%, the highest IBU of 23 IBU units, a pH of 6.4, and the lowest alcohol content of < 0.05% abv. The Heineken 0.0 pilsner was medium in aroma and flavor intensity with a mild skunky, grain, hop, and sweet composition with the lowest PA% of alcohols (10.07%), acids (1.52%), and ketones (0.01%) with the highest peak area percentage for esters (20.67%), furans (0.14%), and sulfurs (0.09%).

Several volatiles identified in all three pilsners contribute to the pilsner's sensory experience and were consistent with literature including ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, 2-phenyl ethyl acetate, however, isobutyl acetate, ethyl decanoate, were found in both Michelob Ultra and Carlsberg but not Heineken 0.0. These esters are important contributions to the classic aroma and flavor of beer (Gonzalez-Viejo et al., 2019; Olaniran et al., 2017; Pires, Teixeira, Branyik, & Vincente, 2014; Saerens, Delvaux, Verstrepen, & Thevelein, 2010; Verstrepen et al., 2003).

Esters

Although Michelob Ultra had a higher percentage of esters to overall volatiles, Heineken. 0.0. had the greatest ester PA% (20.67%) out of all three pilsners. Michelob

Ultra had a 17.57% PA% and Carlsberg a 16.85% PA%. The explanation would have to derive from the unique yeast used exclusively by Heineken (Heineken A- yeast; Heineken, 2020). The Heineken 0.0 is brewed and made the same way as classic Heineken; however, alcohol is removed from the beer by vacuum distillation and is double brewed for aroma, flavor, and mouthfeel. Natural flavorings are also added to the final produced due to some flavor being lost due to alcohol removal (Missbach et al, 2017).

Alcohols

Several alcohols were common amongst the three beers and citied in previous literature including 2-methyl propanol, 3-methyl butanol, 1-hexanol, 2-3 butanediol, phenyl ethyl alcohol, 2,3-butanediol; however, 2-methyl propanol were found in both Michelob Ultra and Carlsberg but not Heineken 0.0 (Gonzalez-Viejo et al., 2019; Olaniran et al., 2017). The Carlsberg pilsner had the highest PA% for alcohols (due to the higher alcohol content) and Heineken 0.0 the lowest. From a sensory perspective, all three pilsners exhibited similar intensities of alcohol taste and aroma.

Terpenes

Terpenes in common amongst the beers and previously cited in literature include D-limonene and linalool. Carlsberg was composed of a greater number of terpenes (0.31% PA%) than the other beers and includes β-myrcene, terpinen-4-ol, nerol, and humulol exclusively. Geranyl acetate and citronellol were terpenes shared between Michelob Ultra and Carlsberg and a-terpineol is a terpene Heineken 0.0 and Carlsberg shared (Holt et al., 2019; Olaniran et al., 2017). The terpene category differentiated the Carlsberg pilsner from the other two beers due to the higher intensity of pine, grass, and hop aroma. Heineken 0.0 had a medium aroma intensity of yeast and grain and was composed of 0.08% PA%. The Michelob Ultra was very light in both aroma and flavor intensity with the lowest PA% for terpenes at 0.05%.

Aldehydes

In previous literature, key aldehydes for beer aroma and flavor that all beers encompassed include acetaldehyde, isovaleric aldehyde, hexanal, nonanal, and decanal (Cramer, Mattinson, Fellman, & Baik, 2005). Heineken 0.0 had the highest PA% of aldehydes (0.59%) and exclusively had the benzaldehyde (almond aroma) volatile; however, it was lacking 2-nonenal which the other two pilsners had. This was a beneficial circumstance in this case since this volatile is linked to beer stalling (Bamforth & Lentini, 2020). Carlsberg was composed of 0.40% PA% and Michelob Ultra the lowest (0.08%). These fresh and green notes were noticeably missing in Michelob Ultra and worked in favor of both the Carlsberg and Heineken 0.0 pilsners.

Acids

Acetic acid, hexanoic acid, octanoic acid, and decanoic acid were all found in the three pilsners and in literature for being contributing aroma and flavor volatiles for beer and naturally produced by yeast during beer maturation (Liu & Quek, 2016). These acids give the beer the slight desirable sourness aroma and flavor that is needed to balance the sweetness from the malted grain and the bitterness from the hops (Holt et al., 2019).

