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The Application of Mathematical Optimization and Flavor-Detection Technologies for Modeling Aroma of Hops

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The Application of Mathematical Optimization and Flavor-Detection Technologies for Modeling Aroma of Hops

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The Application of Mathematical Optimization and Flavor-Detection Technologies for Modeling Aroma of Hops

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In recent years, proprietary hops (Citra, Simcoe, and Mosaic) become the most sought-after hops among brewers due to their excellent aroma. However, they are restricted to the owners unless other growers purchase the costly licensing agreements. Many public hops are available to the growers without any additional costs, but their aroma is difficult to match to the proprietary hops. Although proprietary and public hop varieties are unique in their aroma profiles, all hops varieties contain similar volatile compounds, merely differs in the quantity of different individual compounds. The main objective of this thesis was to investigate the feasibility of matching the aroma of proprietary hops by blending a number of public hops. The aroma profiles of hops were detected by flavor-detection methods including Gas chromatogram-flame ionization detector (GC-FID), Gas chromatography–mass spectrometry (GC-MS), Headspace solid - phase microextraction/gas chromatogram–mass spectrometry (HS-SPME/GC-MS), and sensory evaluation. The modeling was achieved by applying mathematical optimization technique – quadratic programming. For matching proprietary hop aroma, public hop pellets or hop oils were mixed with different percentages in models (e.g., proprietary hop A= x% public hop B + y% public hop C + z% public hop D). The aroma of Citra pellets was closely mimicked by 25.2 % Eureka, 33.2 %

Centennial, and 36.7 % Triple Pearl. The aroma of Simcoe pellets was closely mimicked by 10.0 % Cascade, 50.0 % Us Goldings, 71.1 % Centennial, and 10.4 % Triple Pearl. The aroma of Mosaic pellets was closely mimicked by 6.5 % Eureka, 84.8 % Centennial, and 3.0 % Triple Pearl. In addition, it nearly mimicked the Citra oil with 35.2% Brewers Gold, 5.2 % Cashmere, 32 % Centennial, and 35.7 % Triple Pearl. The obtained aroma of models was validated in the beers with both the high similarity of aroma profiles ($R^2 > 0.90$) and sensory evaluation. This research provided a novel idea on the application of mathematical optimization and flavor-detection technologies for modeling aroma of hops. The success of this project can increase the usage of public hops and extend such an application to other flavor developments.

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Organization

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Chapter 1 Introduction and Thesis Objectives

1.1 Introduction

The chemical compounds extracted from hops strongly impart the aroma of beers. Different hop varieties exhibit distinctive aroma profiles and even the regional factor has the potential contribution on the specific aroma of the same hop variety (Su, & Yin, 2021). Therefore, the chemical composition of hops is an important factor for determining the quality and unique aroma of different hop varieties. Proprietary hops have excellent aroma, which drives them become the most attractive hop varieties. However, they are costly and not available to the farmers without purchasing the license (Worthington, 2010). Public hops are more cost-efficient and easily obtained, but their aroma is less attractive. Since the aroma of proprietary hops cannot be mimicked by public hops individually, it is possible to obtain the unique aroma of proprietary hops by blending a number of different public hops.

Recently, Campden BRI, a nationally recognized food and drink research advisory, set out to assess and blend hops to match the sensory characters of target hops. Campden BRI successfully validated the feasibility of blending hops (Smart, 2016). However, modeling the sensory profiles was subjective and time-consuming as indicated in that study. To date, it is well-known that instrumental analyses, such as gas chromatography (GC) and the combination of headspace solid - phase microextraction and gas chromatography (HS-SPME-GC), are commonly used to identify and distinguish the distinctive aroma of hops (Lafontaine et al., 2019; Brendel, Hofmann, & Granvogl, 2019; Dennenlöhr, Thörner,

Manowski, & Rettberg, 2020). However, how to model the aroma of target hops with these instrumental methods accurately, timely, and objectively hasn't been fully studied.

With the increasing demands of novel food flavor by consumers, artificial intelligence (AI) is attracting more and more attention for flavor development. AI is a powerful tool in optimization, automation, and reduction of human bias in the flavor field. AI technique is able to 'learn' operational human experience from restricted observations, and 'conclude' the interpretable rules to achieve the expected outcomes. AI is important especially in building the bridge between the aroma profiles obtained from instruments and sensory evaluation. Therefore, a mathematical optimization technique - quadratic programming (QP) (Caron, 2021), part of artificial intelligence (AI), offers the idea for modeling (Persson, Fagt, & Nauta, 2019; Xin, Negenborn, & Lin, 2018). The application of QP may help to achieve the objective of modeling the aroma (chemical composition) profiles of proprietary hops with public hops by statistical algorithms, instead of manual processes. The generated models will be further validated by sensory evaluation.

This thesis reports the application of mathematical optimization technique and flavor-detection methods for developing a blended model to match aroma of target product (proprietary hops) using public hops. The blended model for each specific proprietary hop was validated by instrumental analysis and sensory evaluation in both hops and hoppy beers. Also, modeling the full-profile compounds detected by GC of proprietary hops was compared with modeling volatile-compounds detected by HS-SPME-GC. Moreover, when modeling the full-profile aroma, the necessity of focusing on key aroma compounds (reported highly-impact aroma compounds) was investigated in order to obtain a more

successful blended model. Furthermore, the correlation between aroma profile similarity and sensory similarity of different hops was explored.

The success of this project could increase the usage and values of public hops. The study also could be served as an example for matching the aroma of food products or predicting the aroma similarity of blended food models using AI.

1.2 Hypothesis

Since all hop varieties contain similar aroma compounds, merely differ in the quantity of individual compounds. It is possible to blend a number of public hops to match the aroma of proprietary hops by simulating their chemical compositions.

1.3 Thesis objectives

The main objective was to model the aroma of proprietary hops by modeling the profile of volatile compounds (essential oils) in the hops. The specific objectives were to:

1) To model the aroma of proprietary hop (Citra) based on hop oil extracted from fresh hop cones (13 public hops).
2) To model the aroma of proprietary hops (Citra, Simcoe, and Mosaic) based on hop pellets, the most used way of hops by brewers (19 public hops).

1.4 References

Brendel, S., Hofmann, T., & Granvogl, M. (2019). Characterization of key aroma compounds in pellets of different hop varieties (Humulus lupulus L.) by means of the sensomics approach. *Journal of agricultural and food chemistry*, *67*(43), 12044-12053.

Smart, C. (2016). *Blending hops to match flavor*. [online] Available at: https://www.campdenbri.co.uk/news/blending-hops.php. Accessed 7 September 2021.

Caron, S. (n.d.). *Quadratic Programming in Python* [online] Available at: https://scaron.info/blog/quadratic-programming-in-python.html. Accessed 18 January 2021.

Dennenlöhr, J., Thörner, S., Manowski, A., & Rettberg, N. (2020). Analysis of Selected Hop Aroma Compounds in Commercial Lager and Craft Beers Using HS-SPME-GC-MS/MS. *Journal of the American Society of Brewing Chemists*, *78*(1), 16-31.

Lafontaine, S., Varnum, S., Roland, A., Delpech, S., Dagan, L., Vollmer, D., ... & Shellhammer, T. (2019). Impact of harvest maturity on the aroma characteristics and chemistry of Cascade hops used for dry-hopping. *Food chemistry*, *278*, 228-239.

Persson, M., Fagt, S., & Nauta, M. J. (2019). Optimising healthy and safe fish intake recommendations: a trade-off between personal preference and cost. *British Journal of Nutrition*, *122*(2), 206-219.

Su, X., & Yin, Y. (2021). Aroma characterization of regional Cascade and Chinook hops (Humulus lupulus L.). *Food Chemistry*, *364*, 130410.

Worthington, R. (2010). *Why Proprietary Hops?*. [online] Available at: http://inhoppursuit.blogspot.com/2010/01/why-proprietary-hops.html. Accessed 12 May 2020.

Xin, J., Negenborn, R. R., & Lin, X. (2018). Piecewise affine approximations for quality modeling and control of perishable foods. *Optimal Control Applications and Methods*, *39*(2), 860-872.

Chapter 2 Literature Review

2.1 Hops

Hops, the minor ingredient, strongly impart the flavor and quality of beer. The complex chemical composition from different hop varieties contribute to the unique aroma, which play a crucial role during beer brewing. Proprietary hops are the most desired and popular hops due to their outstanding aroma. In the US, approximately 30% of the hops are proprietary hops, exclusively for private use. Amarillo, Ahtanum, Simcoe, Citra, and Mosaic are five common proprietary hop varieties. Amarillo is among the top ten hops grown in the US, according to the USDA estimation (Barnes, 2018). It is distinguished by its citrus aroma, extending to grapefruit, orange, lemon, peach, melon, and apricot. Ahtanum is the aroma and bitterness hops and is well-known for its mixing aromas, including citrusy and floral aromas, earthy, and piney notes (Morebeer.com., 2020). Simcoe is a patented hop that was released in 2000 (Barnes, 2018), which is described as passion fruit, pine, berry, and earthy aromas. Citra was a development from Hallertau Mittelfruh, US Tettnang, Brewer's Gold, and East Kent Golding, released in 2007, and characterized by flavors of grapefruit, melon, lime, gooseberry, passion fruit, and lychee (Morebeer.com., 2020). Mosaic, a crossbreed of Simcoe and Nugget, presents the aromas of mango, lemon, citrus, pine, and blueberry (Barnes, 2018). However, planting proprietary hops requires expensive licenses, which prevent public plantings (Worthington, 2010). Public hops can be grown by any growers, processed and supplied by any suppliers, and brewed without additional cost to breweries (Worthington, 2010). The Irrigated Agriculture Research and Extension Center of USDA Agricultural Research Service has released many hops varieties to public, such as Cascade, Chinook, Centennial, Triple Pearl,

Cashmere, Cluster, Crystal, etc. Each hop variety has its combination of characteristic essential oil, which delivers distinguishable hops aroma (Inui, Tsuchiya, Ishimaru, Oka, & Komura, 2013). However, the sought-after aromas of proprietary hops are irreplaceable. In other words, the aroma of proprietary hops cannot be mimicked or substituted by any public hops alone that are cost-efficient and easily obtained.

Although the dried hop pellets only contain 0.5 – 3.0 % (by mass) of essential oil (Rettberg, Biendl, & Garbe, 2018), it is a complex mixture of hundreds of compounds (Roberts, Dufour, & Lewis, 2004). Almost all components contribute to the aroma profiles of hops varieties. Among all of the known aroma components in hops, only a few uncommon compounds exist distinctively in a few hop varieties (Gros, Nizet, & Collin, 2011). Therefore, different quantities of various aroma compounds differentiate the features of hops varieties (Inui et al., 2013; SALANŢĂ et al., 2018). Simulation of the aroma profiles of proprietary hops using public hops needs to be investigated in order to increase the utilization of public hops.

2.1.1 Role of hops

Hops are primarily valued for their aroma- and bitter-active compounds, which provide the aroma and flavor of beer. Traditionally, the “bittering hops” and “aroma hops” are classified based on the α-acid content. Nevertheless, hops contribute to both the bitter taste and aroma (i.e., hoppy character) during beer brewing. The amounts of bitterness and aromatic tastes that transfer from hops to beer are determined not only by the hop varieties (Dresel et al., 2015) but also by the hopping regimes (Dresel et al., 2013; Sharp, Qian, Shellhammer, & Shellhammer, 2017). The way and timing of hop addition (i.e., hopping

technology) during brewing significantly affect the final formation of essential oils in beer. The early kettle hopped treatment could impact the aroma of beer, however, only a few essential oil compounds remain their native form due to high volatility and poor solubility (Almaguer, Schönberger, Gastl, Arendt, & Becker, 2014). However, their transformations and the final forms are difficult to control. Although early kettle hopping may impact beer aroma (Praet, Van Opstaele, Aerts, & De Cooman, 2015), the dry hopping is a popular aroma hopping regime because it uses a low treatment temperature to minimize the volatilization of hop fragrance (Wolfe, Qian, & Shellhammer, 2012). Different protocols for hopping can lead to isomerization of humulones, but dry hopping reduces the isomerization which in turn leads to a less bitter beer and a more intense aroma (Lafontaine, Pereira, Vollmer, & Shellhammer, 2018). Therefore, dry hopping is selected in this study to increase aroma contribution from hops in the beer as well as to maintain maximum consistency (minimum challenges in tracking the aroma transformations).

2.1.2 Hoppy aroma in beer

The aroma properties of hops in beer are of far greater importance to brewers than they are for hops in raw condition (Schönberger, & Kostelecky, 2011). Moreover, brewing processes affect and differ the way how hops contribute aroma compounds to beers (Vollmer, Lafontaine, & Shellhammer, 2018). According to several studies (Kishimoto, Wanikawa, Kono, & Shibata, 2006; Sharp, Qian, Shellhammer, & Shellhammer, 2017; Wietstock, Gotz, Klie, Methner, & Scheuren, 2015), hops undergo a secondary transformation during the brewing process that alters their aroma characteristics in beers.

The aroma perception of beer is more complex than that of raw hops since some volatiles contribute to only background aromas and do not make a significant contribution to beer flavor (Pinho, Ferreira, & Santos, 2006). The hoppy aroma compounds in beer interact synergistically, additively, or in masked ways, creating negative effects that challenge the sensory perception (Schönberger, & Kostelecky, 2011; Hanke, Herrmann, Rückerl, Schönberger, & Back, 2008; Praet, Van Opstaele, Jaskula-Goiris, Aerts, & De Cooman, 2012). Therefore, the aroma perception of hoppy was investigated in order to assess the hops aroma. However, due to limited published data, it is still challenging to describe briefly and universally how hops transform aroma and affect the aroma of the finished beer. The assessments of aroma of hops and hoppy beer are performed individually, not focusing on the aroma transformation.

2.1.3 Key aroma compounds in hop oils and hoppy beers

Several previously published papers (Brendel, Hofmann, & Granvogl, 2019; Nance, & Setzer, 2011; Inui et al., 2013) have identified the key aroma compound groups of hops that highly contribute to hoppy beer flavor, including terpenes and terpenoids. The qualitative and quantitative compositions of terpenes and terpenoids determine the aroma differences among hops varieties. β-myrcene, α-humulene, and β-caryophyllene, belonging to the major monoterpenes and sesquiterpenes, are the three main constituents of hop oil. β-myrcene, α-humulene, and β-caryophyllene, consisting up to 80% of the total oil (Rettberg, Biendl, & Garbe, 2018), where β-myrcene accounts for around 30 – 60 % of the total oil content (Thompson, Marriott, Dowle, & Grogan, 2010). According to Volatile Compounds in Food (VCF) online database, α-humulene and β-caryophyllene are

described as spicy and woody odors; β-myrcene contributes balsamic, fruit, herb odors in most hops varieties.

Linalool and geraniol, the important terpenoids, are highly detected in hoppy beers (Wang, & Dixon, 2009), which have an approximately 80% transfer rates from hops to beer (Krottenthaler, Hanke, Kappler, and Becker, 2011). They exhibit floral and citrus fruit-like aroma, which are mainly delivered by hops. Linalool serves as an important indicator compound of hop aroma in beer (Hanke et al., 2008). Humans are extremely sensitive to linalool, although it exists at low threshold of around 10 to 100 ppm in hops (Shellhammer, & Peacock, 2021). Thus, linalool is generally considered as a highly-impact aroma compound and flavor-active substance in beer among hop oil components (Peacock, 2010). Geraniol is closely associated with the aroma of several hop varieties, including Citra (Brynildson, 2021). The characteristic aroma of proprietary hops is influenced by their unique chemical compositions, especially the highly aroma impact compounds. However, further research is required to better understand the role of these key aroma compounds when modeling the overall aroma profiles of proprietary hops.

2.2 Sensory evaluation

Sensory evaluation is a science that interprets the response from the sense of human organs to the products. It is a traditional method to evaluate the aroma of beer. A classic method, the Quantitative Descriptive Analysis (QDA), allows the qualitative and quantitative description of beers. Beers are evaluated using the different sensory attributes typically found in beer. These include alcoholic, grassy, malty, floral, fruity attributes, etc. (Meilgaard, Dalgliesh, & Clapperton, 1979). A dynamic method, such as temporal

dominance of sensations (TDS), considers changes of multisensory attributes and interaction among attributes over time (Pineau et al., 2009). Vázquez-Araújo, Parker, and Woods, (2013) reports the comparison of these sensory methods for evaluating beer flavor. Both the QDA (Brendel et al., 2019; Paixão, Tavares Filho, & Bolini, 2020) and TDS (Simioni, Ribeiro, de Souza, Nunes, & Pinheiro, 2018; Silva et al., 2019) are commonly used sensory methods for beer flavor evaluation. However, in this study, the overall aroma evaluation and their similarity among beers are the focuses, not the specific aroma attributes. Therefore, the sensory analysis with 9 points scale is carried out. Additionally, discrimination testing is applied to determine if detectable differences between the proprietary hoppy beer and its blended model for each variety exist.

2.3 Flavor-detection methods of hop oils and hoppy beers

Flavor is a perceptive expression of food products, involving smell and taste. A smell that triggers flavor perception is one of the important factors consumers assess before tasting a food product. Followed by the tasting process, an array of chemical stimuli is revealed, including not only the volatiles but also the non-volatile components. The conventional sensory evaluation is the most employed method for flavor assessment. However, it is usually subjective, irreproducible, and time-consuming, even for trained panelists or the flavor experts. Therefore, more accurate instrumental analysis method has been developed to overcome the human bias, owing to the reason that the sense of smell contributes much more than the sense of taste and touch in flavor evaluation (Foodpairing.com, 2009).

Analyzing food aroma by instruments has been developed and evolved rapidly. Gas chromatography (GC) is a well-documented and sensitive flavor-detection method for hop oils (Eri, Khoo, Lech, & Hartman, 2000; Kaškonas et al., 2016; SALANŢĂ et al., 2018) and hoppy beers (Ceola, Huelsmann, Da-Col, & Martendal, 2019; da Silva et al., 2015; Alves et al., 2020). It is efficient to separate individual aroma compounds with low sample requirement and high detection sensitivity. Therefore, GC is a wildly used flavor detection method in evaluating the odors of hops and obtaining the aroma profiles based on qualitative and quantitative flavor analysis. However, the nonvolatile compounds in beer may contaminate the instrument via direct liquid injection, which causes the low reproducibility of results (Charry-Parra, DeJesus-Echevarria, & Perez, 2011). Aroma extraction in the headspace using solid phase microextraction (SPME) is a widely used technique in the flavor field. SPME can detect low levels (ppb and ppm) of aroma compounds and prevent the contamination of nonvolatile substances. Also, headspace-based flavor extraction is crucial for obtaining highly reliable aroma profiles, because it focuses on volatiles and exposed them directly. Several papers have reported on the use of SPME to extract the volatiles from the headspace of hoppy beers (Giannetti, Mariani, Torrelli, & Marini, 2019; Dennenlöhr, Thörner, Manowski, & Rettberg, 2020; Alves et al., 2020; Rodrigues, Caldeira, & Câmara, 2008; Hrivňák, Šmogrovičová, Nádaský, & Lakatošová, 2010).