Michelob Ultra had the highest PA% for acids (3.81%) and Heineken 0.0 the lowest (1.52%).

Phenols

Phenols contribute astringency and bitter flavor to the beer. Concentrations of phenols depend on beer variety, the ingredients used, and the environmental conditions during cultivation (Humia et al., 2019). The most notoriable phenol amongst the beer samples and in literature was 4-vinyl-guaiacol (Vanbeneden, Saison, Delvaux, F. & Delvaux, F. R., 2008). 4-vinyl-guaiacol is an aromatic phenol that has a strong flavorful and aroma influence on beer of smoky, spicy, nutty, woody at low concentrations and medicinal and unpleasant at high concentrations. Carlsberg had the highest 4-vinyl-guaiacol phenol PA% (0.021%) and Heineken 0.0 and Michelob Ultra (0.006%) the lowest (0.017%). The Carlsberg sensory profile echoed the results from SPME-GC-MS having characteristics of grain and spice.

Ketones

Ketones were a very small category for the pilsners with Michelob Ultra having the highest PA% (0.03%) and Heineken and Carlsberg the lowest, both obtaining 0.01% PA% of ketones. The one ketone volatile all three pilsners shared was tetramethyl acetyloctahydronaphthalenes (also known as amberonne or iso E super gamma). This volatile has an amber and woody aroma (Stepanyuk & Kirschning, 2019). The amber and woody volatiles were very limited in the noticeable aroma for all three pilsners.

Sulfurs

Naturally occurring sulfurs from yeast metabolism, along with Maillard reaction products including furans amongst the three beers consist of furfural, 2-3 furanone, 2-3 benzofuran, 3-(Methylthio)propyl acetate, and 3-(methylthio)-propanol. (Bamforth, 2003; Humia et al., 2019) Studies at the Carlsberg research center have shown that a genetically modified yeast strain is used to produce the relatively large amount of sulfur dioxide the Carlsberg pilsner encompasses (Hansen & Kielland-Brandt, 1996; Stewart, 2017). This is confirmed in our study as well shown by the Carlsberg pilsner had the greatest amount of sulfur volatiles and PA% (0.06%).

Refreshing Aromas and Flavors

Fresh and refreshing aromas have been described as citrus, specifically orange and lemon, peach, green, watery, and aldehydic (Zarzo, 2012). These aromas are all very high in terpenes (d-limonene, o-cymene, p-cymene, myrcene, linalool, citronellol, and valencene), esters (ethyl butyrate, ethyl isobutyrate, ethyl isovalerate, ethyl acetate, ethyl hexanoate, ethyl octanoate, 2-phenyl ethyl acetate, and propyl butyrate) and aldehydes (decanal and hexanal; Fenko, Schifferstein, Huang, & Hekkert, 2009; Labbe et al., 2009b; Martin, Gartenmann, & Cartier, 2005; Shui et al., 2019; Zellner & Durlach, 2003). Other aroma and flavors that have been associated with refreshment include peppermint and those related to foods with high water content such as melons (watermelon, honeydew, cantaloupe), cucumbers, fresh berries, grapefruit, and kiwi (Allwood et al., 2014; Brookie, Best, & Conner, 2018; Maoto, Beswa, & Jideani, 2019).

Esters, terpenes, aldehydes are the functional groups that are the most influential on refreshing perception derived from beer consumption. A potential reason contributing to beer refreshment is that these pilsners are composed primarily of esters along with being 90-95% water (Thesseling, Bircham, Mertens, Voordeckers, & Verstrepen, 2019). Terpenes and aldehydes are included in the pilsners; however, at a lower PA% to fit within an appealing threshold. The balance between citrus, fruity, hoppy, green, and lightness is what an ideal refreshing beer has perfected.

Carlsberg encompassed the largest composition of terpenes cited as refreshing while Heineken 0.0 had the least. Both Carlsberg and Michelob Ultra had more refreshing esters than Heineken 0.0. However, all three pilsners were compromised of the same amount of refreshing aldehydes, both hexanal and decanal. Comparatively both Carlsberg and Michelob Ultra are to be considered more refreshing than Heineken 0.0 due to embracing more refreshing volatiles. However, refreshment is a multi-dimensional concept that is based on consumer opinion. A consumer who prefers more vegetal, yeast, and grain aroma-forward beer would find the Carlsberg pilsner the most refreshing while a consumer that prefers light and sweet aroma-forward beer would find Michelob Ultra the most refreshing.