In addition, the selection of fiber coating may optimize aroma extraction, since the precision and content of extracted volatile compounds are affected by the polarity and volatility characteristics of fiber coating (Charry-Parra, DeJesus-Echevarria, & Perez, 2011). The commonly used fiber coatings include polydimethylsiloxane (PDMS),

polyacrylate (PA), carboxen/polydi-methylsiloxane (CAR-PDMS), carbowax-polyethylene glycol (PEG), polydimethyl-siloxane/divinylbenzene (PDMS/DVB) and divinylbenzene/carboxen/polydimethyl-siloxane (DVB/CAR/PDMS). Selection of a suitable coating and optimized processing parameters could increase the extraction efficiency of volatiles. Overall, in flavor fields, headspace solid-phase microextraction combined with GC and or mass spectrometry (HS-SPME-GC/MS) is a well-known analytical method to qualify and quantify the aroma compounds for flavor development and prediction.

2.4 Artificial intelligence techniques for flavor development

With the increasing demands of food flavor by consumers, artificial intelligence (AI) attracts more and more attention for flavor development. AI offers more choices to optimize and automate the flavor development processes and reduce human bias in the flavor field. The major benefit of AI is that it simplifies problems and speeds up the analysis of multiple datasets. Although the analytical instruments detect the quantities and compositions of aroma profiles, the instruments are not able to predict flavor perceptions. The instrumental analytical data are still essential to be analyzed and related with the sensory evaluation or the key flavor characteristics. AI techniques can bridge instrumental analysis with sensory evaluation in many flavor fields. Recently, there are some platforms successfully implementing AI technology for flavor pairing (Foodpairing.com., 2009), preferences (Flavorwiki.com., 2018), improvement (Johnson et al., 2019), and innovation (Faridi, Goodwin, Lougee, & Martin, 2019), which improve the acceptability of flavor. Besides, quality control (Bi, Zhang, Qiu, & Huang, 2020) and flavor prediction (Wu, Lei,

Zhu, & Luo, 2016; Wu, Lei, Zhu, & Luo, 2016) are other main application directions using AI techniques as the predictive tool. The main principle of machine learning (ML) algorithms is based on: 1) 'learn' operational human experience from limited observations, 2) 'conclude' interpretable rules based on specific experimental purposes to distinguish samples, and 3) 'decide' the contribution of each objective feature of assessed product to the final decision (Sharma, 2020). The main applications are for 1) understanding consumer's sensory perception that is affected by the chemical compositions of products' flavor, applying the effects of chemical information on sensory assessment in models, and finally predicting flavor preference of consumers (Yu, Low, & Zhou, 2018), 2) seeking non-obvious relations among ingredients based on flavor pairing principles, creating new flavor by pairing ingredients, and understanding the interval combination influences (Sharma, 2020), 3) assessing the effects of variables (such as regions, varieties, processing processes) on food quality, aiming to predict sensory assessments or quality parameters, and even monitoring processes (Aguilera, Lozano, Paredes, Alvarez, & Suárez, 2012), and so on. Nowadays, flavor science is embracing AI technologies to solve some edge-cutting problems and make food development more diversified and personalized. Some examples include the development of new product, the improvement of existing food products in the market, and the enhancement of the speed and effectiveness of product development.

The relationship between the similarity of aroma profiles and sensory evaluation for hops are still needed to be explored in this thesis. If we could successfully validate the feasibility of predicting sensory similarity of hops by simulating their aroma profiles, the full AI processes could be applied to achieve the purpose of predicting similarity. Overall,

the applications of AI in flavor offer the novel idea that model the aromas by using specific algorithms to shorten modeling time and avoid the subjective bias.

2.4.1 Quadratic programming (QP)

To simulate the aroma profiles of proprietary hops efficiently and objectively, the mathematical optimization - quadratic programming (QP) (Caron, 2021), a part of AI technology, was applied by outputting blended model with minimizing the sum of the differences of individual aroma compounds. QP is a linearly constrained quadratic optimization problem, which optimizes several quadratic functions affected by linear variables. Optimization the objective function in such a way that minimizes or maximizes the given constraints for the optimal variable (Persson et al., 2018). The special property of QP problem, convex optimization over convex set, ensures the output of global minimum or maximum rather than local minimum or maximum (Boyd, Boyd, & Vandenberghe, 2004). It is an efficient tool to achieve the optimal results, such as the optimum beer fermentation that maximizes ethanol production with the fixed time (Ramirez, & Maciejowski, 2007), the optimum kefir candy formula that maximizes viabilities of lactic acid bacteria and yeasts (Chen, Kuo, Shiu, & Chen, 2011), and the personalized fish intake recommendation that maximizes the health effects and minimizes the contaminant risks (Persson et al., 2018). Specifically, in this thesis, with the help of QP, the precision of blended models is under control and the time for matching the flavor is shortened dramatically by avoiding plenty of manual blending. In addition, to focus on the key aroma compounds in hops when modeling the overall aroma profiles, the weights could be easily added on them in QP optimization processes. However, studies about modeling

aroma profiles with the focus on key aroma compounds are not clear, so there is a need for more research to further validate the weighted models at the level of hops and hoppy beer.

2.5 References

Alves, V., Gonçalves, J., Figueira, J. A., Ornelas, L. P., Branco, R. N., Câmara, J. S., & Pereira, J. A. (2020). Beer volatile fingerprinting at different brewing steps. *Food Chemistry*, *326*, 126856.

Almaguer, C., Schönberger, C., Gastl, M., Arendt, E. K., & Becker, T. (2014). Humulus lupulus–a story that begs to be told. A review. *Journal of the Institute of Brewing*, *120*(4), 289-314.

Aguilera, T., Lozano, J., Paredes, J. A., Alvarez, F. J., & Suárez, J. I. (2012). Electronic nose based on independent component analysis combined with partial least squares and artificial neural networks for wine prediction. *Sensors*, *12*(6), 8055-8072.

Brendel, S., Hofmann, T., & Granvogl, M. (2019). Characterization of Key Aroma Compounds in Pellets of Different Hop Varieties (Humulus lupulus L.) by Means of the Sensomics Approach. *Journal of agricultural and food chemistry*, *67*(43), 12044-12053.

Brynildson, M. (n.d.). *The Oxford Companion To Beer Definition Of Geraniol*. [online] Craft Beer & Brewing. Available at: https://beerandbrewing.com/dictionary/euk63bzlWH/ Accessed 18 January 2021.

Bi, K., Zhang, D., Qiu, T., & Huang, Y. (2020). GC-MS Fingerprints Profiling Using Machine Learning Models for Food Flavor Prediction. *Processes*, *8*(1), 23.

Boyd, S., Boyd, S. P., & Vandenberghe, L. (2004). *Convex optimization*. Cambridge university press.

Barnes, C. (2018). *A Look at Designer Hops*. [online] The Full Pint - Craft Beer News. Available at: https://thefullpint.com/editorial/look-designer-hops/ Accessed 13 October 2021.

Charry-Parra, G., DeJesus-Echevarria, M., & Perez, F. J. (2011). Beer volatile analysis: optimization of HS/SPME coupled to GC/MS/FID. *Journal of food science*, *76*(2), C205-C211.

Chen, K. N., Kuo, C. Y., Shiu, J. S., & Chen, M. J. (2011). Process optimization for a novel kefir candy with high probiotic viability. *Journal of Food Process Engineering*, *34*(2), 427-443.

Caron, S. (n.d.). *Quadratic Programming in Python* [online] Available at: https://scaron.info/blog/quadratic-programming-in-python.html. Accessed 18 January 2021.

Ceola, D., Huelsmann, R. D., Da-Col, J. A., & Martendal, E. (2019). Headspace-solid phase microextraction and GC-MS followed by multivariate data analysis to study the effect of hop processing type and dry hopping time on the aromatic profile of top-fermented beers. *Separation Science Plus*, *2*(7), 245-252.

Dennenlöhr, J., Thörner, S., Manowski, A., & Rettberg, N. (2020). Analysis of Selected Hop Aroma Compounds in Commercial Lager and Craft Beers Using HS-SPME-GC-MS/MS. *Journal of the American Society of Brewing Chemists*, *78*(1), 16-31.

Dresel, M., Praet, T., Van Opstaele, F., Van Holle, A., Naudts, D., De Keukeleire, D., ... & Aerts, G. (2015). Comparison of the analytical profiles of volatiles in single-hopped worts and beers as a function of the hop variety. *Brewing Science*, *68*(1-2), 8-28.

da Silva, G. C., da Silva, A. A., da Silva, L. S., Godoy, R. L. D. O., Nogueira, L. C., Quitério, S. L., & Raices, R. S. (2015). Method development by GC–ECD and HS-SPME–GC–MS for beer volatile analysis. *Food chemistry*, *167*, 71-77.

Dresel, M., Van Opstaele, F., Praet, T., Jaskula-Goiris, B., Van Holle, A., Naudts, D., ... & Aerts, G. (2013). Investigation of the impact of the hop variety and the hopping technology on the analytical volatile profile of single-hopped worts and beers. *BrewingScience*, *66*(11-12), 162-175.

Eri, S., Khoo, B. K., Lech, J., & Hartman, T. G. (2000). Direct thermal desorption− gas chromatography and gas chromatography− mass spectrometry profiling of hop (Humulus lupulus L.) essential oils in support of varietal characterization. *Journal of agricultural and food chemistry*, *48*(4), 1140-1149.

Flavorwiki.com. (2018). *Flavor Wiki*. [online] Available at: https://flavorwiki.com Accessed 10 May 2020.

Faridi, H., Goodwin, R., Lougee, R., & Martin, J. (2019). Artificial Intelligence and the Future of Flavor Innovation. *Cereal Foods World, 64(6).*

Foodpairing.com. (2009). *Science Behind - Foodpairing*. [online] Available at: https://www.foodpairing.com/en/science-behind Accessed 12 May 2020.

Giannetti, V., Mariani, M. B., Torrelli, P., & Marini, F. (2019). Flavour component analysis by HS-SPME/GC–MS and chemometric modeling to characterize Pilsner-style Lager craft beers. *Microchemical Journal*, *149*, 103991.

Gros, J., Nizet, S., & Collin, S. (2011). Occurrence of odorant polyfunctional thiols in the super alpha Tomahawk hop cultivar. Comparison with the thiol-rich Nelson Sauvin bitter variety. *Journal of agricultural and food chemistry*, *59*(16), 8853-8865.

Hrivňák, J., Šmogrovičová, D., Nádaský, P., & Lakatošová, J. (2010). Determination of beer aroma compounds using headspace solid-phase microcolumn extraction. *Talanta*, *83*(1), 294-296.

Hanke, S., Herrmann, M., Rückerl, J., Schönberger, C., & Back, W. (2008). Hop volatile compounds (Part II): Transfer rates of hop compounds from hop pellets to wort and beer. *Brew. Sci*, *61*, 140-147.

Inui, T., Tsuchiya, F., Ishimaru, M., Oka, K., & Komura, H. (2013). Different beers with different hops Relevant compounds for their aroma characteristics. *Journal of agricultural and food chemistry*, *61*(20), 4758-4764.

Johnson, A. J., Meyerson, E., de, La, Parra, J., Savas, TL., Miikkulainen, R., & Harper, CB. (2019). Flavor-cyber-agriculture: Optimization of plant metabolites in an open-source control environment through surrogate modeling. *PLoS ONE* 14(4): e0213918.

Kaškonas, P., Stanius, Ž., Kaškonienė, V., Obelevičius, K., Ragažinskinė, O., & Maruška, A. (2016). Clustering analysis of different hop varieties according to their essential oil composition measured by GC/MS. *Chemical Papers*, *70*(12), 1568-1577.

Krottenthaler, M., Hanke, S., Kappler, S., & Becker, T. (2011). Influences to the transfer rate of hop aroma compounds during dry-hopping of lager beers. In *33rd European Brewery Convention Congress*.

Kishimoto, T., Wanikawa, A., Kono, K., & Shibata, K. (2006). Comparison of the odor-active compounds in unhopped beer and beers hopped with different hop varieties. *Journal of agricultural and food chemistry*, *54*(23), 8855-8861.

Lafontaine, S. R., Pereira, C. B., Vollmer, D. M., & Shellhammer, T. H. (2018). The effectiveness of hop volatile markers for forecasting dry-hop aroma intensity and quality of Cascade and Centennial hops. *Brew. Sci*, *71*, 116-140.

Morebeer.com. (2020) *The Largest List of Brewing Hops*. [online] Available at: https://www.morebeer.com/articles/homebrew_beer_hops/ Accessed 13 October 2021.

Meilgaard, M. C., Dalgliesh, C. E., & Clapperton, J. F. (1979). Beer flavour terminology 1. *Journal of the Institute of Brewing*, *85*(1), 38-42.

Nance, M. R., & Setzer, W. N. (2011). Volatile components of aroma hops (Humulus lupulus L.) commonly used in beer brewing. *Journal of Brewing and Distilling*, *2*(2), 16-22.

Persson, M., Fagt, S., Pires, S. M., Poulsen, M., Vieux, F., & Nauta, M. J. (2018). Use of mathematical optimization models to derive healthy and safe fish intake. *The Journal of nutrition*, *148*(2), 275-284.

Peacock, V. E. (2010). The value of linalool in modeling hop aroma in beer. Tech. Q. *Master Brew. Assoc. Am*, *47*(4), 29-32.

Praet, T., Van Opstaele, F., Aerts, G., & De Cooman, L. (2015). Sesquiterpene oxidation products as key impact compounds for "kettle hop" aroma. In *ASBC Annual Meeting, La Quinta, CA*.

Pinho, O., Ferreira, I. M., & Santos, L. H. (2006). Method optimization by solid-phase microextraction in combination with gas chromatography with mass spectrometry for analysis of beer volatile fraction. *Journal of chromatography A*, *1121*(2), 145-153.

Praet, T., Van Opstaele, F., Jaskula-Goiris, B., Aerts, G., & De Cooman, L. (2012). Biotransformations of hop-derived aroma compounds by Saccharomyces cerevisiae upon fermentation. *Cerevisia*, *36*(4), 125-132.

Pineau, N., Schlich, P., Cordelle, S., Mathonnière, C., Issanchou, S., Imbert, A., ... & Köster, E. (2009). Temporal Dominance of Sensations: Construction of the TDS curves and comparison with time–intensity. *Food Quality and Preference*, *20*(6), 450-455.

Paixão, J. A., Tavares Filho, E., & Bolini, H. M. A. (2020). Investigation of Alcohol Factor Influence in Quantitative Descriptive Analysis and in the Time-Intensity Profile of Alcoholic and Non-Alcoholic Commercial Pilsen Beers Samples. *Beverages*, *6*(4), 73.

Rettberg, N., Biendl, M., & Garbe, L. A. (2018). Hop aroma and hoppy beer flavor: chemical backgrounds and analytical tools—a review. *Journal of the American Society of Brewing Chemists*, *76*(1), 1-20.

Rodrigues, F., Caldeira, M., & Câmara, J. D. S. (2008). Development of a dynamic headspace solid-phase microextraction procedure coupled to GC–qMSD for evaluation the chemical profile in alcoholic beverages. *Analytica Chimica Acta*, *609*(1), 82-104.

Ramirez, W. F., & Maciejowski, J. (2007). Optimal beer fermentation. *Journal of the Institute of Brewing*, *113*(3), 325-333.

Roberts, M. T., Dufour, J. P., & Lewis, A. C. (2004). Application of comprehensive multidimensional gas chromatography combined with time-of-flight mass spectrometry (GC× GC-TOFMS) for high resolution analysis of hop essential oil. *Journal of separation science*, *27*(5-6), 473-478.

Shellhammer, T. & Peacock, V. (n.d.). *The Oxford Companion To Beer Definition Of Linalool*. [online] Craft Beer & Brewing. Available at: https://beerandbrewing.com/dictionary/Rp7oFjrGLU/ Accessed 18 January 2021.

Sharp, D. C., Qian, Y., Shellhammer, G., & Shellhammer, T. H. (2017). Contributions of select hopping regimes to the terpenoid content and hop aroma profile of ale and lager beers. *Journal of the American Society of Brewing Chemists*, *75*(2), 93-100.

Simioni, S. C. C., Ribeiro, M. N., de Souza, V. R., Nunes, C. A., & Pinheiro, A. C. M. (2018). Multiple-sip temporal dominance of sensations associated with acceptance test: a study on special beers. *Journal of food science and technology*, *55*(3), 1164-1174.

SALANŢĂ, L. C., Socaci, S. A., TOFANĂ, M., Mudura, E., Pop, C. R., Nagy, M., & Odagiu, A. (2018). Characterization of volatile components in hop pellets using in-tube extraction GC-MS analysis. *Romanian Biotechnological Letters*, *23*(2), 13541.

Sharma, M. (2020). Machine Learning and Artificial Intelligence in the Food Industry. *Purakala with ISSN. 0971-2143 is an UGC CARE Journal*, *31*(4), 950-954.

Sharp, D. C., Qian, Y., Shellhammer, G., & Shellhammer, T. H. (2017). Contributions of select hopping regimes to the terpenoid content and hop aroma profile of ale and lager beers. *Journal of the American Society of Brewing Chemists*, *75*(2), 93-100.

Schönberger, C., & Kostelecky, T. (2011). 125th anniversary review: The role of hops in brewing. *Journal of the Institute of Brewing*, *117*(3), 259-267.

Silva, A. P., Voss, H. P., van Zyl, H., Hogg, T., de Graaf, C., Pintado, M., & Jager, G. (2019). Effect of adding hop aroma in beer analysed by temporal dominance of sensations and emotions coupled with temporal liking. *Food Quality and Preference*, *75*, 54-63.

Thompson, M. L., Marriott, R., Dowle, A., & Grogan, G. (2010). Biotransformation of β-myrcene to geraniol by a strain of Rhodococcus erythropolis isolated by selective enrichment from hop plants. *Applied microbiology and biotechnology*, *85*(3), 721-730.

Vollmer, D. M., Lafontaine, S. R., & Shellhammer, T. H. (2018). Aroma extract dilution analysis of beers dry-hopped with Cascade, Chinook, and Centennial. *Journal of the American Society of Brewing Chemists*, *76*(3), 190-198.

Vázquez-Araújo, L., Parker, D., & Woods, E. (2013). Comparison of temporal–sensory methods for beer flavor evaluation. *Journal of Sensory Studies*, *28*(5), 387-395.

Wang, G., & Dixon, R. A. (2009). Heterodimeric geranyl (geranyl) diphosphate synthase from hop (Humulus lupulus) and the evolution of monoterpene biosynthesis. *Proceedings of the National Academy of Sciences*, *106*(24), 9914-9919.

Wu, Z. Y., Lei, X. J., Zhu, D. W., & Luo, A. M. (2016). Investigating the variation of volatile compound composition in maotai-flavoured liquor during its multiple fermentation steps using statistical methods. *Food technology and biotechnology*, *54*(2), 243-249.

Wu, Z. Y., Lei, X. J., Zhu, D. W., & Luo, A. M. (2016). Prediction of key aroma development in coffees roasted to different degrees by colorimetric sensor array. *Food chemistry*, *240*, 808-816.

Worthington, R. (2010). *Why Proprietary Hops?*. [online] Available at: http://inhoppursuit.blogspot.com/2010/01/why-proprietary-hops.html. Accessed 12 May 2020.

Wolfe, P., Qian, M. C., & Shellhammer, T. H. (2012). The effect of pellet processing and exposure time on dry hop aroma extraction. In *Flavor chemistry of wine and other alcoholic beverages* (pp. 203-215). American Chemical Society.

Wietstock, P. C., Gotz, F., Klie, R., Methner, F. J., & Scheuren, H. (2015). Feasibility study using a novel desorption/absorption process for transferring hop volatiles to beer. *Brewing science*, *68*(1-2), 1-7.

Yu, P., Low, M. Y., & Zhou, W. (2018). Development of a partial least squares-artificial neural network (PLS-ANN) hybrid model for the prediction of consumer liking scores of ready-to-drink green tea beverages. *Food Research International*, *103*, 68-75.

Chapter 3 Mathematical modeling of the aroma profiles of hops with the focus on highly-impact aroma compounds

Abstract

Citra is one of the most sought-after proprietary hops among brewers due to its excellent aroma. To find the substitution of Citra aroma, a mathematical optimization method - quadratic program (QP) was used in this study to model the aroma profile detected from gas chromatography (GC) or headspace solid-phase microextraction with GC (HS-SPME/GC), by blending public hops, with a focus on the key aroma compounds, namely β-myrcene, α-humulene, β-caryophyllene, linalool, and geraniol. By validating the models in hops and hoppy beer, the full-profile models based on GC data were more reliable and similar than the volatile-compound models based on HS-SPME/GC data. The higher similarity was validated in both aroma profiles analysis ($R^2 > 0.95$) and sensory evaluation. Moreover, the weighing of key aroma compounds in beer did not significantly improve the similarity, although it was in hops. The results showed that the aroma of Citra can be nearly replaced by 35.2% Brewers Gold, 5.2 % Cashmere, 32 % Centennial, and 35.7 % Triple Pearl. An integrated approach using mathematical optimization and flavor-detection techniques for modeling the aroma of target products (hops) has been developed in this study, providing a novel approach to flavor development in food products.

Keywords: Flavor models, Hop and beer aroma, Quadratic program, GC-MS/FID, HS-SPME-GC-MS/FID, Sensory evaluation, PCA

3.1 Introduction

Among various hop varieties, the distinct aromas of proprietary hops make them a favorite of brewers. However, compared with public hops, they are costly and license restricted access to the public (Worthington, 2010). Besides, the aroma of proprietary hop cannot be duplicated by individual public hop, since each hop variety, even the same hop variety from different origin, has its typical essential oil pattern (Kovačevič & Kač, 2002). Nevertheless, almost all hop varieties contain similar volatile compounds, merely differing in the quantity of individual compounds (Inui, Tsuchiya, Ishimaru, Oka, & Komura, 2013). These observations allow us to propose that the aroma of proprietary hops can be matched by blending public hops to model their unique aroma profiles. Recent progress for modeling the aroma of hops was reported by Campden BRI, which aimed to match the specific sensory characters by blending hops (Smart, 2016). However, sensory profiles that assessed by human are subjective and their manual modeling method is time-consuming. A time-efficient and accurate modeling method, as well as objective aroma representation, should be investigated in this study. Moreover, to date, it is evident from previous studies (Brendel, Hofmann, & Granvogl, 2019; Nance, & Setzer, 2011; Inui et al., 2013), key aroma compound, β-myrcene, α-humulene, β-caryophyllene, linalool and geraniol, that highly contribute to hoppy beer flavor were identified. Obviously, the characteristic aromas of proprietary hops are influenced by their unique chemical combinations of compounds, especially the highly impact aroma compounds. However, their combined contribution to the overall aroma, especially their contribution to aroma similarity was unclear. Therefore, these knowledges allow us to discover the feasibility of modeling the overall aroma profiles of proprietary hops with a focus on key aroma compounds.

Brewers generally place far more value on the aroma properties of hops in beer than they do on the aroma of raw hops (Schönberger, & Kostelecky, 2011). However, not as clear as the aroma detection of hops, the aroma substances that highly contribute to beer characteristic aroma are so complex due to the fact that some volatiles only contribute the background aroma and lack the significant contribution to beer flavor (Pinho, Ferreira, & Santos, 2006) and their complicated additive, synergistic or masking effects among plenty of beer aroma substances (Praet, Van Opstaele, Jaskula-Goiris, Aerts, & De Cooman, 2012). Therefore, understanding hop aroma attributes and evaluating the aroma similarity require not only a focus on the hop aroma profiles, but also the aroma characters in hoppy beer. Both the aroma similarity of hops and hoppy beer need to be investigated in this study.

To determine the aroma objectively and improve the modeling accuracy, gas chromatography with flame ionization detection (GC-FID) and gas chromatography with mass spectrometry (GC-MS) are the well-documented and sensitive flavor-detection methods for hop oils and hoppy beers (Ceola, Huelsmann, Da-Col, & Martendal, 2019; Forteschi et al., 2019; Mongelli et al., 2016; Killeen et al., 2017), focusing on quantification and qualification, respectively. They are efficient to separate individual aroma compounds with lower sample requirement and higher detection sensitivity. With a more focus on volatile compounds of hop oil, solid - phase microextraction (SPME) is a preferred aroma extraction approach in headspace with low detection levels (ppb and ppm). It coupled with GC is undoubtedly the choice of analytical method for the volatiles from hop oil (Ligor et al., 2014; Van Opstaele, Praet, Aerts, & De Cooman, 2013). However, there is no previous research for comparing the aroma sensory representation and modeling accuracy between volatile profiles extracted via SPME and the full profiles detected by GC directly.

Additionally, the use of SPME in beer can prevent the contamination of instruments with nonvolatile substances via direct liquid injection, which causes poor reproducibility (Charry-Parra, DeJesus-Echevarria, & Perez, 2011). Several papers (Charry-Parra et al., 2011; Rodrigues, Caldeira, & Câmara, 2008; Pinho, Ferreira, & Santos, 2006) have reported the use of headspace solid-phase microextraction combined with gas chromatography and/ or mass spectrometry (HS-SPME-GC/MS) to obtain aroma profiles of beer. Therefore, both the HS-SPME-GC and GC technologies are used to identify aroma profiles of hops and further create the volatile-compounds models and full-profile models, respectively, while the aroma profiles of hoppy beer are determined by HS-SPME-GC technology.

As a convenient and accurate modeling method, the quadratic programming (QP) is the powerful mathematical optimization method to simulate the aroma profiles of proprietary hops with minimizing the sum of the differences of individual aroma compounds (Caron, 2021). With the help of mathematical optimization, the precision of blended models is under control (minimize the difference between the blended aroma profiles and anticipated aroma profiles) and the time for matching the flavor is shortened dramatically (avoid plenty of manual blending). QP is successfully applied in some food fields, such as new dairy tofu development (Chen, Chen, & Lin, 2004), food nutrient values estimation (Westrich, Altmann, & Potthoff, 1998), diets optimization (Van Dooren, 2018), fish intake recommendations (Persson, Fagt, & Nauta, 2019; Persson et al., 2018), perishable foods control (Xin, Negenborn, & Lin, 2018).

The purpose of this study was to: 1) investigate the feasibility of matching the aromas of proprietary hops by blending public hops with the combination of QP and flavor-

detection techniques; 2) assess the effectiveness of modeling volatile profiles and full profiles detected by HS-SPME-GC and GC techniques, respectively; 3) explore the usefulness of key aroma compounds centric overall aroma profile modeling of hops. Overall, to model the volatile profiles and full profiles of hops by QP, we weighted the key aroma compounds scalarly and optimized the weighted models at the level of hop oil by sensory evaluation. The optimal weighted models and non-weighted models were validated and compared their aroma similarity in hop oil and hoppy beer through aroma profile analysis and sensory evaluation (Fig. 3.1)

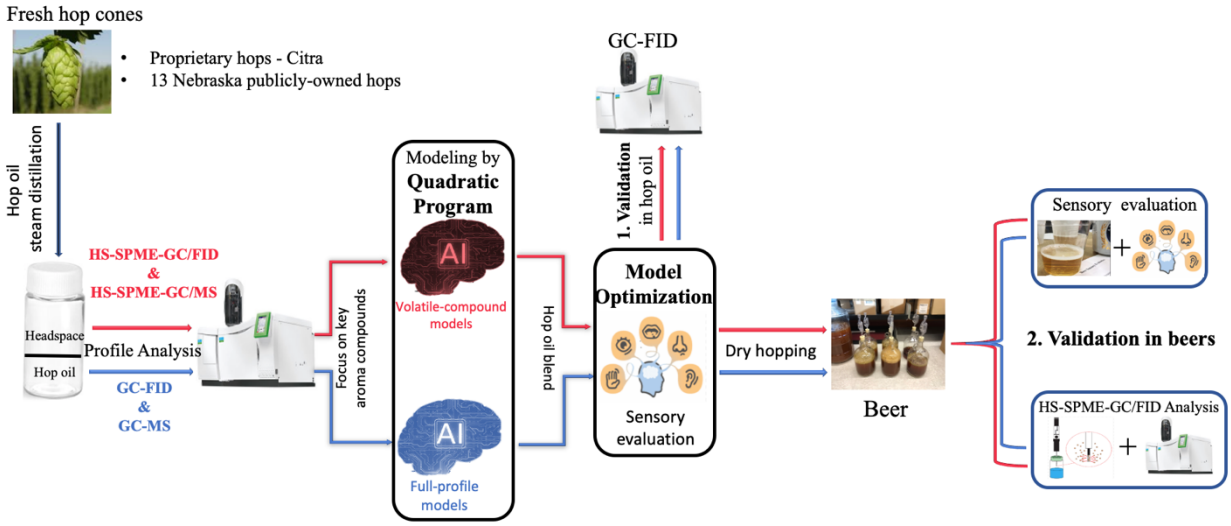

Fig. 3.1. Experiment design of the objective 1.

3.2 Materials and methods

3.2.1 Materials

The proprietary hop cones, Citra, was purchased from Yakima Valley Hops (Yakima, WA, USA). Thirteen Nebraska public fresh hop cones, Cascade, Chinook, Centennial, Cashmere, Tahoma, Saaz, Triple Pearl, Columbus, Brewers Gold, Galena,

Newport, Nugget, and Crystal were purchased from Midwest Hop Producers (Plattsmouth, Nebraska, USA) in year 2020. Briess Gold Malt Extract Syrup, LalBrew® BRY-97 American West Coast Ale Dry Yeast, and Cascade hopshot™ hops extract (bitterness acids) were purchased from Northern Brewer (Roseville, MN, USA) for brewing beer. Food grade ethanol (200 proof, undenatured) supplied by Decon Labs (King of Prussia, PA, USA) and deionized water were used to make hop perfume for sensory evaluation of hop oil. All other reagents used were of analytical grade.

3.2.2 Fresh hop oils preparation

The hop oils were isolated by steam distillation using a Clevenger apparatus according to a method developed in-house. Specifically, 1 lb. of fresh hop cones (ground in a smash machine) were weighted into a 5 gallons pot stills with gasket less seal. The volume of deionized water 1.5 gallons was added in ahead, and the distillation lasted for 3 h. The hop oils obtained from the condenser were collected and stored at -20 °C until use.

3.2.3 Fresh hop oils analysis

3.2.3.1 Volatile aroma profile analysis by HS-SPME-GC/MS method

A 50 μL each hop oil placed in a 15 mL glass vial was equilibrated for 30 min at 20 °C in an ultrasonic bath followed by solid phase microextraction (SPME) fiber exposure to the headspace for 30 min. The fiber of SPME used for the extraction of the volatile compounds was 75 μm carboxen/polydimethylsiloxane (CAR-PDMS) (Supelco, Bellefonte, PA, USA). The extraction for each sample was replicated three times. The trapped aroma compounds were desorbed by maintaining the fiber exposes to injection port

of GC system for 10 min, in a splitless mode. The quantification analyses were performed on an HP Agilent 6890 Series GC system (Agilent Technologies Co. Ltd., Santa Clara, CA, USA) equipped with automatic sampler and flame ionization detector (FID). Helium was used as the carrier gas with a flow rate of 2.9 mL/min. The components were separated on a DB-Wax UI column (30 m × 0.25 mm × 0.25 μm). The injector and detector temperatures were set at 250 and 260 °C, respectively. The chromatographic elution was temperature programmed as follows: isothermal at 50 °C (1 min), then increased at a rate of 8°C /min to 200 °C. All chemicals used in the analyses were of chromatographic grade.

The hop oil composition was qualified by GC coupled with mass spectrometry (MS) using a Thermo Scientific™ ISQ™ 7000 Single Quadrupole GC-MS System equipped with a DB-Wax UI column (30 m × 0.25 mm × 0.25 μm). The ion source temperature was set at 200 °C and the spectra was operated in the electron impact mode at 70 eV over a scan range of 50–500 m/z. Compound identifications were made by searching MAIN MS data library (a match quality of 95% minimum was used as a criterion) and comparison of their retention indexes (RIs) with the published data. Other parameters were same as that used for quantification.

3.2.3.2 Full aroma profile analysis by GC/MS method

50 μL hop oil and 1 mL hexane were placed in a 1.5 mL GC vial. The hop oil composition was quantified by gas chromatography (GC) analysis. Each sample was analyzed in triplicate with independent preparation. The injection volume was 3 μL. The inlet mode is spitless. The quantification and qualification of aroma compounds were performed under the same types and conditions of the GC and GC-MS system. The

chromatographic elution was temperature programmed as follows: isothermal at 50 °C (1 min), then increased at a rate of 8 °C /min to 200 °C, and then increased at a rate of 12 °C /min to 240 °C and hold for 6 mins.

3.2.4 Volatile-compound models

3.2.4.1 Weight the key aroma compounds

The key aroma compounds, β-myrcene, α-humulene, β-caryophyllene, geraniol, and linalool, were considered as the first rank compounds to add the weights in quadratic programming (QP) to model the aroma of Citra. The rest of the compounds were considered as the second rank compounds without inputting weights. The weight ratios were described as follows: n: 1, where n and 1 were the weights for the first and second rank compounds, respectively. A total of eight weight ratios, namely 1:1, 5:1, 10:1, 15:1, 20:1, 25:1, 30:1, and 35:1, were investigated in this study to make blended models.

3.2.4.2 Model the aroma of proprietary hops

Quadratic program (QP) was performed in Python using CVXOPT and QUADPROG solvers to model the aroma of proprietary hops based on modeling the peak area of individual aroma compounds (Caron, 2021). The specific weights were assigned to each individual key aroma compound outside the squared differences between the peak area of target proprietary hops and mixed public hops. The QP minimized the sum of squared differences between the peak areas of individual aroma compounds from proprietary hops and the mixture of public hops after inputting the aroma profiles of public and proprietary hops for modeling. The blended model was outputted as the mixture of

percentages of different public hops. Specifically, QP minimized a multivariate quadratic function in which the difference between target aroma profiles and the sum of the percentage of inputted aroma profiles, subject to linear constraints on the individual peak area.

3.2.4.3 Model optimization by sensory evaluation

To prepare oil perfume, 200 μL of each hop oil was infused with 8 ml ethanol and 2 mL deionized water, followed by preparing the blended models through mixing oil perfumes and then optimizing models based on sensory evaluation. 30 μL each oil perfume sample, including individual hop oil perfume and blended models, was placed into the perfume test paper and sealed in the plastic cups with covers to serve directly. Panelists were well-trained, showing the good distinction ability of Citra aroma. Coffee beans were used to refresh the nose during evaluation.

Weights for the first rank compounds in volatile-compound models ranged from 1 to 35 with 8 levels. The second rank compounds were non-weighted. The volatile-compound models with different weight ratios, including 1:1, 5:1, 10:1, 15:1, 20:1, 25:1, 30:1, and 35:1, were performed to assess the difference from Citra perfume by well-trained panelists to optimize the models with the lowest aroma difference.

3.2.5 Full-profile models

3.2.5.1 Weight the key aroma compounds

The key aroma compounds, β-myrcene, α-humulene, β-caryophyllene, geraniol, and linalool, were weighted as the first rank compounds. The compounds with aroma

intensities (relatively peak areas) over 0.2 % were considered as the second rank compounds to add weights. The rest compounds were regarded as the third rank compounds without adding weights. The weight ratios inputted in QP for full-profile models were as follows: m: n: 1, where m, n, and 1 were the weights for the first, second and third rank compounds, respectively. Weights for the first two rank compounds were performed at five levels (**Table 3.1**) to make the full-profile models. Each blended model with different weight ratios was carried out based on the experiment created by central composite design (CCD) (**Table 3.2**).

Table 3.1. Five levels (coded and actual values) CCD for two weight variables on aroma compounds in full-profile models.

Variable	**Symbol**	**Level**				
		-1.41	**-1**	**0**	**1**	**1.41**
First rank weights	X_1	3.85	10	25	40	46.15
Second rank weights	X_2	5.9	10	20	30	34.1

Table 3.2. The sensory scores for the similarity of hops essential oil based on different rank weights combination.

Run	**Independent variable**		**Response**
	X_1	**X_2**	**Sensory evaluation (scores) [c]**
Factorial design			
1	10	10	5.93 ± 1.12 [a]
2	40	10	4.20 ± 2.02 [a]
3	10	30	5.90 ± 2.72 [a]
4	40	30	4.93 ± 2.64 [a]
Axial points			
5	3.85	20	6.53 ± 1.64 [a]
6	46.15	20	5.20 ± 2.95 [a]
7	25	5.9	6.83 ± 2.10 [a]
8	25	34.1	6.47 ± 1.68 [a]
Central point			
9	25	20	6.60 ± 1.96 [a]

10	25	20	7.07 ± 1.66 [a]
11	25	20	7.20 ± 1.56 [a]
Optimum	19.54	-	7.11

X1 (correct the format), weights inputted into the quadratic program for first rank compounds;

X2 weights inputted into the quadratic program for second rank compounds;

[c] Sensory attributes (n = 5 panelists with three replicates) were expressed as mean ± standard deviation and the samples with the same letter are not significantly different at $p > 0.05$.

3.2.5.2 Model the aroma of proprietary hops

Modeling the full profiles of proprietary hops was engineered using the same technology and principles as 3.2.4.2.

3.2.5.3 Model optimization by response surface methodology

Preparation of the oil perfume and its sensory evaluation were conducted under the same conditions as those used for volatile compounds models. To model the full-profile aroma, the weights for the first and second rank compounds shown in **Table 3.2** were conducted. Total eleven blended models with weights and one non-weighted model were evaluated the aroma difference from Citra perfume by well-trained panelists. The full-profile models were optimized to obtain the lowest aroma difference by response surface methodology (RSM).

A 2^2 full-fraction CCD was applied for response surface fitting. This experimental design was set with two independent variables: weights for first rank compounds, weights for second rank compounds at five levels, including three replicates at the center point using R studio (R version 4.0.3) (**Table 3.1**). The total experimental runs generated by the CCD was 11 ($2^k + 2k + c_p$, where k is the factor number (k = 2) and c_p is the number of replicates at the center point ($c_p = 3$)) (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008). Total 11 experimental runs were replicated three times to obtain reliable data for the

response. The second order polynomial model in two factor CCD: x_1, x_2, was explained by Eq. (1):

$$y=ß_0+ß_1x_1+ß_2x_2+ß_{11}x_1^2+ß_{22}x_2^2+ß_{12}\,x_1\,x_2$$

where y is a response variable (sensory scores); x_1 and x_2 are the independent variables (weights for first rank compounds and weights for second rank compounds, respectively); $ß_0$, $ß_{1,2}$, $ß_{11,22}$, $ß_{12}$ are represented the intercept, linear coefficient, quadratic coefficient, and interaction coefficient, respectively.