To conclude, there was a relation between beer volatiles with acceptance and refreshment. In order for a beer to be accepted and considered refreshing, there must be the presence of significant esters (ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, 2-phenyl ethyl acetate, isobutyl acetate, ethyl decanoate), alcohols (2-methyl propanol, 3-methyl butanol, 1-hexanol, 2-3 butanediol, phenyl ethyl alcohol, 2,3-

butanediol), terpenes (D-limonene, linalool), aldehydes (acetaldehyde, isovaleric aldehyde, hexanal, nonanal, decanal), acids (acetic acid, hexanoic acid, octanoic acid, decanoic acid), phenol (4-vinyl-guaiacol), ketone (tetramethyl acetyloctahydronaphthalenes), and sulfurs (furfural, 2-3 furanone, 2-3 benzofuran, 3-(methylthio)propyl acetate, and 3-(methylthio)propanol).

CHAPTER VI

CONCLUSION

The preliminary survey (*n* = 1,050) affirmed refreshment derived from beer consumption was high (≥ 8 out of 10 on a hedonic scale) according to a majority of participants (56%). Participants stated they consumed beer due to flavor enjoyment, the feeling of refreshment, and alcohol effects on the body. Perceived refreshment was linked to a lighter mood and quenched thirst, while the two most impactful factors of perceived refreshment were flavor and temperature. Refreshing beer flavors were described as lime, lemon, and orange, while refreshing beer flavor profiles were deemed crisp/clean and fruity. Lighter varieties such as hefeweizen, blonde ales, and American lagers were the frequently chosen refreshing beer varieties.

Based on the survey results, a consumer study of 322 participants was conducted to investigate how model beers with three different flavors and five various alcohol levels impacted sensory attributes as well as refreshment. Perceived refreshment was significantly correlated with the hedonic and intensity scores of overall beer flavor, tested flavor, alcohol, carbonation, acidity, and bitterness according to Pearson correlation analysis. For all flavors, the 2.5% abv. samples were rated the highest amongst perceived refreshment due to the mild intensity of the alcohol taste, while the most preferred flavor was citrus, followed by cucumber and lime. The factor of alcohol levels demonstrated significant differences amongst all sensory attributes. However, the flavor type had significant impact on all sensory attributes except bitterness liking. Beer alcohol levels and flavor types significantly impacted perceived refreshment post-beer consumption and should be investigated thoroughly in future research.

There is a relation between volatiles and refreshment, and this remains true in beer as well. For a beer to be deemed acceptable and considered refreshing, there must be the presence of significant esters (ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, 2-phenyl ethyl acetate, isobutyl acetate, ethyl decanoate), alcohols (2-methyl propanol, 3-methyl butanol, 1-hexanol, 2-3 butanediol, phenyl ethyl alcohol, 2,3-butanediol), terpenes (D-limonene, linalool), aldehydes (acetaldehyde, isovaleric aldehyde, hexanal, nonanal, decanal), acids (acetic acid, hexanoic acid, octanoic acid, decanoic acid), phenol (4-vinyl-guaiacol), ketone (tetramethyl acetyloctahydronaphthalenes), and sulfurs (furfural, 2-3 furanone, 2-3 benzofuran, 3-(methylthio)propyl acetate, and 3-(methylthio)propanol). Michelob Ultra, Heineken 0.0, and Carlsberg were chosen for volatile analysis based on data from the survey where they were identified as commonly consumed beers.

In conclusion, beer flavors (lime, citrus, and cucumber) and alcohol contents (from 0%-7.5% abv.) significantly impacted the refreshing perception of beer. Factors such as flavor and alcohol levels should be overstated in the imperative justification to get the level JAR for consumers during the design of a new or existing beer. The chemical composition of pilsners and their functional groups should include specific volatiles to be acceptable amongst consumers. This study provided important contributions to the alcoholic beverage industries in the development of new, refreshing beers with a focus on flavor and ideal alcohol level.

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APPENDIX A.

IRB Approval Letter

Texas Woman's University

Institutional Review Board (IRB)

irb@twu.edu

https://www.twu.edu/institutional-review-board-irb/

July 18, 2019

Amy Hampton
Nutrition and Food Sciences

Re: Exempt - IRB-FY2019-346 Refreshment Related to Beer Flavor

Dear Amy Hampton,

The above referenced study has been reviewed by the TWU IRB - Denton operating under FWA00000178 and was determined to be exempt on July 18, 2019.