3.2.6 Beer preparation

Hoppy beer of Citra, blended models (optimal and non-weighted full-profile models and volatile-compound models), several public hops (the main components for blended models), and un-hopped beer were prepared as follows.

1) Mashing: adding 5.5 lbs. malt extract syrup to 5 gallons water to adjust the brix^o equals to 10.1 at 152 °F.
2) Kettle: adding 3 mL hopshot^{TM} to achieve approximate 30 IBUs based on Cascade hopshot^{TM} instruction (10 IBU/ml is needed for 60 min boiling addition for 5 gallons of beer at 1.04 specific gravity) and boiling for 60 min. Finally, adjusting the brix^o of wort equaled to 10.46.
3) Fermentation: adding American ale dry yeast (2 g/gallon wort) and storing at 18 °C for 7 days and then at 4 °C for 4 days.
4) Dry hopping: fermented wort was separated into several 500 mL batches, 50 mL hop oil of each hop variety was added to fermented wort, no hop oil required for un-hoppy beer, and then the beers were stored at 18 °C for 7 days.

5) Bottling: the beers were stored in 1L brown bottles for posterior analysis at 4 °C.

3.2.7 Beer analysis

3.2.7.1 Aroma profile analysis by HS-SPME-GC/MS method

The volatile composition of beer was quantified in triplicate by HS-SPME coupled to GC-FID based on the method of Pinho, Ferreira, and Santos (2006). The extraction of volatile compounds from headspace was performed using HS-SPME technique with 75 μm CAR-PDMS fiber (Supelco, Bellefonte, PA, USA). The selection of fiber is in order to concentrate the sample effectively and extract the higher variety of volatile compounds. 5 g each beer sample and 2 g NaCl in a 15 mL vial were equilibrated for 30 min at 20 °C in an ultrasonic bath followed by SPME fiber exposure to the headspace for 30 min. The trapped aroma compounds were desorbed by maintaining the fiber exposes to injection port of GC system for 10 min, in a splitless mode. The chromatographic analyses were performed on an HP Agilent 6890 Series GC system (Agilent Technologies Co. Ltd., Santa Clara, CA, USA). Helium was used as the carrier gas with a flow rate of 2.9 mL/min. The components were separated on a DB-Wax UI column (30 m × 0.25 mm × 0.25 μm). The injector and detector temperatures were set at 250 and 260 °C, respectively. The chromatographic elution was temperature programmed as follows: isothermal at 50°C (1min), then increased at a rate of 8°C /min to 200°C. All chemicals used in the analyses were of chromatographic grade.

3.2.7.2 Sensory evaluation of beer

For all sensory tests, beer samples (10 mL) was served in the 60 mL plastic cups with covers and labeled using 3-digit random codes to avoid bias for posterior uses. Before evaluation, all beer samples were equilibrated at room temperature (around 20 °C) for 15 min to maximize the aroma and minimize the effects of temperature. Five well-trained panelists were asked to assess the aroma difference of beer samples from Citra hoppy beer (control).

The optimal-weight blended models (full-profile model and volatile-compound model), non-weighted blended models, public hoppy beers (the main component hops varieties for blended models) and un-hoppy beer were evaluated the overall aroma difference from Citra hoppy beer (control) using the 10 – point difference from control scale: 1 = completely different, 10 = same (Compusense.com., 2020). The evaluation was undertaken by each panelist triplicated using different beer batches. Coffee beans were used to refresh the nose between sample evaluation.

3.2.8 Statistical procedures

Analysis of variance (ANOVA) with judge as a fixed factor and product as a random factor was used to compare the means of the sensory results and determine the difference. Tukeys' HSD (honestly significant difference) posthoc analyses (at a 5% significance level) were conducted to identify significant differences among the similarity aroma evaluation. R squared value was used to evaluate the positive correlation of the aroma profiles of different hop varieties to indicate the similarity of the aroma profiles for both hop oil and hoppy beer. These statistical analyses were performed by R studio version 4.0.3.

3.3 Results and Discussions

3.3.1 Hop oil profile and selection of key aroma compounds

3.3.1.1 Volatile aroma profile of hop oil and key aroma compounds

Table 3.3 shows volatile compounds and their peak areas detected in the headspace of hop oil from different hops varieties by HS-SPME/GC-MS method. Besides a few aromatic compounds only detected in certain varieties, almost all aroma compounds could be detected in hops, which is in agreement with the statement reported by Inui et al. (2013). For instance, β-ocimene detected in Citra could only be identified in Galena, Triple pearl, and Tahomn among the 13 public hops varieties. **Table 3.4** shows the volatile compounds commonly qualified in Citra hop oil by HS-SPME/GC-MS system. Total 24 compounds were identified in the headspace and listed in the order of retention time. The main aroma compounds: β-myrcene, linalool, β-caryophyllene, α-humulene and geraniol represented the major peaks, which led to the average relative contents: 93.882%, 0.227%, 1.005%, 0.841%, and 0.013%, respectively. The relative contents of the main aroma compounds in Citra accounted for 95.964% of the total hop oil content, with a range of 95.400% to 96.701%. The remaining 19 compounds accounted for 2.779% of the total content, ranging from 2.137% to 3.315%. The higher relative contents of terpenes in hop oil have also detected by Almaguer, Schönberger, Gastl, Arendt, and Becker (2014). Among terpenes, β-myrcene, β-caryophyllene, and α-humulene were the main terpenes, playing the important roles in hop aroma, which were reported by Duarte, Amorim, Grazul, and de Oliveira (2020). Moreover, Kishimoto, Wanikawa, Kagami, and Kawatsura (2005) published that terpenes are the main class of volatile components of hops that contribute the aroma characters to beer. Despite accounting for only a small fraction of total oil

Table 3.3 Volatile compounds and their peak areas detected in the headspace of hop oil from different hops varieties by HS-SPME/GC-MS method.

Volatile compounds [a]	Peak area [b]													
	Proprietary hops	Public hops												
	Citra	Brewers Gold	Cascade	Cashmere	Centennial	Chinook	Colombus	Crystal	Galena	Newport	Nugget	Saaz	Triple pearl	Tahomn
1,4-Pentadiene	172.02	11.30	67.21	39.75	90.34	215.68	50.43	36.35	12.13	70.45	54.13	39.44	3.09	45.68
a-Pinene	359.49	255.89	193.82	133.59	256.73	160.74	170.94	84.32	113.83	175.53	180.88	215.04	200.18	171.30
camphene	333.87	202.47	101.34	159.03	115.03	226.66	203.74	75.89	99.75	267.21	241.36	122.27	130.62	125.60
ß-Pinene	2011.18	1535.90	1245.96	1163.41	1183.74	1273.66	1109.93	795.58	834.37	1332.89	1135.82	1342.03	1170.00	1361.54
ß-Myrcene	150182.64	117845.26	97023.61	64019.16	144447.73	83225.58	81758.14	71427.21	70276.82	87806.04	86046.31	113497.26	108850.52	98638.58
β-ocimene	133.65	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	43.99	n.d.	n.d.	n.d.	73.61	116.05
p-cymene	539.90	873.86	534.86	1288.96	597.68	457.53	517.20	205.55	2146.13	737.97	3039.63	549.55	793.46	1634.83
methyl heptanoate	332.35	522.04	320.46	792.31	975.89	849.04	198.01	318.94	696.24	900.56	1142.38	432.46	813.25	586.00
Methyl 6-methylheptanoate	216.08	193.85	190.00	484.37	439.03	1246.62	349.67	334.62	878.22	1200.60	1477.78	218.02	377.57	183.93
Cyclohexene, 1,5,5-trimethyl-3-methylene-	38.36	15.70	26.27	35.68	22.80	n.d.	18.74	17.58	41.26	26.68	42.34	20.87	25.41	30.68
methyl octanoate	596.33	333.38	215.27	579.57	628.29	698.06	242.49	601.62	828.53	763.47	1234.08	252.29	715.15	807.78
1,3,5-undecatriene	319.64	57.45	71.82	71.58	n.d.	n.d.	75.68	197.87	n.d.	57.33	n.d.	52.60	82.79	0.00
methyl nonanoate	311.32	208.05	194.72	914.84	213.83	549.32	452.15	537.32	1068.40	743.57	867.64	228.57	699.93	526.58
Methyl 13,16-octadecadiynoate	30.07	39.68	61.26	159.96	126.52	70.78	77.33	129.00	191.66	150.82	159.56	75.83	125.01	79.68
linalool	363.21	73.95	160.39	326.74	310.51	439.98	170.41	316.89	537.58	473.58	810.43	122.70	214.46	221.30
β-caryophyllene	1607.22	1828.67	1800.56	6709.49	1570.13	2340.56	3217.85	3308.12	5537.91	2885.88	3841.80	1500.24	1339.97	1988.57
methyl 4-decenoate	355.73	241.95	202.83	686.10	481.93	737.38	516.74	733.15	1022.88	1036.87	1581.52	217.55	682.72	567.00
α-humulene	1345.79	1923.83	3144.18	10313.91	2120.07	3330.60	3698.85	7495.12	5033.38	3160.57	4870.37	2328.33	1482.69	3823.04
cis-Geranic acid, methyl ester	441.17	381.96	397.30	469.15	1244.41	696.11	337.53	621.98	527.98	485.82	1001.25	314.06	600.06	1022.18
Guaia-1(10),11-diene	159.79	162.59	406.41	455.25	63.90	491.91	438.93	1581.33	286.95	205.95	312.95	349.79	1130.24	1576.38
ß-copaene	64.78	107.46	307.68	98.41	137.95	396.22	353.90	259.03	326.01	236.61	186.91	206.64	288.02	487.12
trans-calamenene	19.67	31.47	95.69	n.d.	52.96	82.09	99.94	91.75	66.13	27.32	100.21	67.16	18.91	81.77
Geraniol	20.56	9.80	18.85	21.17	183.15	103.50	n.d.	50.02	28.40	32.54	16.55	n.d.	60.86	22.27
Octanoic acid	15.08	13.39	n.d.	28.27	n.d.	n.d.	25.51	95.96	16.98	n.d.	14.59	n.d.	n.d.	44.14

[a] Compounds detected from the headspace of the hop oil are listed in the order of retention time. [b]Mean of triplicates; standard deviations were <20% . n.d.: not detected

Table 3.4 Volatile compounds detected in the headspace of Citra hop oil by HS-SPME/GC-MS method.

No.	Compound*	RI	Relative content (%)		No.	Compound	RI	Relative content (%)	
			Mean	Range				Mean	Range
1	1,4-Pentadiene	-	0.108	0.017 - 0.162	13	methyl nonanoate	1485	0.195	0.168 – 0.225
2	a-Pinene	1024	0.225	0.180 - 0.264	14	Methyl 13,16-octadecadiynoate	-	0.019	0.012 – 0.029
3	camphene	1068	0.209	0.168 - 0.236	15	linalool	1542	0.227	0.218 – 0.244
4	ß-Pinene	1109	1.257	1.163 - 1.339	16	β-caryophyllene	1596	1.005	0.780 – 1.239
5	ß-Myrcene	1161	93.882	93.387 - 94.225	17	methyl 4-decenoate	1632	0.222	0.191 – 0.278
6	β-ocimene	1235	0.084	0.000 – 0.092	18	α-humulene	1668	0.841	0.625 – 1.076
7	p-cymene	1267	0.338	0.218 – 0.422	19	cis-Geranic acid, methyl ester	-	0.276	0.258 – 0.314
8	methyl heptanoate	1287	0.208	0.116 – 0.273	20	Guaia-1(10),11-diene	-	0.100	0.084 – 0.133
9	Methyl 6-methylheptanoate	1338	0.135	0.103 – 0.165	21	ß-copaene	-	0.040	0.025 – 0.054
10	Cyclohexene, 1,5,5-trimethyl-3-methylene-	-	0.024	0.014 – 0.034	22	trans-calamenene	1818	0.012	0.008 – 0.018
11	methyl octanoate	1390	0.373	0.355 – 0.400	23	Geraniol	1837	0.013	0.012 – 0. 15
12	1,3,5-undecatriene	1386	0.200	0.168 – 0.235	24	Octanoic acid	2054	0.009	0.000 – 0.009
Frist rank compounds (5)					**Second rank compounds (The rest of the 19)**			**2.779**	**2.137 – 3.315**
ß-Myrcene			93.882	93.387 – 94.225					
linalool			0.227	0.218 – 0.244					
β-caryophyllene			1.005	0.780 – 1.239					
α-humulene			0.841	0.625 – 1.076					
Geraniol			0.013	0.012 – 0. 15					
Total			**95.964**	**95.400 – 96.701**					

* Compounds are listed in the order of retention time. RI, retention indices are determined on DB-Wax UI column.

content, geraniol and linalool provide significant aromas to hops (Fritsch, & Schieberle, 2005; Vollmer, Lafontaine, & Shellhammer, 2018), playing the key role in hoppy beer due to their good solubility and the efficient extraction rate (Dresel et al., 2015). Therefore, under the main purpose of modeling the overall aroma profiles of hops, these key aroma compounds were emphasized by inputting the weights on them in the modeling program. In one word, to model the overall aroma

characters of Citra, these key aroma compounds were added the weights, while the rest of the compounds were non-weighted.

3.3.1.2 Full aroma profile of hop oil and key aroma compounds

Table 3.5 shows aroma compounds and their peak areas detected in the hop oil from different hops varieties by GC-MS method. **Table 3.6** shows the aroma compounds commonly detected in Citra hop oil that were aimed to model. GC-MS is an extremely powerful method that offers high separation efficiency and fast processing speeds to characterize aroma compounds. In contrast to aroma extraction from the headspace of hop oil, which focuses on volatile compounds, the GC-MS system detects the full aroma profile, resulting in more complex aroma profiles that are more difficult to model. A total of 31 compounds were identified in the full aroma profile. The main aroma compounds contributed about 94.738% to the total content: 87.847% (β-myrcene), 0.434% (linalool), 3.037% (β-caryophyllene), 3.385% (α-humulene), and 0.035% (geraniol). For modeling more complex full aroma profiles, the modeling program not only considers the main aroma compounds, but also the compounds with higher relative contents in Citra, under the requirement of modeling overall full profiles. In other words, simulating these substances preferentially without sacrificing the overall full profile similarity. Therefore, in this study, aroma compounds with relative contents exceeding 0.200 % were selected to add weights for the modeling program, including β-ocimene (0.289%), β-pinene (1.025%), methyl octanoate (0.302%), methyl nonanoate (0.268%), methyl 4-decenoate (0.552%), cis-geranic acid methyl ester (0.892%), β-chamigrene (0.776%), and α-muurolene (0.270%). Overall, the main aroma compounds that have been added the weights were considered as the first rank compounds, 13 compounds with the second higher relative contents (> 0.200%) were weighted as the second rank compounds, while the rest of the

Table 3.5. Aroma compounds and their peak areas detected in the hop oil from different hops varieties by GC-MS method.

Aroma compounds [a]	Peak area [b]													
	Proprietary hops	Public hops												
	Citra	Brewers Gold	Cascade	Cashmere	Centennial	Chinook	Colombus	Crystal	Galena	Newport	Nugget	Saaz	Triple pearl	Tahoma
β-pinene	1813.65	1701.46	1953.76	1213.22	2142.32	1024.33	1592.12	1392.08	1537.02	1179.26	1447.93	1905.09	1765.07	1935.71
β-myrcene	155413.94	136062.73	137252.28	85846.823	155749.79	97932.66	117085.11	132416.80	125475.82	87704.40	107650.98	145861.80	149107.54	142690.15
β-ocimene	510.60	705.45	807.18	1490.85	941.68	272.24	540.87	358.60	3226.04	569.61	3016.02	820.80	842.36	2050.87
methyl heptanoate	193.53	371.42	594.95	574.42	658.69	324.22	212.98	385.62	759.80	652.50	862.76	359.94	677.48	662.49
Methyl 6-methylheptanoate	212.74	162.63	462.65	494.24	343.35	1252.51	439.15	179.81	1116.79	1175.08	1908.54	243.45	314.42	200.23
methyl octanoate	534.77	296.23	397.98	643.93	208.46	528.93	260.15	522.06	712.91	636.65	1246.76	264.97	501.58	770.02
1,3,5-undecatriene	287.68	82.09	177.20	136.59	191.09	56.58	132.43	129.45	84.92	74.31	202.97	88.00	84.87	99.17
methyl nonanoate	474.20	335.77	444.92	1448.60	168.63	918.02	551.51	537.76	1012.12	870.04	1005.45	299.80	710.76	545.39
Methyl 13,16-octadecadiynoate	22.51	32.31	93.30	181.47	112.44	65.06	61.06	84.06	145.57	137.30	155.65	65.60	95.60	61.45
linalool	768.37	165.07	806.62	649.43	603.19	819.26	270.10	906.46	756.98	829.77	1405.67	265.56	375.11	440.53
β-caryophyllene	5372.68	5833.08	6347.25	21439.67	3105.24	6792.68	6663.78	6514.18	14085.19	8003.24	8017.52	4195.59	2728.36	3657.88
methyl 4-decenoate	976.96	550.82	887.82	1531.27	758.96	1369.03	792.18	1006.09	1783.27	2161.67	2863.71	456.08	901.88	767.05
α-humulene	5987.75	8213.18	17650.99	n.d.	5631.35	13237.81	10248.83	25745.71	18288.08	13665.18	14633.78	11494.04	4233.31	8829.26
cis-Geranic acid, methyl ester	1577.52	1177.53	n.d.	43907.78	2187.63	2318.22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1383.91	2035.87
β-chamigrene	1373.73	1008.87	1744.75	2853.40	539.10	2908.23	1592.74	2659.11	1432.38	1067.71	2074.85	1919.67	4796.45	5400.73
Muurolene	477.59	872.71	2275.60	3927.37	662.14	3432.84	1782.74	1398.30	2403.92	1612.43	1619.46	1265.71	1651.98	1817.23
α-ylangene	24.10	63.67	90.72	227.99	130.93	1641.66	631.48	91.29	119.02	98.05	138.44	58.59	83.81	116.95
geraniol vinyl ether	224.65	185.99	970.38	799.85	330.26	254.42	409.83	219.90	240.82	381.64	1833.64	684.43	213.71	145.95
Geraniol	62.77	38.85	97.89	310.84	485.76	457.16	319.78	181.14	132.82	166.57	305.82	n.d.	30.47	29.78
Ethyl 6,9,12-hexadecatrienoate	44.04	n.d.	115.50	260.75	48.49	323.49	95.28	63.20	165.94	250.65	1218.07	62.57	70.96	72.30
2-(Octadec-9-enyloxy) ethanol	17.54	28.79	193.03	166.34	n.d.	38.41	105.40	84.74	58.21	135.53	183.69	62.29	34.65	29.26
caryophyllene oxide	14.45	n.d.	58.83	147.80	26.31	136.26	33.15	33.33	70.21	93.04	556.82	28.36	22.93	18.05
2-pentadecanone	70.18	194.73	283.46	565.59	n.d.	103.83	370.13	209.37	270.84	241.40	592.28	185.68	122.75	127.30
humulene epoxide II	180.72	432.43	364.21	1162.64	31.25	191.08	724.66	555.76	468.36	469.78	985.62	302.97	315.69	315.45
Gamolenic Acid	41.32	28.81	222.24	583.02	39.05	107.54	235.51	181.91	118.63	343.96	393.95	153.20	128.19	51.29
Linoleic Acid ethyl ester	31.71	12.48	111.62	228.03	n.d.	203.97	69.54	98.66	53.50	86.41	191.20	102.55	57.27	84.66
decanoic acid	53.56	n.d.	18.79	44.41	20.43	44.08	23.44	54.06	28.25	22.85	63.92	30.14	52.04	117.89
9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	26.92	12.43	14.06	46.34	n.d.	38.23	24.69	10.55	9.40	19.02	55.48	n.d.	n.d.	n.d.
1-Monolinoleoylglycerol trimethylsilyl ether	68.07	71.84	24.68	194.53	n.d.	256.19	22.31	73.94	23.88	n.d.	46.77	23.19	11.35	50.97
(2S,2'S)-2,2'-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	24.13	18.67	56.49	59.81	n.d.	n.d.	37.90	49.40	26.63	n.d.	60.59	23.66	21.91	27.61
Octaethylene glycol monododecyl ether	31.63	63.20	27.84	90.79	27.31	34.29	79.04	36.73	55.06	59.89	91.31	50.00	48.12	42.20

[a] Compounds detected from the hop oil are listed in the order of retention time. [b]Mean of triplicates; standard deviations were <20% . n.d.: not detected

Table 3.6. Aroma compounds detected in Citra hop oil by GC-MS method.

No.	Compound *	RI	Relative content (%)		No.	Compound	RI	Relative content (%)	
			Mean	Range				Mean	Range
1	β-pinene	1109	1.025	0.997 – 1.065	19	Geraniol	1837	0.035	0.035 – 0.036
2	β-myrcene	1161	87.847	86.329 –87.918	20	Ethyl 6,9,12-hexadecatrienoate	-	0.025	0.024 – 0.026
3	β-ocimene	1235	0.289	0.206 – 0.445	21	2-(Octadec-9-enyloxy) ethanol	-	0.010	0.009 – 0.010
4	methyl heptanoate	1287	0.109	0.070 – 0.184	22	caryophyllene oxide	1979	0.008	0.008 – 0.009
5	Methyl 6-methylheptanoate	1338	0.120	0.116 – 0.123	23	2-pentadecanone	2017	0.040	0.038 – 0.041
6	methyl octanoate	1390	0.302	0.295 – 0.313	24	humulene epoxide II	2066	0.102	0.099 – 0.107
7	1,3,5-undecatriene	1394	0.163	0.157 – 0.170	25	Gamolenic Acid	-	0.023	0.023 – 0.024
8	methyl nonanoate	1485	0.268	0.262 – 0.277	26	Linoleic Acid ethyl ester	-	0.018	0.017 – 0.019
9	Methyl 13,16-octadecadiynoate	-	0.013	0.012 – 0.013	27	decanoic acid	2272	0.030	0.030 – 0.031
10	linalool	1542	0.434	0.424 – 0.451	28	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	-	0.015	0.015 – 0.016
11	β-caryophyllene	1596	3.037	2.968 – 3.148					
12	methyl 4-decenoate	1632	0.552	0.539 – 0.573					
13	α-humulene	1668	3.385	3.306 – 3.511	29	1-Monolinoleoylglycerol trimethylsilyl ether	-	0.038	0.038 - 0.040
14	cis-Geranic acid, methyl ester	-	0.892	0.873 – 0.923					
15	β-chamigrene	1724	0.776	0.759 – 0.805	30	(2S,2'S)-2,2'-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	-	0.014	0.013 - 0.014
16	α-Muurolene	-	0.270	0.264 – 0.280					
17	α-ylangene	-	0.014	0.013 – 0.015	31	Octaethylene glycol monododecyl ether	-	0.018	0.000 – 0.018
18	geraniol vinyl ether	-	0.127	0.124 – 0.130					
Frist rank compounds (5)					**Second rank compounds (8)**				
β-myrcene			87.847	86.329 –87.918	methyl octanoate			0.302	0.295 – 0.313
linalool			0.434	0.424 – 0.451	methyl nonanoate			0.268	0.262 – 0.277
β-caryophyllene			3.037	2.968 – 3.148	methyl 4-decenoate			0.552	0.539 – 0.573
α-humulene			3.385	3.306 – 3.511	cis-Geranic acid, methyl ester			0.892	0.873 – 0.923
Geraniol			0.035	0.035 – 0.036	β-chamigrene			0.776	0.759 – 0.805
Total			**94.738**	**93.063 – 95.064**	α-Muurolene			0.270	0.264 – 0.280
					β-ocimene			0.289	0.206 – 0.445
					β-pinene			1.025	0.997 – 1.065
Third rank compounds (18)			**0.887**	**0.466 – 3.016**	**Total**			**4.374**	**4.237 – 4.452**

* Compounds are listed in the order of retention time. RI, retention indices are determined on DB-Wax UI column.

18 compounds were non-weighted. For full profile of Citra hop oil, the aroma compounds (the first and second rank compounds) needed to be weighted account for 99 % of the total contents.

3.3.2 Blending model optimization

3.3.2.1 Model optimization based on volatile compounds of hop oils

The volatile compounds extracted from the headspace of hop oil avoid the extraneous nonvolatile residues, which might affect adversely the GC columns (Eri, Khoo, Lech, & Hartman, 2000). In addition, the odor-active properties of volatiles that contribute to the specific smell of hops have been reported in several papers (Brendel, Hofmann, & Granvogl, 2019; Neiens, & Steinhaus, 2018; Eyres, Marriott, & Dufour, 2007). The models that are focused on volatiles are therefore taken into account in this research. **Table 3.7** represents the volatile-compound models for Citra with different weight ratios on volatile compounds, including 1:1, 5:1, 10:1, 15:1, 20:1, 25:1, 30:1, and 35:1. For each model, QP outputs the hops combination and their percentage. The model combinations shown in **Table 3.7** are optimal results with minimizing the sum of squared differences between the peak areas of individual aroma compounds from proprietary hops and the mixture of public hops. Among them, Brewers Gold, Centennial, Saaz and Triple Pearl were used to mix on a percentage basis. There was only a small amount (0.1%) of Saaz used in 1:1 or 25:1 model. With increasing weight ratios, except for the non-weighted model, Centennial in blended models decreased dramatically from 67.6% to 13.0%, while the amount of Triple Pearl increased dramatically from 47.8% to 120.8%. The total amount of hop oil correlated closely with the use of Triple Pearl, indicating the same change rule in blended models. In addition, as shown in **Table 3.7**, the higher percentage of hop oil was also found in blended models at 30:1 and 35:1 (131.8%

and 133.8%, respectively), while the lower was found in non-weighted (1:1) or 5:1 model (119.6% and 115.9%, respectively).

Table 3.7. Volatile-compound models for proprietary hops (Citra) with different weight ratios on volatile compounds.

Weight ratios for volatile-compound models [a]	Nebraska public hops				
	Brewers Gold (%)	**Centennial (%)**	**Saaz (%)**	**Triple Pearl (%)**	**Total (%)**
1:1	0.719	0.381	0.001	0.095	1.196
5:1	0.005	0.676	0.000	0.478	1.159
10:1	0.018	0.538	0.000	0.646	1.202
15:1	0.002	0.434	0.000	0.801	1.237
20:1	0.000	0.341	0.000	0.927	1.268
25:1	0.016	0.257	0.001	1.019	1.293
30:1	0.000	0.191	0.000	1.127	1.318
35:1	0.000	0.130	0.000	1.208	1.338

a Weight ratios represent the ratio of the weights on key aroma compounds (β-myrcene, α-humulene, β-caryophyllene, geraniol, and linalool) and the rest of the volatile compounds in modeling program, where 1:1 = all volatile compounds have weights of 1; 5:1 = key aroma compounds have weights of 5, while rest compounds have weights of 1; …; 35:1 = key aroma compounds have weights of 35, while rest compounds have weights of 1.

To optimize the effect of weights on key aroma compounds for the volatile-compound models, difference testing was performed for several hop oil models by a well-trained panel. In **Figure 3.2 (a),** the panelists demonstrated their good distinction ability for the Citra hop aroma by distinguishing the difference of hop oils, showing that they have been well-trained. Panelists identified the Citra aroma with statistical significance ($p < 0.05$) among Brewers Gold, Centennial, and Triple Pearl hop oil. It is shown in **Figure 3.3** that the volatile-compound model with a weight ratio of 30:1 achieved the lowest difference from Citra and differed significantly from the public hops group. The 10:1, 15:1, 20:1, 25:1, and 35:1 volatile-compound models were not significantly different from public hops, although they did not differ significantly from Citra aroma. Moreover, their standard deviations were higher than those in 30:1 model, which was unexpected. Models

with no weight (1:1) and with less weight (5:1) were significantly different from Citra Aroma. Therefore, the 30:1 model, which represents 19.1% Centennial and 112.7% Triple Pearl for modeling 100% unit of Citra aroma, was the optimum model need to verify the difference scale at both sensory evaluation and aroma profile levels.

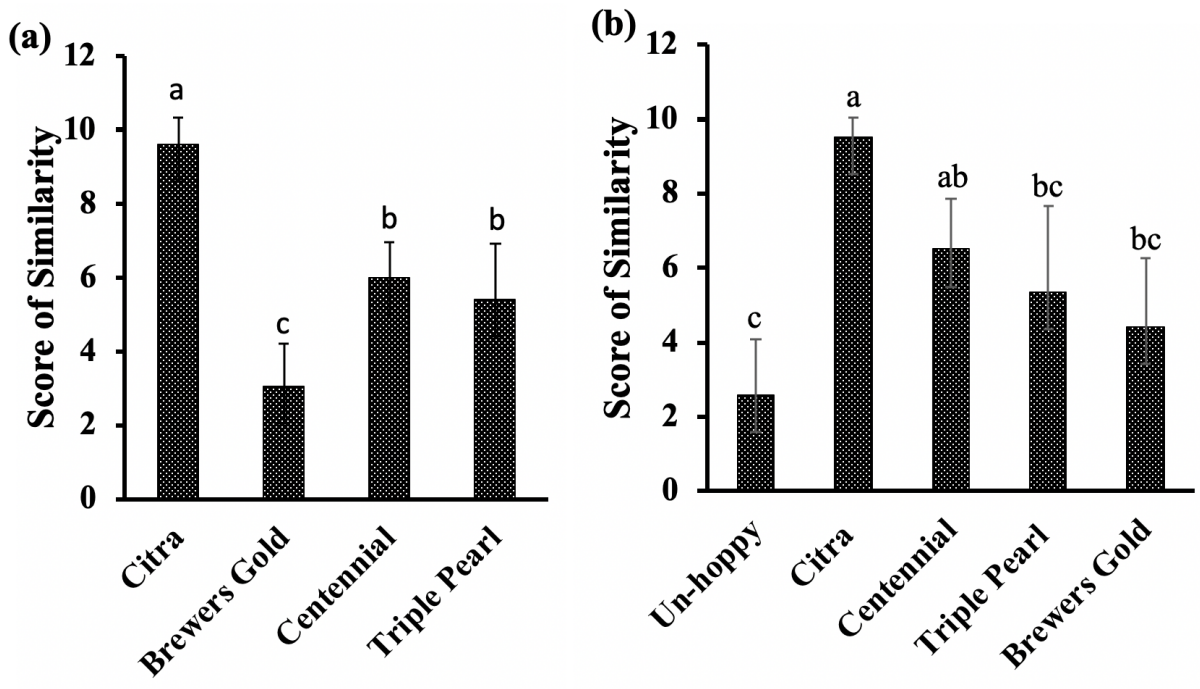

Fig. 3.2. Trained panel (n = 5) of difference testing for Citra (a – hop oil; b - hoppy beer) (Citra was considered as the control). Data are mean ± standard deviation (SD) of triplicate analyses. Different letters represent significant differences among the evaluation group ($p < 0.05$).

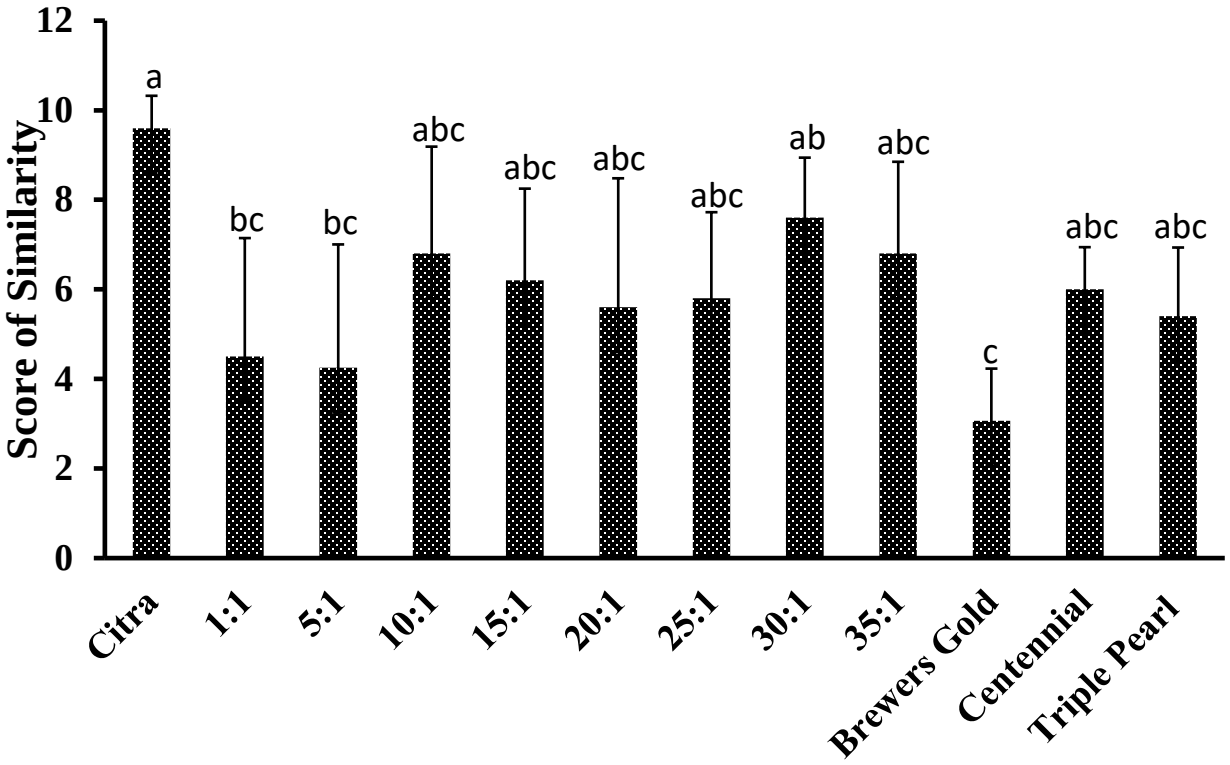

Fig. 3.3. Degree of Difference test (n = 5) for Citra hop oil (1:1, 5:1, 10:1, …, 35:1 are blended models that refer to Table 3.3; Brewers Gold, Centennial, and Triple Pearl are main components for models; Citra was considered as the control). Data are mean ± SD of triplicate analyses. Different letters represent significant differences among the evaluation group ($p < 0.05$).

3.3.2.2 Model optimization based on full profile of hop oils

Although it is clear that the volatiles play the major roles in hop aroma and driving the aromas in beer, both the volatile and nonvolatile compounds could be extracted from hops to hydroalcoholic wort by dry hopping (Schönberger, & Kostelecky, 2011). The nonvolatile compounds may impact the concentration of volatiles in beer, thus playing partly in hop aroma (Goldstein, Ting, Navarro, & Ryder, 1999). Full profiles that include both volatile and nonvolatile components offer the most complete information regarding the hop oils. Therefore, the models based on the full profiles of hop oils have also been conducted.

Table 3.8. Summary of regression analysis.

Variables	Regression coefficient	Response
		Sensory evaluation [d]
Intercept	**ß$_0$**	6.9596 ***
linear		
x_1 [a]	**ß$_1$**	-0.5742 [ns]
x_2	**ß$_2$**	0.0229 [ns]
Quadratic		
x_1^2	**ß$_{11}$**	-0.8044*
x_2^2	**ß$_{22}$**	-0.4103 [ns]
Cross product		
$x_1 x_2$ [c]	**ß$_{12}$**	0.1917 [ns]
Model		P = 0.156
R^2 (%)		72.5
R^2_{adj} (%)		45.0
Lack of fit [e]		insignificant

*** $p < 0.001$ highly significant. * $0.01 < p < 0.05$ significant; ns: $0.05 < p$ not significant. [a] x_1 = weights of first rank compounds. [b] x_2 = weights of second rank compounds. [c] x_1x_2 = Interaction term of the weights of first and second rank compounds [d] The degree of difference test. [e] Lack of fit is model error

To optimize the effect of weights of different rank compounds on the lowest difference of full-profile models, sensory evaluation was carried out by trained panelists. **Table 3.2** shows the experimental results of the difference from control (Citra) observed under the investigated weights for both first and second ranks compounds of full-profile models. The experimental data were fitted to second-order polynomial model in two factor CCD described in Eq. (1). The regression analysis of model parameters is summarized in **Table 3.8**. The *p* value related to the F test for regression was not highly significant ($p = 0.156$). However, as seen in **Table 3.8**, the coefficients for quadratic variable, namely, weights on the first rank compounds (key aroma compounds) had the significant influence on the sensory evaluation (higher scores), while all of the linear terms for variable, weights on the second rank compounds (second intense compounds), had no significant influence. The difference before and after weighing the first rank compounds based on RSM design

can also be seen in **Figure 3.4**. The weights on the first rank compounds indicated negative effects on the sensory evaluation, which meant the maximum degree (lowest difference) reached with increasing weights, and then decreased. The quadratic model for similarity evaluation of full-profile models could be expressed as following equation:

$$Y=6.9596 - 0.5742\ x_1 - 0.8044\ x_1^2 \quad (2)$$

Therefore, there was no need to weigh the second rank compounds, since they showed insignificant influence. According to the model, the optimal weights for the first rank compounds were 19.54, the optimal sensory degree could be found as 7.11. But there was still a need to validate the optimal parameters of the obtained model for practical application of hop oils in beer.