Note that any modifications to this study must be submitted for IRB review prior to their implementation, including the submission of any agency approval letters, changes in research personnel, and any changes in study procedures or instruments. Additionally, the IRB must be notified immediately of any adverse events or unanticipated problems. All modification requests, incident reports, and requests to close the file must be submitted through Cayuse.

On December 7, 2020, this approval will expire and the study must be renewed or closed. A reminder will be sent 45 days prior to this date.

If you have any questions or need additional information, please contact the IRB analyst indicated on your application in Cayuse or refer to the IRB website at http://www.twu.edu/institutional-review-board-irb/.

Sincerely,

TWU IRB - Denton

APPENDIX B.

Beer Survey Recruitment Scripts

Please help the TWU *NFS Food Science and Flavor Chemistry* program investigate if BEER FLAVOR has an impact on your perception of *refreshment*.

We all know that *carbonation* and *cold temperature* plays a part in feeling refreshed but does FLAVOR too?

ALL beer consumers AGED 21+ are invited to take an online survey for a study about BEER REFRESHMENT.

In order to participate you must

-BE 21+

-Must consume beer RESPONSIBLY

-Must consume beer RESPONSIBLY

-Consume beer 1-2 times per week or more

-Do not have to be a TWU student or staff so feel free to share this survey!

By taking this survey,
you will have the option to be entered to WIN 1 OF 10 AMAZON
GIFT CARDS! ($10 each)

Purpose

The TWU NFS Food Science and Flavor Chemistry program is investigating if beer flavor has an impact on perceived beer refreshment. Other than carbonation, cold temperature, and alcohol effects on the body, does *flavor* itself make a beer more or less refreshing? The purpose of this study is to research if there's a relationship between *beer flavor* and *feeling refreshed.*

Procedure

This survey is for **all beer consumers 21 years old or older**. Please help us in gathering data on beer refreshment so we can all benefit! Simply, take this short online survey using the link provided below. The survey should take around 10 to 15 minutes to complete.

Survey Link

Compensation

Upon completion of this survey, you will have the option to be entered to win 1 of 10 Amazon gift cards ($10 each).

Contact Information

Please contact myself (ahampton4@twu.edu) or my faculty advisor Dr. Du (xdu@twu.edu) if you have any questions about this survey.

Thank you for your time and assistance.

There is a potential risk of loss of confidentiality in all email, downloading, and internet transactions.

Amy Hampton
M.S. student
Texas Woman's University
Food Science and Flavor Chemistry

APPENDIX C.

Preliminary Beer Survey Form

General Questions	Answer Options
Reasons why consumers drink beer:	
1. Which of these factors do you consider when you choose a beer to drink? Check all boxes that may apply.	❑ Flavor ❑ Price ❑ Brand ❑ Coming from a local brewery ❑ Alcohol percentage ❑ Beer variety ❑ Other __________
2. Why do you drink beer? Check all boxes that may apply.	❑ Alcohol effects on the body ❑ Taste of the beer ❑ Feeling refreshed after consumption ❑ Prefer beer over liquor ❑ Social pressure ❑ Other __________
Consumers opinions on beer refreshment:	
3. How would you rate the *refreshment of beer* on a scale from 1-10 (1 is a very low refreshing perception and 10 is a very refreshing perception)?	1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8 ❑ 9 ❑ 10❑
4. When perceiving refreshment from beer consumption, do you feel… Check all boxes that may apply.	❑ Quenched thirst ❑ Re-energized ❑ Lightened mood ❑ Invigorated ❑ Other __________
Internal factors associated with beer refreshment:	
5. Which beer factors impact your perception of beer refreshment? Check all boxes that may apply	❑Temperature (Cold/Chilled) ❑Carbonation ❑Flavor ❑Alcohol effects on the body ❑ Variety of beer ❑ Matrix the beer is presented in (glass, plastic, can, etc.) ❑ Other __________
6. What type(s) of beer FLAVOR do you find	❑ Lemon ❑ Lime ❑ Orange