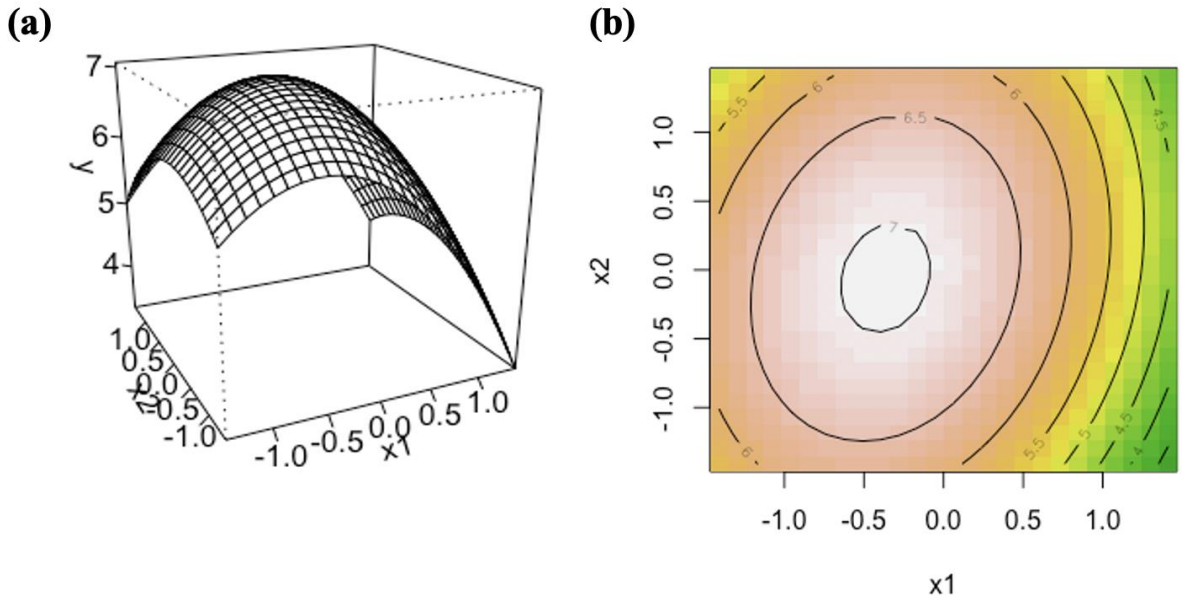

Fig. 3.4. (a) Response surface plot and (b) contour plot for sensory responses as a function of the weights for full-profile models on first rank compounds (x_1) and second rank compounds (x_2).

3.3.3 Validating optimized models based on aroma profile of hop oil and hoppy beer by GC analysis

To evaluate the similarity in the overall aroma of different hop oils or hoppy beer, aroma profile analyses were carried out by GC systems. Although different hop varieties have unique aroma profiles, they all contain similar volatile compounds, differing in quantity of each compound and having specific aroma patterns, apart from a few compounds found only in specific hop varieties (Gros, Nizet, & Collin, 2011). The comparison of overall aroma profiles may represent the differences in aroma characters of selected samples (Inui et al., 2013), thus, the closer the aroma profiles are, the more similar between overall aroma. In addition to visual comparisons, a coefficient of determination (R^2 value) was calculated for selected aroma profiles based on their overall similarity. The R^2 value indicates how well the fitted regression model approximates the real data, in this case, how well the tested aroma profiles approximate Citra. Weighted full-profile and volatile-compound models that optimized in hop oil by difference testing, namely 19.54:1:1 and 30:1, were performed to validate the similarity of aroma profiles in hop oil and hoppy beer. Additionally, the non-weighted models were also carried out to validate the effectiveness of weighting key aroma compounds. The comparison of their aroma profiles with Citra, indicated by the R^2 value, revealed that how similar they are to Citra. The resulting similarity parameters for hop oil and hoppy beer are presented in **Table 3.9** and **Table 3.10**, respectively. The adequacy of the fit of the models was validated by the high R^2 for all blended models in hop oil ($R^2 > 0.99$). Moreover, the application of the models in hoppy beer was also confirmed by a high R^2 (> 0.98) and significant improvements in similarity in comparison with public hop components (Brewers Gold, Centennial, and Triple Pearl). However, the aroma profile similarity of the non-weighted volatile-compound model ($R^2 = 0.8224$) was poor in hoppy beer, even lower than that of

Centennial (R^2 = 0.8966), the public hop. This might be attributed to the fact that hop oil was 12.2 % lower in the non-weighted model than in the optimal weighted model, as illustrated in **Table 3.7**. The aroma intensity of the non-weighted volatile-compound model was lower. Further, the volatile-compound models tended to focus on volatile compounds extracted from headspace, which detected fewer compounds' varieties. In general, the results showed that the models achieved the goal of improving the aroma similarity with Citra hops better than the public hop varieties. Additionally, the full-profile models were found to be more similar and stable than volatile-compound models.

Table 3.9. R squared values for aroma profile similarity of hop oil between blended models (Full-profile and Volatile-compound models) and Citra hop oil.

Proprietary hops	**Full-profile model [a]**		**Volatile-compound model [b]**	
	Non-weighted (1:1:1)	**Optimal weighted (19.54:1:1)**	**Non-weighted (1:1)**	**Optimal weighted (30:1)**
Citra	0.9962	0.9534	0.9903	0.9927

[a]The optimal weighted full-profile model (19.54:1:1) refers to Table 3.5, obtaining the highest sensory scores (lowest difference degree); non-weighted model (1:1:1) means all the aroma compounds detected from full profile are non-weighted.

[b]The optimal weighted volatile-compound model (30:1) refers to Figure 3.3, obtaining the highest sensory scores (lowest difference degree); non-weighted model (1:1) means all the aroma compounds detected from the volatile profile are non-weighted.

Table 3.10. R squared values for aroma profile similarity of hoppy beers between blended models (Full-profile and Volatile-compound models), public hoppy beers and Citra hoppy beer.

Proprietary hops	**Full-profile model [a]**		**Volatile-compound model [b]**		**Public hoppy beer [c]**		
	1:1:1	**19.54:1:1**	**1:1**	**30:1**	**Brewers gold**	**Centennial**	**Triple Pearl**
Citra	0.9807	0.9941	0.8224	0.9977	0.3130	0.8966	0.4267

[a]The optimal weighted full-profile model (19.54:1:1) refers to Table 3.5, obtaining the highest sensory scores (lowest difference degree); non-weighted model (1:1:1) means all the aroma compounds detected from full profile are non-weighted.

[b]The optimal weighted volatile-compound model (30:1) refers to Figure 3.3, obtaining the highest sensory scores (lowest difference degree); non-weighted model (1:1) means all the aroma compounds detected from the volatile profile are non-weighted.

[c]The public hops, Brewers Gold, Centennial, and Triple Pearl, are hops varieties for the main components of blended models.

3.3.4 Validating optimized models based on sensory evaluation of hoppy beer

3.3.4.1 Hopping regimes and sensory training for beer

It is clear that volatiles derived from hops strongly contribute the "hoppy" aroma to beer by dry hopping or late hopping, not only the bitterness contribution (Rettberg, Biendl, & Garbe, 2018). Humulones can be isomerized under different hopping regimes, but cold brewing by dry hopping reduces the level of isomerization, which results in a less bitterness and more intense aroma (Lafontaine, Pereira, Vollmer, & Shellhammer, 2018). Thus, dry hopping that prevents fermentation processes or hops volatiles from evaporating was carried out to compare hop aromas in hoppy beers (Fritsch, & Schieberle, 2003). The wort temperature, scale, and contact time were controlled while hop oil was added at the same level to the fermented wort. Aside from the similarity analyzed by instruments, the difference testing can be used to determine the difference of hoppy beers from Citra. Five panelists were well-trained, showing the good identification ability for Citra aroma and "hoppy" aroma in beer (Figure 3.2(b)). Panelists were capable to distinguish Citra aroma and recognize the un-hoppy beer as significant difference ($p < 0.05$) from "hoppy" aroma.

3.3.4.2 Sensory results analysis

Four beer samples, including optimal weighted and non-weighted volatile-compound models (30:1 and 1:1) and optimal weighted and non-weighted full-profile models (19.54:1:1 and 1:1:1), were evaluated the difference from Citra hoppy beer (**Table 3.11**). The overall average of all evaluations for each model was not significant different as the large standard deviation. However, it is clear that the full-profile models obtained the relatively higher scores (lower difference) and smaller standard deviations. Furthermore, the non-weighted full-profile model

(1:1:1) achieved the highest scores and lowest standard deviation (7.93 ± 1.10). Due to the fact that the overall average of all evaluations may mask the characteristics of the assessment and dramatically increase the standard deviation, we also showed the sensory results for each panelist. Compared with volatile-compound models, the panelists rated full-profile models with a lower degree of difference. Four panelists selected the non-weighted full-profile model for lower difference, while three selected the weighted full-profile model, and only two selected the volatile-compound model. The full-profile models achieved lower difference to some extent. Moreover, the models with weights on key aroma compounds did not greatly decrease the difference of aroma characters in hoppy beer, even though they were optimized and confirmed in hop oil by sensory tests. A possible reason could be the secondary transformation of hops during the brewing process which alters the aroma characteristics of hops differently than those of beers, as observed in several papers (Kishimoto, Wanikawa, Kono, & Shibata, 2006; Sharp, Qian, Shellhammer, & Shellhammer, 2017; Wietstock, Gotz, Klie, Methner, & Scheuren, 2015). Transformation may result in different sensory perception and thresholds; besides, the final concentrations of some compounds may be below the odor threshold that could be captured (Hanke, Herrmann, Rückerl, Schönberger, & Back, 2008). Additionally, additive, synergistic or masking effects on the interactions of some of the aroma compounds challenge the sensory impression (Schönberger, & Kostelecky, 2011; Hanke et al., 2008). Overall, non-weighted full-profile model produced the lowest difference, as confirmed by not only instrumental analyses but also sensory evaluation of overall aroma characters. The best model for modeling aromatic character profiles of Citra can be described by 35.2% Brewers Gold, 5.2 % Cashmere, 32 % Centennial, and 35.7 % Triple Pearl (the model formulae was not included in table).

Table 3.11. Sensory evaluation (n= 5) of hoppy beer for optimal weighted models and non-weighted models with Citra hoppy beer.

Panelists	Similarity evaluation				Evaluation results (Scores > 7) [a]			
	Full-profile model [b]		Volatile-compound model [c]		Full-profile model		Volatile-compound model	
	1:1:1	19.54:1:1	1:1	30:1	1:1:1	19.54:1:1	1:1	30:1
1	6.67 ± 0.57	8.00 ± 0.00	6.33 ± 0.58	7.67 ± 0.58		√		√
2	7.00 ± 0.00	8.00 ± 0.00	6.00 ± 0.00	5.00 ± 0.00	√	√		
3	8.00 ± 0.00	5.67 ± 0.58	6.00 ± 1.00	5.00 ± 1.00	√			
4	9.33 ± 0.58	5.67 ± 0.58	9.33 ± 0.58	6.33 ± 0.58	√		√	
5	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	9.33 ± 1.15	√	√	√	√
Overall	7.93 ± 1.10 [a]	7.20 ± 1.37 [a]	7.27 ± 1.58 [a]	6.67 ± 1.84 [a]				

[a]The selected models were evaluated with lower difference for each panelist.

[b]The optimal weighted full-profile model (19.54:1:1) refers to Table 3.5, obtaining the highest sensory scores (lowest difference degree); non-weighted model (1:1:1) means all the aroma compounds detected from full profile are non-weighted.

[c]The optimal weighted volatile-compound model (30:1) refers to Figure 3.3, obtaining the highest sensory scores (lowest difference degree); non-weighted model (1:1) means all the aroma compounds detected from the volatile profile are non-weighted.

Sensory data for each panelist are mean ± SD of triplicate analyses.

Overall data for models are mean ± SD of each panelist and analyses triplicated. The same letter represents non-significant differences among different models ($p>0.05$).

3.4 Conclusions

The results of aroma profile similarity and sensory evaluation suggested that the non-weighted full-profile model was optimal for modeling the aroma characteristics of Citra hops. In general, the non-weighted full-profile model achieved better similarity in hoppy beer by well-trained panelists as well as higher R^2 values that indicated aroma profile similarity in hop oil and hoppy beer. Furthermore, the GC-based full-profile model had a higher stability and better effectiveness than the HS-SPME-GC based model. This indicates that full profiles captured by the GC techniques could adequately portray hops' aroma characteristics. In addition, results have shown that when modeling hops' overall aroma profiles, there is no need to focus on the key aroma compounds (ß-Myrcene, linalool, β-caryophyllene, α-humulene, and geraniol). Although the approval of optimal weighted models in hop oil demonstrated that key aroma compounds contribute to the optimal profiles, the validation of these models in beer did not support the

effectiveness of weighting key aroma compounds. This shows that weights do not need to be input in the QP program for key aroma compounds to model the aroma due to the making or additive effects of beer on some aroma compounds of hops. In the future, understanding the transformation of aroma compounds during brewing processes and the degrees of aroma contribution in hops and beer may aid in improving the modeling accuracy and focusing on shortages. With expanding the pool of public hops, the aroma characters of proprietary hops can be modeled with improved accuracy using the combination of mathematical optimization method and flavor-detection technology.

3.5 References

Almaguer, C., Schönberger, C., Gastl, M., Arendt, E. K., & Becker, T. (2014). Humulus lupulus–a story that begs to be told. A review. *Journal of the Institute of Brewing*, *120*(4), 289-314.

Brendel, S., Hofmann, T., & Granvogl, M. (2019). Characterization of Key Aroma Compounds in Pellets of Different Hop Varieties (Humulus lupulus L.) by Means of the Sensomics Approach. *Journal of agricultural and food chemistry*, *67*(43), 12044-12053.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*, *76*(5), 965-977.

Caron, S. (n.d.). *Quadratic Programming in Python* [online] Available at: https://scaron.info/blog/quadratic-programming-in-python.html. Accessed 18 January 2021. Accessed 18 January 2021.

Compusense.com. (2020). *Quality Assurance with Difference from Control Testing.* [online] Available at: http://compusense.com/wp-content/uploads/2020/03/Difference_from_Control_Testing_White_Paper.pdf Accessed 12 September, 2021.

Ceola, D., Huelsmann, R. D., Da-Col, J. A., & Martendal, E. (2019). Headspace-solid phase microextraction and GC-MS followed by multivariate data analysis to study the effect of hop processing type and dry hopping time on the aromatic profile of top-fermented beers. *Separation Science Plus*, *2*(7), 245-252.

Chen, M. J., Chen, K. N., & Lin, C. W. (2004). Sequential Quadratic Programming for Development of a New Probiotic Dairy Tofu with Glucono-δ-Lactone. *Journal of food science*, *69*(7), 344-350.

Charry-Parra, G., DeJesus-Echevarria, M., & Perez, F. J. (2011). Beer volatile analysis: optimization of HS/SPME coupled to GC/MS/FID. *Journal of food science*, *76*(2), C205-C211.

Duarte, L. M., Amorim, T. L., Grazul, R. M., & de Oliveira, M. A. L. (2020). Differentiation of aromatic, bittering and dual-purpose commercial hops from their terpenic profiles: An approach involving batch extraction, GC–MS and multivariate analysis. *Food Research International*, *138*, 109768.

Dresel, M., Praet, T., Van Opstaele, F., Van Holle, A., Naudts, D., De Keukeleire, D., ... & Aerts, G. (2015). Comparison of the analytical profiles of volatiles in single-hopped worts and beers as a function of the hop variety. *Brewing Science*, *68*(1-2), 8-28.

Eri, S., Khoo, B. K., Lech, J., & Hartman, T. G. (2000). Direct thermal desorption− gas chromatography and gas chromatography− mass spectrometry profiling of hop (Humulus lupulus L.) essential oils in support of varietal characterization. *Journal of Agricultural and Food Chemistry*, *48*(4), 1140-1149.

Eyres, G. T., Marriott, P. J., & Dufour, J. P. (2007). Comparison of odor-active compounds in the spicy fraction of hop (Humulus lupulus L.) essential oil from four different varieties. *Journal of agricultural and food chemistry*, *55*(15), 6252-6261.

Fritsch, H. T., & Schieberle, P. (2003). Changes in key aroma compounds during boiling of unhopped and hopped wort. In *Proc. 29th EBC Congress*.

Fritsch, H. T., & Schieberle, P. (2005). Identification based on quantitative measurements and aroma recombination of the character impact odorants in a Bavarian Pilsner-type beer. *Journal of Agricultural and Food Chemistry*, *53*(19), 7544-7551.

Forteschi, M., Porcu, M. C., Fanari, M., Zinellu, M., Secchi, N., Buiatti, S., ... & Pretti, L. (2019). Quality assessment of Cascade Hop (Humulus lupulus L.) grown in Sardinia. *European Food Research and Technology*, *245*(4), 863-871.

Gros, J., Nizet, S., & Collin, S. (2011). Occurrence of odorant polyfunctional thiols in the super alpha Tomahawk hop cultivar. Comparison with the thiol-rich Nelson Sauvin bitter variety. *Journal of agricultural and food chemistry*, *59*(16), 8853-8865.

Goldstein, H., Ting, P., Navarro, A., & Ryder, D. (1999). Water-soluble hop flavor precursors and their role in beer flavor. 27th European Brewery Convention; Cannes, France.

Hanke, S., Herrmann, M., Rückerl, J., Schönberger, C., & Back, W. (2008). Hop volatile compounds (Part II): Transfer rates of hop compounds from hop pellets to wort and beer. *Brew. Sci*, *61*, 140-147.

Inui, T., Tsuchiya, F., Ishimaru, M., Oka, K., & Komura, H. (2013). Different beers with different hops. Relevant compounds for their aroma characteristics. *Journal of agricultural and food chemistry*, *61*(20), 4758-4764.

Kishimoto, T., Wanikawa, A., Kagami, N., & Kawatsura, K. (2005). Analysis of hop-derived terpenoids in beer and evaluation of their behavior using the stir bar− sorptive extraction method with GC-MS. *Journal of agricultural and food chemistry*, *53*(12), 4701-4707.

Kishimoto, T., Wanikawa, A., Kono, K., & Shibata, K. (2006). Comparison of the odor-active compounds in unhopped beer and beers hopped with different hop varieties. *Journal of agricultural and food chemistry*, *54*(23), 8855-8861.

Kovačevič, M., & Kač, M. (2002). Determination and verification of hop varieties by analysis of essential oils. *Food Chemistry*, *77*(4), 489-494.

Killeen, D. P., Watkins, O. C., Sansom, C. E., Andersen, D. H., Gordon, K. C., & Perry, N. B. (2017). Fast sampling, analyses and chemometrics for plant breeding: bitter acids, xanthohumol and terpenes in lupulin glands of hops (Humulus lupulus). *Phytochemical Analysis*, *28*(1), 50-57.

Lafontaine, S. R., Pereira, C. B., Vollmer, D. M., & Shellhammer, T. H. (2018). The effectiveness of hop volatile markers for forecasting dry-hop aroma intensity and quality of Cascade and Centennial hops. *Brewing Science*, *71*, 116.

Ligor, M., Stankevičius, M., Wenda-Piesik, A., Obelevičius, K., Ragažinskienė, O., Stanius, Ž., ... & Buszewski, B. (2014). Comparative gas chromatographic–mass spectrometric evaluation of hop (Humulus lupulus L.) essential oils and extracts obtained using different sample preparation methods. *Food Analytical Methods*, *7*(7), 1433-1442.

Mongelli, A., Rodolfi, M., Ganino, T., Marieschi, M., Caligiani, A., Dall'Asta, C., & Bruni, R. (2016). Are Humulus lupulus L. ecotypes and cultivars suitable for the cultivation of aromatic hop in Italy? A phytochemical approach. *Industrial crops and products*, *83*, 693-700.

Neiens, S. D., & Steinhaus, M. (2018). Odor-active compounds in the special flavor hops Huell Melon and Polaris. *Journal of agricultural and food chemistry*, *66*(6), 1452-1460.

Nance, M. R., & Setzer, W. N. (2011). Volatile components of aroma hops (Humulus lupulus L.) commonly used in beer brewing. *Journal of Brewing and Distilling*, *2*(2), 16-22.

Pinho, O., Ferreira, I. M., & Santos, L. H. (2006). Method optimization by solid-phase microextraction in combination with gas chromatography with mass spectrometry for analysis of beer volatile fraction. *Journal of chromatography A*, *1121*(2), 145-153.

Praet, T., Van Opstaele, F., Jaskula-Goiris, B., Aerts, G., & De Cooman, L. (2012). Biotransformations of hop-derived aroma compounds by Saccharomyces cerevisiae upon fermentation. *Cerevisia*, *36*(4), 125-132.

Persson, M., Fagt, S., & Nauta, M. J. (2019). Optimising healthy and safe fish intake recommendations: a trade-off between personal preference and cost. *British Journal of Nutrition*, *122*(2), 206-219.

Persson, M., Fagt, S., Pires, S. M., Poulsen, M., Vieux, F., & Nauta, M. J. (2018). Use of mathematical optimization models to derive healthy and safe fish intake. *The Journal of nutrition*, *148*(2), 275-284.

Rettberg, N., Biendl, M., & Garbe, L. A. (2018). Hop aroma and hoppy beer flavor: chemical backgrounds and analytical tools—a review. *Journal of the American Society of Brewing Chemists*, *76*(1), 1-20.

Sharp, D. C., Qian, Y., Shellhammer, G., & Shellhammer, T. H. (2017). Contributions of select hopping regimes to the terpenoid content and hop aroma profile of ale and lager beers. *Journal of the American Society of Brewing Chemists*, *75*(2), 93-100.

Schönberger, C., & Kostelecky, T. (2011). 125th anniversary review: The role of hops in brewing. *Journal of the Institute of Brewing*, *117*(3), 259-267.

Vollmer, D. M., Lafontaine, S. R., & Shellhammer, T. H. (2018). Aroma extract dilution analysis of beers dry-hopped with Cascade, Chinook, and Centennial. *Journal of the American Society of Brewing Chemists*, *76*(3), 190-198.

Van Dooren, C. (2018). A review of the use of linear programming to optimize diets, nutritiously, economically and environmentally. *Frontiers in nutrition*, *5*, 48.

Van Opstaele, F., Praet, T., Aerts, G., & De Cooman, L. (2013). Characterization of novel single-variety oxygenated sesquiterpenoid hop oil fractions via headspace solid-phase microextraction and gas chromatography–mass spectrometry/olfactometry. *Journal of agricultural and food chemistry*, *61*(44), 10555-10564.

Vázquez-Araújo, L., Parker, D., & Woods, E. (2013). Comparison of temporal–sensory methods for beer flavor evaluation. *Journal of Sensory Studies*, *28*(5), 387-395.

Wietstock, P. C., Gotz, F., Klie, R., Methner, F. J., & Scheuren, H. (2015). Feasibility study using a novel desorption/absorption process for transferring hop volatiles to beer. *BREWING SCIENCE*, *68*(1-2), 1-7.

Westrich, B. J., Altmann, M. A., & Potthoff, S. J. (1998). Minnesota's Nutrition Coordinating Center uses mathematical optimization to estimate food nutrient values. *Interfaces*, *28*(5), 86-99.

Worthington, R. (2010). *Why Proprietary Hops?*. [online] Available at: http://inhoppursuit.blogspot.com/2010/01/why-proprietary-hops.html. Accessed 12 May 2020.

Xin, J., Negenborn, R. R., & Lin, X. (2018). Piecewise affine approximations for quality modeling and control of perishable foods. *Optimal Control Applications and Methods*, *39*(2), 860-872.

Chapter 4 Mathematical modeling of the aroma profiles of hops: An approach integrated instrumental analysis, sensory evaluation and quadratic programming

Abstract

The aroma profiles isolated from proprietary hops, Citra, Simcoe and Mosaic, and characterized by gas chromatography with flame-ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS), were subjected to modeling with blended public hops. Based on instrumental analysis with GC and sensory evaluation, the obtained models were validated in hops and hoppy beer, showing significantly improved sensory similarity and a good degree of aroma profile similarity ($R^2 > 0.93$). The aroma profile of the hoppy beer and hop oil was significantly positive related to their smell flavor, which resulted in the R^2 factor range of 61.4% to 87.6%, indicating the more similar aroma profiles lead to closer smell flavor. All three blended models exhibited higher similarity to proprietary hop aromas than individual hop, confirming the feasibility of aroma modeling through the combination of mathematical optimization and flavor-detection techniques.

Keywords: Hop aromas, Beer, Flavor models, Quadratic program, GC-MS, HS-SPME-GC-FID, Sensory evaluation

4.1 Introduction

Hops are wildly used to produce the attractive aromas of beer during the brewing process. Currently, Citra, Simcoe and Mosaic are three of the most coveted dual-purpose hops among craft brewers and homebrewers for their excellent aroma. Recently, numerous researches have been explored the aroma of hops and the hop-derived aromas in beer, as a function of cultivar (Vázquez-Araújo, Rodríguez-Solana, Cortés-Diéguez, & Domínguez, 2013; Dong et al., 2015), geographic region (Forster, & Gahr, 2014), environment and weather (Forteschi et al., 2019), planting processes (Sharp, Townsend, Qian, & Shellhammer, 2014). Additionally, it has been widely reported that hop aroma can be detected using GC systems that have high sensitivity and capacity. However, there is no previous research focusing on exploring the blended combination to match the specific hops aroma according to their unique aroma profiles.

The aroma contribution of hops itself is not the same as that in hoppy beer. Pinho, Ferreira, and Santos (2006) have found that some volatiles contribute the background aroma of beer and do not significantly contribute to the beer flavor, which make the complexity of the characteristic aroma of beer. In addition, the additive, synergistic or masking effects that exist among the beer aroma substances (Praet, Van Opstaele, Jaskula-Goiris, Aerts, & De Cooman, 2012) may affect the evaluation of hop-derived aromas. Therefore, it is crucial to understand how hop aroma attributes affect beer. Headspace Solid-phase microextraction and Gas Chromatography (HS-SPME-GC) is a well-documented method to analyze beer aromas, preventing contamination of instruments with nonvolatile substances (Charry-Parra, DeJesus-Echevarria, & Perez, 2011). Regarding modeling techniques, the method QP was used to model the aroma profiles of proprietary hops obtained from GC.

Several studies have extensively examined the relationship between analytical and sensory aspects of hoppy aroma, mainly according to hop aromatization technology (Van Opstaele, De Rouck, De Clippeleer, Aerts, & De Cooman, 2010) and hop varieties (Mikyška et al., 2018). Their correlations were successfully explored and defined. However, there was no discovery focusing on the relationship between the similarity of analytical and sensory evaluation of hoppy beer. Therefore, the possible relationship between the similarity of analytical profiles of hops, in addition to hoppy beer, and sensory of hoppy beer, was investigated in this study. Moreover, the reveal of the potential correlation may support the feasibility of modeling aromas.

This study aimed to (1) create the blended models through the combination of QP and flavor-detection techniques for matching the aromas of proprietary hops (Citra, Simcoe, Mosaic), (2) validate the models at the level of hops and hoppy beer by sensory evaluation and aroma profile analysis, (3) investigate the relationship between the results of sensory evaluation and aroma profiles analysis, and (4) explore the potential highly-impact aroma compounds for specific proprietary hops that help to understand their characters in the future. Overall, we conducted the aroma profiles of hops by GC to model the aroma profiles of proprietary hops by QP, and then validated the results in hops and hoppy beer through aroma profile analysis and sensory evaluation (**Fig. 4.1**). This study is important for exploring the future replacement of proprietary hops and guiding the novel direction of modeling knowledge for aromas through the combination of mathematical optimization techniques and flavor-detection methods.

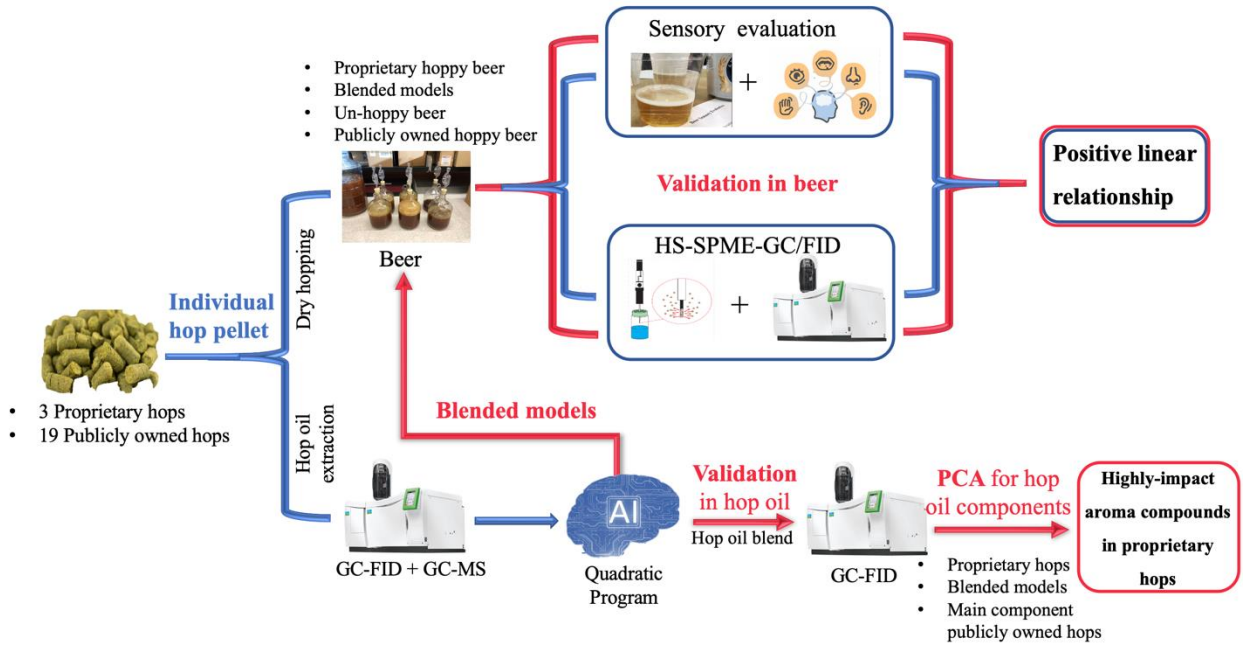

Fig. 4.1. Experiment design of the objective 2.

4.2 Materials and methods

4.2.1 Materials

Hop varieties used for investigating were shown in **Table 4.1**. Three proprietary hop varieties, Simcoe, Citra, and Mosaic were purchased from Yakima Valley Hops (Yakima, WA, USA). Nineteen public hop varieties were purchased from the retail markets, including eight varieties, Apollo, Calypso, Us Goldings, Eureka!™, Vanguard, Us Fuggle, Centennial that were purchased from Northern Brewer (Minnesota, USA), Summit was purchased from Yakima Valley Hops (Yakima, WA, USA), and eleven varieties, Cascade, Chinook, Cluster, Tahoma, Saaz, Triple Pearl, Columbus, Brewers Gold, Galena, Nugget, and Crystal that were purchased from Midwest Hop Producers (Nebraska, USA). Briess Gold Malt Extract Syrup, LalBrew® BRY-97 American West Coast Ale Dry Yeast, and Cascade hopshot™ hops extract were purchased from Northern Brewer (Roseville, MN, USA) for brewing beer.

Table 4.1 Hop varieties used for investigating in this study

Variety	Alpha acid	Beta acid	Year	Purpose	Aroma characters	Retailers or Wholesalers
Proprietary hops						
Simcoe®	13.3%	NA	2019	Dual-Purpose	Passionfruit, pine, earthy, citrus	Yakima Valley Hops., LLC
Citra®	18.5%	NA	2019	Dual-Purpose	Citrus, lime, gooseberry, passionfruit, grapefruit	Yakima Valley Hops., LLC
Mosaic®	17.5%	NA	2019	Dual-Purpose	Citrus, pine, earth, herbal, blueberry, peach, tropical fruit	Yakima Valley Hops., LLC
Public hops						
Apollo	12.1%	NA	2019	Bittering	Lime, grapefruit, pine, resin	Northern Brewer., LLC
Calypso	12.0%	NA	2019	Dual-Purpose	Florals, fruity (lemon zest, dried apple and pear), citrus	Northern Brewer., LLC
Us Goldings	4.3%	NA	2019	Aroma	Mild, delicate with sweet floral	Northern Brewer., LLC
Eureka!™	18.0%	NA	2019	Dual-Purpose	Dark fruits, strong herbal notes, pine tree, resin	Northern Brewer., LLC
Vanguard	5.4%	NA	2019	Aroma	Herbal and floral tones	Northern Brewer., LLC
Us Fuggle	4.9%	NA	2019	Aroma	Mild woody, floral, grassy, herbal	Northern Brewer., LLC
Summit	15.0%	NA	2019	Bittering	Spicy, herbal, citrus, onion/garlic, grassy	Yakima Valley Hops., LLC
Centennial	8.6%	NA	2019	Dual-Purpose	Pine, earthy, floral and citrus (lemon)	Northern Brewer., LLC
Cluster	5.33%	3.18%	2020	Dual-Purpose	Spicy, floral, fruity, earthy	Midwest Hop Producers., LLC
Cascade	5.75%	5.15%	2020	Dual-Purpose	Floral, zesty grapefruit, spicy citrus	Midwest Hop Producers., LLC
Chinook	10.31%	2.54%	2020	Dual-Purpose	Citrus, spicy, piney	Midwest Hop Producers., LLC
Tahoma	3.19%	3.86%	2020	Aroma	Lemon citrus, orange, wood, spicy	Midwest Hop Producers., LLC
Saaz	5.60%	4.62%	2020	Aroma	Mild, earthy, herbal, spicy	Midwest Hop Producers., LLC
Triple Pearl	8.16%	2.17%	2020	Dual-Purpose	Orange citrus, melon, resin, spicy, black pepper	Midwest Hop Producers., LLC
Columbus	8.95%	2.95%	2020	Dual-Purpose	Earthy, spicy, black pepper, subtle citrus	Midwest Hop Producers., LLC
Brewers Gold	5.20%	2.67%	2020	Bittering	Spicy, blackcurrant, fruity	Midwest Hop Producers., LLC
Galena	8.77%	5.53%	2020	Aroma	Spicy, citrus, blackcurrant, fruity, herbal	Midwest Hop Producers., LLC
Nugget	7.77%	4.88%	2020	Bittering	Mild, pleasant, spicy, herbal	Midwest Hop Producers., LLC
Crystal	3.91%	4.82%	2020	Aroma	Woody, pine, citrus, spicy, floral	Midwest Hop Producers., LLC

NA: Not available

4.2.2 Reagents

β-pinene, β-myrcene, β-caryophyllene, α-humulene, linalool, geraniol, methyl octanoate, methyl nonanoate and 3-methylbutanoic acid standards, with a purity above 95%, were supplied by Sigma-Aldrich (St. Louis, MO, USA). Standards were used to establish a calibration curve to transfer the peak area of individual aroma compounds to concentration united as μg/mL, and

further calculate the analyte concentration in hop oils. All analytes were dissolved in hexanes for quantifying the volatile composition in hop oils. All other reagents used were of analytical grade. Hexane (HEX) and sodium chloride were acquired from Sigma-Aldrich (St. Louis, MO, USA). As external standards, β-myrcene was used for quantifying of β-myrcene; β-pinene was used for other monoterpenes; β-caryophyllene was used for quantifying of β-caryophyllene and its oxidation products; α-humulene was used for other sesquiterpenes; geraniol was used for quantifying of geraniol; linalool was used for other terpene alcohols; methyl octanoate was used for quantifying of methyl octanoate; 3-methylbutanoic acid was used for acids; methyl nonanoate was used for all other compounds.

4.2.3 Hop oils preparation

Hop pellets were grounded using a small analytical mill. The extractions were carried out with hexane at a hop:hexane ratio of 1:2 (w:v) for 12 hours in 50 mL centrifuge tubes. At the end of extraction, the upper liquid was separated, centrifuged and then filtered to obtain pure liquid. All the extracted hop oils were stored in auto-sampler vials at -20 °C for posterior analysis. Each extraction was undertaken in triplicate.

4.2.4 Hop oils analysis

The hop oil composition was quantified by gas chromatography (GC) analyses. Each analysis was undertaken in triplicate using different vials. The quantification analyses were performed on an HP Agilent 6890 Series GC system (Agilent Technologies Co. Ltd., Santa Clara, CA, USA) equipped with automatic sampler and flame ionization detector (FID). Helium was used as the carrier gas with a flow rate of 1 mL/min. The components were separated on a DB-Wax UI

column (30 m × 0.25 mm × 0.25 μm). The injector and detector temperatures were set at 250 and 260 °C, respectively. The injection volume is 3 μL. The inlet mode is splitless. The chromatographic elution was temperature programmed as follows: isothermal at 50 °C (1 min), then increased at a rate of 5 °C/min to 240 °C and hold 5 min.

The hop oil composition was qualified by GC coupled with mass spectrometry (MS) using Thermo Scientific™ ISQ™ 7000 Single Quadrupole GC-MS System equipped with a DB-Wax UI column (30 m × 0.25 mm × 0.25 μm). The ion source temperature was set at 200 °C and the spectra was operated in the electron impact mode at 70 eV over a scan range of 50–500 m/z. Compound identifications were made by searching MAIN MS data library (a match quality of 95% minimum was used as a criterion) and comparison of their retention indexes (RIs) with the published data. Other parameters were same as that used for quantification.

4.2.5 Model the aroma of proprietary hops

Quadratic program (QP) was performed in Python using CVXOPT and QUADPROG solvers to build blended models for proprietary hops based on modeling the concentration of individual aroma compounds (Caron, 2021). Blended models made from hops pellets (by mass) were formulated by combining the percentages of public hops (e.g. proprietary hop A = x% public hop B + y% public hop C + z% public hop D). The QP minimized the sum of squared difference between analyte concentrations from proprietary hops and the mixture of public hops when the aroma profiles of public hops and proprietary hops were input in QP. The blended models were outputted as the mixture of percentages of different public hops. Generally, QP minimized a quadratic function based on the difference between target aroma profiles and the sum of input aroma profile percentages, with linear constraints applied to individual analyte concentrations.

4.2.6 Beer preparation

Three proprietary hoppy beers, three blended model hoppy beers, un-hoppy beer and several public hoppy beers (the main components for blended models) were prepared as follows.

1) Mashing: adding 5.5 lbs. malt extract syrup to 5 gallons water to adjust the brix^{o} equals to 10.1 at 152 °F.
2) Kettle: adding 3 mL hopshot^{TM} to achieve approximate 30 IBUs based on Cascade hopshot^{TM} instruction (10 IBU/mL is needed for 60 min boiling addition for 5 gallons of beer at 1.04 specific gravity) and boiling for 60 min. Finally, adjusting the brix^{o} of wort equaled to 10.46.
3) Fermentation: adding American ale dry yeast (2 g/gallon wort) and storing at 18 °C for 7 days and then at 4 °C for 4 days.
4) Dry hopping: fermented wort was separated into several 500 mL batches, 10g hop pellets of each hop variety were added to fermented wort, no hop pellets required for un-hoppy beer, and then the beers were stored at 18 °C for 7 days.
5) Bottling: the hop pellets were filtered, and the filtered beers were stored in 1 L brown bottles for posterior analysis at 4 °C.