refreshing? Check all boxes that may apply.	❑ Grapefruit ❑ Honey ❑ Strawberry ❑ Raspberry ❑ Blueberry ❑ Blackberry ❑ Bitter ❑ Hoppy ❑ Amber ❑ Pumpkin/cloves/cinnamon ❑ Chocolate ❑ Other ___________
7. What type(s) of FLAVOR PROFILES do you find refreshing? Check all boxes that may apply.	❑ Crisp/Clean ❑ Hoppy/Bitter ❑ Fruity ❑ Malty/Sweet ❑ Sour/Tart ❑ Spice ❑ Deep/Chocolate/Coffee ❑ I am not sure ❑ Other ___________
8. What type(s) of beer do you find the most *refreshing?* Check all boxes that may apply.	❑American Lager (ex. Budweiser, Coors, Bud Light, Miller/Miller Lite) ❑German Pilsner (ex. Carlsberg) ❑Blonde Ale (ex. Deep Ellum Dallas Blonde ale, Alaskan Blonde Ale) ❑American Pale Wheat (ex. Revolver Blood and Honey, Samuel Adams Summer Ale) ❑American IPA (ex. Dogfish Head 60 Minute IPA, Lagunitas IPA) ❑American Pale Ale (ex. Sierra Nevada Pale Ale, Oskar Blues Dale's Pale Ale) ❑Vienna Lager (ex. Dos Equis Amber Lager, Abita Amber Lager) ❑None of the above ❑ Other ___________
9. What type(s) of beer do you find the most *refreshing?* Check all boxes that may apply.	❑ Hefeweizen (ex. Blue Moon Belgian White, Paulaner Hefeweizen) ❑ Belgian Witbier (ex. Hoegaarden, Shock Top Belgian White, Allagash White)

	❑ Belgian Tripel (ex. New Belgium tripel, Real Ale Devil's Backbone tripel) ❑ Amber American Lager (ex. Shiner Bock, Sam Adams Boston Lager) ❑ Oktoberfest (ex. Paulaner Oktoberfest-Märzen, Spaten Oktoberfest) ❑ English Brown Ale (ex. Newcastle Brown Ale) ❑ Stouts (ex. Oatmeal stouts, Milk stouts, Irish stouts-Guinness) ❑ None of the above ❑ Other ___________
External factors related to refreshment:	
10. Do different seasons make a difference in which beer you find refreshing?	❑ Yes, WINTER beers are the most refreshing (bocks, stouts, porters) ❑ Yes, SPRING beers are the most refreshing (pilsners, lambics, sours, wheat beers like Kolsch, Hefeweizen, and Belgian Witbier) ❑ Yes, SUMMER beers are the most refreshing (citrus-forward beers, fruit-forward beers) ❑ Yes, FALL beers are the most refreshing (IPA's, pale ales, amber lagers, spice-forward beers) ❑ No, I find the same beers refreshing all year round
11. Does pairing food with beer have an impact on beer refreshment?	❑ Yes ❑ No
Demographics	
1. What gender do you identify with?	❑ Male ❑ Female ❑ Prefer not to say ❑ Other
2. What age range are you?	❑ 21-25 ❑ 26-35 ❑ 36-50 ❑ 50-65 ❑ 65+
3. How often do you consume beer?	❑ 5 or more times per week ❑ 1-3 times per week ❑ Once every 2 weeks ❑ Once a month ❑ A few times a year

	❑ Never
4. What is your highest level of education?	❑ Less than a high school diploma ❑ High school degree or equivalent ❑ Some college ❑ Associates Degree ❑ Bachelor's Degree ❑ Master's Degree ❑ Doctorate ❑ Other ___________
5. What is your employment status?	❑ Unemployed ❑ Part-time (less than 40 hours per week) ❑ Full-time (40 hours per week O) ❑ Self-employed ❑ Student ❑ Military ❑ Retired ❑ Other ___________

APPENDIX D

Consumer Study Ballot

Please Rate Sample _________

1. **How much do you LIKE the refreshing taste of this beer?**

   □Dislike extremely

   □Dislike very much

   □Dislike moderately

   □Dislike slightly

   □Neither like nor dislike

   □Like slightly

   □Like moderately

   □Like very much

   □Like extremely

2. **How REFRESHING do you find this beer?**

   □Not refreshing at all

   □Not very refreshing

   □Moderately not refreshing

   □Slightly not refreshing

   □Neutral

   □Slightly refreshing

   □Moderately refreshing

   □Very refreshing

   □Extremely refreshing

3. **How much do you LIKE the overall BEER FLAVOR?**

   □Dislike extremely

   □Dislike very much

   □Dislike moderately

   □Dislike slightly

- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

4. **How INTENSE is the overall BEER FLAVOR?**
   - ❑ No flavor at all
   - ❑ Not enough flavor
   - ❑ Slightly not enough flavor
   - ❑ Just about right
   - ❑ Slightly too much flavor
   - ❑ Too much flavor
   - ❑ Way too much flavor
5. **What are the major flavor CHARACTERISTICS you perceive for the beer? (Click all answers applied to you):**
   - ❑ Clean
   - ❑ Green
   - ❑ Lemon
   - ❑ Lime
   - ❑ Cucumber
   - ❑ Citrus (orange)
   - ❑ Astringent
   - ❑ Candy
   - ❑ Crisp
   - ❑ Other ________________

8. **How much do you like the ALCOHOL taste of this beer? Do you think the consumer can understand Alcohol taste? Should we use another word?**

- ❑ Dislike extremely
- ❑ Dislike very much
- ❑ Dislike moderately
- ❑ Dislike slightly
- ❑ Neither like nor dislike
- ❑ Like slightly
- ❑ Like moderately
- ❑ Like very much
- ❑ Like extremely

9. How intense is the ALCOHOL FLAVOR?

- ❑ No alcohol flavor at all
- ❑ Not enough flavor
- ❑ Slightly not enough flavor
- ❑ Just about right
- ❑ Slightly too much flavor
- ❑ Too much flavor
- ❑ Way too much flavor

10. How much do you like the CARBONATION of this beer?

- ❑ Dislike extremely
- ❑ Dislike very much
- ❑ Dislike moderately
- ❑ Dislike slightly
- ❑ Neither like nor dislike
- ❑ Like slightly
- ❑ Like moderately
- ❑ Like very much
- ❑ Like extremely

11. How intense is the CARBONATION?

- ❑ No carbonation at all
- ❑ Not enough carbonation
- ❑ Slightly not enough carbonation
- ❑ Just about right
- ❑ Slightly too much carbonation
- ❑ Too much carbonation
- ❑ Way too much carbonation

12. How much do you like the ACIDITY of this beer?

- ❑ Dislike extremely
- ❑ Dislike very much
- ❑ Dislike moderately
- ❑ Dislike slightly
- ❑ Neither like nor dislike
- ❑ Like slightly
- ❑ Like moderately
- ❑ Like very much
- ❑ Like extremely

13. How intense is the ACIDITY?

- ❑ No acidity at all
- ❑ Not enough acidity
- ❑ Slightly not enough acidity
- ❑ Just about right
- ❑ Slightly too much acidity
- ❑ Too much acidity
- ❑ Way too much acidity

14. How much do you like the BITTERNESS of this beer?

- ❑ Dislike extremely
- ❑ Dislike very much

- ❑ Dislike moderately
- ❑ Dislike slightly
- ❑ Neither like nor dislike
- ❑ Like slightly
- ❑ Like moderately
- ❑ Like very much
- ❑ Like extremely

15. How intense is the BITTERNESS?

- ❑ No bitterness at all
- ❑ Not enough bitterness
- ❑ Slightly not enough bitterness
- ❑ Just about right
- ❑ Slightly too much bitterness
- ❑ Too much bitterness
- ❑ Way too much bitterness

------Repeats for all 5 samples with time breaks (15 seconds) included for palate cleansers------

Demographics

What gender do you identify with?

- ❑ Female
- ❑ Male
- ❑ Prefer not to say
- ❑ Gender fluid

What age range are you?

- ❑ 21-25
- ❑ 26-35

- ❑ 36-50
- ❑ 50-65
- ❑ 65+

How often do you consume beer?

- ❑ 5 or more times per week
- ❑ 1-3 times per week
- ❑ Once every 2 weeks
- ❑ A few times a year
- ❑ Never

What is your highest level of education?

- ❑ Less than high school
- ❑ High school degree
- ❑ Trade school degree
- ❑ Associate degree
- ❑ Bachelor's degree
- ❑ Master's degree
- ❑ Doctorate degree

What is your employment status?

- ❑ Unemployed
- ❑ Part-time (less than 40 hours per week)
- ❑ Full-time (40 or more hours per week)
- ❑ Self-employed
- ❑ Student
- ❑ Student and employed
- ❑ Military
- ❑ Retired