Each beer with different hop varieties was undertaken using the same wort under the same kettle and fermentation conditions in a 500 mL bottle.

4.2.7 Beer analysis

4.2.7.1 Aroma profile analysis by HS-SPME-GC-FID method

The volatile composition of beer was quantified by headspace solid phase microextraction (HS-SPME) coupled to GC-FID based on the method of Pinho et al. (2006). The extraction of volatile compounds from headspace was performed using HS-SPME technique with 75 μm carboxen/polydimethylsiloxane (CAR-PDMS) fiber (Supelco, Bellefonte, PA, USA). The selection of fiber was in order to concentrate the sample effectively and extract the higher variety of volatile compounds. 5 g each beer sample and 2 g NaCl in a 15 mL vial were equilibrated for 30 min at 20 °C in an ultrasonic bath followed by SPME fiber exposure to the headspace for 30 min. The trapped aroma compounds were desorbed by maintaining the fiber exposes to injection port of GC system for 10 min, in a splitless mode. The chromatographic analyses were performed on an HP Agilent 6890 Series GC system (Agilent Technologies Co. Ltd., Santa Clara, CA, USA). Helium was used as the carrier gas with a flow rate of 1 mL/min. The components were separated on a DB-Wax UI column (30 m × 0.25 mm × 0.25 μm). The injector and detector temperatures were set at 250 and 260 °C, respectively. The chromatographic elution was temperature programmed as follows: isothermal at 50 °C (1 min), then increased at a rate of 5 °C /min to 200 °C.

4.2.7.2 Sensory evaluation of beer

Sensory evaluation was implemented by five well-trained panelists to assess the difference between proprietary hops and their blended models. A total of 10 ml of each beer sample was served in the plastic cups with covers and labeled using 3-digit random codes to avoid bias for posterior uses. All beer samples were equilibrated at room temperature (around 20 °C) for 15 minutes before evaluation to maximize the aroma and minimize temperature effects.

Difference test

The blended model hoppy beer, public hoppy beer (the main components for blended models) and un-hoppy beer were evaluated the overall aroma difference from their proprietary hoppy beer (control) using 10 – point difference from control scale: 1 = completely different and 10 = same (Compusense.com., 2020). Additionally, panelists were asked to rate the similarity of beer samples with proprietary hoppy beers using a rank scale that depended on the number of samples. The evaluation for each proprietary hop was undertaken using different beer batches triplicated. Coffee beans were used to refresh the nose between two sample evaluations.

Discrimination test

Triangle testing was performed posterior to difference from control evaluation to determine if the detectable aroma differences were present between the proprietary hoppy beers and their blended models. The beer samples were served as the possible arrangements that presented randomly for each panelist: AAB, ABA, BAA, BBA, BAB and ABB, where A is proprietary hoppy beer and B is their blended model, given the same beer samples different random codes. The well-trained panelists smelled three samples at a time from left to right and picked the sample that was different from the others. Panelists were allowed to re-evaluate samples only in accordance with their assigned presentation order. Coffee beans were used to refresh the nose between two sample evaluations. The results were assessed based on the ratio of total right judgments and total judgments according to the 5% statistical significance ($p \leq 0.05$).

4.2.8 Statistical procedures

Analysis of variance (ANOVA) with judge as a fixed factor and product as a random factor was used to compare the means of the sensory results and determine the difference. Tukeys' HSD

(honestly significant difference) *posthoc* analyses (at a 5% significance level) were conducted to identify significant differences among the evaluations for each proprietary hop. R squared value was used to evaluate the positive correlation between the aroma profiles of different hop varieties to indicate the similarity of the aroma profiles for both hop oil and hoppy beer. The least-squares regression was used to model the relationship between sensory difference degrees for hoppy beer with aroma-profiles similarity for hoppy beer and hop oil by fitting a linear equation to observed data. The aroma-profiles similarity for hoppy beer and hop oil was considered as the explanatory variables, comparing with the sensory difference degree that was considered as the response variable. ANOVA was performed to identify the significant positive-linear relationship (at a 5% significance level) and adjusted R squared value was carried out to indicate the how much of the total response variables can be explained by the explanatory variables. Discrimination test data were processed using the R version 4.0.3 with SensoMineR Package. Principal component analysis (PCA) was used to visualize the arrangements of the proprietary hops, their blended models and main components for models in an n-dimensional space by identifying and separating the directions of hops varieties to show the highly-impact aroma compounds for each proprietary hop. Other statistical analyses were performed by R studio version 4.0.3.

4.3 Results and Discussions

4.3.1 Aroma profile of hop oil

Table 4.2 shows total 41 compounds identified in hop oil by GC-MS. The hop oil is the mixture of aroma compounds, where include terpenes (mono- or sesqui-terpenes) that accounts for around 70 % of total oil (Duarte, Amorim, Grazul, & de Oliveira, 2020) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, and acids) (Gonçalves, Figueira,

Rodrigues, & Camara, 2012). Each hop variety has its own specific compound composition, resulting in a distinctive aroma. Comparing the aroma profiles of hops may therefore help distinguish the characteristic odors. The 41 representative hop oil components were classified into 9 groups (**Table 4.2**), including monoterpenes (3), sesquiterpenes (9), terpene alcohols (3), alcohols (1), esters (10), ethers (4), aldehydes (1), ketones (1), acids (6), and unknown (4). Analyte concentrations in hop oil were quantified in groups, with reference standards selected within each group. Other words, the quantification of sesquiterpenes was calibrated by the reference standard, which was a sesquiterpene, and the concentration was the unit amount of this reference. **Table 4.3** summarizes aroma compounds and their analytical concentrations detected in the hop pellets of 3 proprietary hops and 19 public hops varieties by GC-MS method.

Table 4.2. The classification of aroma compounds detected in hop pellets.

	Compound [a]	**t_R (min)** [b]	**RI** [c]	**ID method** [d]	**Odor description** [e]
Monoterpenes	β-pinene	5.17	1109	MS, RI, CI	pine, resin, wood
	β-myrcene	6.28	1161	MS, RI, CI	balsamic, fruit, herb
	β-ocimene	7.01	1235	MS, RI	citrus, flower, herb
Sesquiterpenes	Copaene	14.29	1488	MS	spice, wood
	β-caryophyllene	16.64	1596	MS, RI, CI	spice, wood
	α-humulene	18.23	1668	MS, RI, CI	balsamic, hop, spice, wood
	β-chamigrene	19.72	1724	MS, RI	-
	α-Muurolene	20.03	-	MS	wood
	α-guaiene	20.51	-	MS	balsamic, wood
	trans-calamenene	21.19	1818	MS	-
	caryophyllene oxide	23.03	1979	MS, RI	herb, spice, sweet, wood
	humulene epoxide II	26.36	2066	MS, RI	floral, spice
Terpene alcohols	3-(4-methyl-3-pentenyl) furan (=perillene)	11.94	1412	MS, RI	mold, paint, strong, wood
	linalool	14.98	1542	MS, RI, CI	grape, lavender, rose
	Geraniol	21.45	1837	MS, RI, CI	lemon peel, passion fruit
Alcohols	2-(Octadec-9-enyloxy) ethanol	22.55	-	MS	-
Esters	methyl heptanoate	8.18	1281	MS, RI	-

	2-Methylbutyl isovalerate (2-methylbutyl 3-methylbutanoate)	8.98	1286	MS, RI	-
	Methyl 6-methylheptanoate	10.37	1338	MS, RI	-
	methyl octanoate	11.51	1390	MS, RI, CI	fruit, orange, sweet
	methyl nonanoate	13.95	1485	MS, RI, CI	coconut, floral, fruit, sweet
	cis-Geranic acid, methyl ester (Methyl nerolate)	19.32	-	MS	-
	Ethyl 6,9,12-hexadecatrienoate	22.28	-	MS	-
	Linoleic Acid ethyl ester	29.10	-	MS	-
	Diisooctyl phthalate	43.69	-	MS	-
Ethers	1-Monolinoleoylglycerol trimethylsilyl ether	31.01	-	MS	-
	(2S,2'S)-2,2'-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	37.98	-	MS	-
	Heptaethylene glycol monododecyl ether	38.30	-	MS	-
	Octaethylene glycol monododecyl ether	39.25	-	MS	-
Aldehydes	vanillin	41.51	2577	MS, RI	chocolate, sweet, vanilla
Ketones	2-pentadecanone	25.66	2017	MS, RI	herb, spice
Acids	3-Methylbutanoic acid (isovaleric acid)	17.17	1665	MS, RI, CI	cheese, fecal, putrid fruit, rancid, sweat
	Gamolenic Acid	27.25	-	MS	-
	decanoic acid	30.01	-	MS	-
	2-phenylacetic acid	40.43	2561	MS, RI	beeswax, caramel, floral, honey
	humulone	42.52	-	MS	-
	Lupulone	43.02	-	MS	-
Unknown	unknown	15.38	-	-	-
	unknown	23.84	-	-	-
	unknown	25.27	-	-	-
	unknown	36.82	-	-	-

[a] Compounds are listed in the order of retention time. [b] Retention time. [c] RI, retention ind**ices** are determined on DB-Wax UI column. [d] Method of identification: MS, identification by comparison with mass spectra stored in MAIN library; RI, identification by retention index and comparison with those reported in the published literatures; CI, chemical standard identification using pure analytical standards. [e] Odor description is searched from VCF online database

Table 4.3. Aroma compounds and their analytical concentrations detected in the hop pellets from different hops varieties by GC-MS method.

Compound [a]	Unit (µg/mL)	Concentration [b]										
		Proprietary hops			Public hops							
		Citra	Simcoe	Mosaic	Cascade	Triple Pearl	Us Goldings	Centennial	Eureka	Chinook	Columbus	Crystal
β-pinene	β-pinene	127.39	96.68	113.64	63.77	66.47	37.76	98.51	112.47	81.23	72.27	259.13
myrcene	Myrcene	2623.02	2488.44	2603.01	902.95	2356.66	728.85	2553.91	2986.81	1187.05	1042.63	1009.09
β-ocimene	β-pinene	83.53	100.39	103.90	16.71	40.89	16.71	16.71	103.11	156.85	29.39	16.71
methyl heptanoate	methyl nonanoate	17.78	6.95	4.77	4.04	13.06	47.31	n.d.	32.46	n.d.	7.52	2.87
2-Methylbutyl isovalerate	methyl nonanoate	4.73	5.98	6.41	2.86	4.97	2.98	13.36	9.38	3.49	3.50	2.38
Methyl 6-methylheptanoate	methyl nonanoate	8.99	9.04	9.98	7.05	4.21	5.42	16.07	13.86	15.59	12.38	8.40
methyl octanoate	methyl octanoate	9.65	12.20	11.38	n.d.	1.65	n.d.	8.52	2.64	n.d.	n.d.	3.51
3-(4-methyl-3-pentenyl) furan	linalool	5.81	4.05	2.47	2.06	n.d.	n.d.	2.24	6.68	1.77	2.08	2.21
methyl nonanoate	methyl nonanoate	17.45	13.36	13.66	2.92	n.d.	2.63	3.96	7.96	n.d.	n.d.	4.95
Copaene	α-humulene	0.63	n.d.	6.28	n.d.	30.82	n.d.	3.33	24.19	34.06	7.45	n.d.
linalool	linalool	38.33	19.19	16.55	8.47	29.31	10.10	14.98	44.81	8.60	8.82	19.25
Unknown	methyl nonanoate	13.16	14.46	14.50	6.85	3.59	5.56	9.12	11.02	10.39	4.85	4.06
β-caryophyllene	β-caryophyllene	320.87	225.50	298.52	73.12	132.75	173.36	257.42	584.57	212.20	128.88	156.43
3-Methylbutanoic acid	3-Methylbutanoic acid	34.21	35.01	29.58	28.23	20.58	7.54	46.02	4.81	16.84	21.04	24.17
α-humulene	α-humulene	653.17	597.26	822.94	336.50	361.82	661.58	530.81	1587.25	764.55	317.33	765.82
cis-Geranic acid, methyl ester	methyl nonanoate	210.84	44.37	73.69	218.33	788.41	41.71	131.95	146.07	244.95	243.64	213.68
β-chamigrene	α-humulene	9.99	n.d.	n.d.	14.47	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-Muurolene	α-humulene	41.06	53.24	82.09	33.80	191.49	45.20	94.42	158.43	187.21	70.46	53.91
α-guaiene	α-humulene	n.d.	0.27	74.67	n.d.	n.d.	n.d.	25.11	n.d.	99.88	32.58	n.d.
trans-calamenene	α-humulene	21.67	70.95	35.98	0.43	1.23	n.d.	7.04	10.87	n.d.	5.68	n.d.
Geraniol	Geraniol	22.06	20.35	42.64	8.47	8.86	1.98	62.47	16.82	15.50	8.64	13.42
Ethyl 6,9,12-hexadecatrienoate	methyl nonanoate	85.84	117.02	101.22	42.14	83.31	56.21	133.15	156.73	61.76	88.24	58.51
2-(Octadec-9-enyloxy) ethanol	linalool	5.53	2.86	1.41	1.42	1.40	n.d.	3.26	2.39	5.89	2.04	n.d.
caryophyllene oxide	β-caryophyllene	217.99	289.45	242.67	88.52	206.76	117.37	323.17	328.29	147.45	183.07	117.81
unknow	methyl nonanoate	21.56	24.95	29.76	37.45	26.47	18.80	22.79	12.37	36.24	26.42	34.97
unknow	methyl nonanoate	24.71	30.08	19.74	12.80	11.69	5.36	4.63	20.52	11.40	n.d.	13.60
2-pentadecanone	methyl nonanoate	46.75	45.78	29.13	33.62	6.64	30.93	5.75	109.37	8.97	6.59	70.13
humulene epoxide II	α-humulene	11.03	60.90	36.28	15.91	11.84	n.d.	5.00	89.33	2.73	35.19	9.71
Gamolenic Acid	3-Methylbutanoic acid	16.86	11.32	13.09	6.66	14.75	6.85	7.31	11.25	7.87	7.12	8.87
Linoleic Acid ethyl ester	methyl nonanoate	28.53	12.32	31.33	15.28	17.33	9.68	25.54	34.38	52.35	21.78	8.48

decanoic acid	3-Methylbutanoic acid	24.05	42.19	41.54	27.12	20.27	18.43	9.10	64.24	31.10	21.20	20.37
1-Monolinoleoylglycerol trimethylsilyl ether	methyl nonanoate	177.82	127.81	140.97	28.38	133.48	45.74	111.13	87.58	94.21	221.30	153.21
unknown	methyl nonanoate	57.25	100.03	171.14	479.52	97.42	106.60	190.88	130.65	49.63	102.33	86.80
(2S,2'S)-2,2'-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	methyl nonanoate	132.73	185.69	240.21	325.01	125.70	96.83	231.49	195.65	78.70	151.80	98.62
Heptaethylene glycol monododecyl ether	methyl nonanoate	490.92	868.79	1149.41	1431.17	527.89	335.88	849.88	710.40	292.06	688.30	390.49
Octaethylene glycol monododecyl ether	methyl nonanoate	199.30	204.22	484.83	1238.35	238.93	190.02	183.73	207.31	228.96	271.12	178.14
2-phenylacetic acid	3-Methylbutanoic acid	59.19	88.88	53.42	38.22	27.78	34.14	60.67	36.11	42.75	79.09	40.26
vanillin	methyl nonanoate	1146.44	1211.85	1390.89	2091.97	911.90	690.39	1462.77	1803.18	1187.67	1873.55	2088.40
humulone	3-Methylbutanoic acid	115.99	173.48	190.49	201.83	150.24	82.70	148.60	191.83	105.93	188.48	376.85
Lupulone	3-Methylbutanoic acid	496.20	589.38	839.00	1310.21	497.30	369.75	517.06	529.17	522.48	628.24	2111.48
Diisooctyl phthalate	methyl nonanoate	140.12	162.60	407.36	556.66	118.93	128.90	177.93	173.51	155.22	127.29	70.00

Table 4.3. (continued)

Compound [a]	**Unit (µg/mL)**	**Concentration** [b]										
		Public hops										
		Galena	**Nugget**	**SAAZ**	**Summit**	**Tahoma**	**Brewers Gold**	**Calypso**	**Us Fuggle**	**Cluster**	**Apollo**	**Vanguard**
β-pinene	β-pinene	55.95	101.33	42.17	95.65	43.46	63.58	65.68	39.96	67.67	120.07	34.12
myrcene	Myrcene	1368.43	1887.46	719.81	2514.12	1423.34	796.97	430.50	776.46	594.41	1323.69	468.09
β-ocimene	β-pinene	67.50	89.76	20.57	16.71	65.32	54.86	71.80	22.94	42.32	46.22	16.71
methyl heptanoate	methyl nonanoate	31.35	19.72	3.31	94.69	13.53	n.d.	n.d.	n.d.	5.90	14.85	110.00
2-Methylbutyl isovalerate	methyl nonanoate	3.84	4.19	n.d.	4.01	2.52	2.86	8.67	4.60	2.65	5.96	4.77
Methyl 6-methylheptanoate	methyl nonanoate	15.73	10.61	12.91	11.27	4.71	5.62	27.55	4.59	5.83	13.03	4.70
methyl octanoate	methyl octanoate	3.52	2.21	n.d.	6.76	6.55	n.d.	7.45	2.40	4.48	7.62	5.10
3-(4-methyl-3-pentenyl) furan	linalool	1.80	5.39	1.86	n.d.	2.06	1.18	6.44	1.50	1.82	6.60	2.31
methyl nonanoate	methyl nonanoate	6.35	5.54	n.d.	n.d.	5.85	n.d.	9.67	4.39	n.d.	9.01	5.14
Copaene	α-humulene	4.10	1.50	n.d.	39.13	4.35	10.05	4.80	n.d.	n.d.	17.22	2.66
linalool	linalool	7.63	44.34	9.14	14.53	14.41	14.55	40.02	15.87	6.96	12.38	5.22
Unknown	methyl nonanoate	15.11	8.17	3.13	9.22	3.77	11.09	18.72	4.55	5.77	10.48	5.52
β-caryophyllene	β-caryophyllene	238.22	331.49	49.51	580.93	77.88	140.46	305.29	194.88	80.21	549.80	196.25
3-Methylbutanoic acid	3-Methylbutanoic acid	19.72	34.83	22.65	10.69	16.28	29.77	58.41	20.64	18.71	7.43	31.52

α-humulene	α-humulene	478.59	920.34	255.48	1213.96	363.61	458.09	741.97	889.21	211.28	1355.04	942.90
cis-Geranic acid, methyl ester	methyl nonanoate	68.91	212.98	92.71	249.40	365.38	126.50	68.06	64.74	32.25	51.81	70.73
β-chamigrene	α-humulene	n.d.	n.d.	n.d.	1.33	6.58	n.d.	n.d.	n.d.	n.d.	46.79	n.d.
α-Muurolene	α-humulene	74.68	63.68	15.21	222.71	74.65	83.39	104.77	53.32	17.76	129.50	73.57
α-guaiene	α-humulene	n.d.	n.d.	n.d.	76.32	n.d.	45.34	n.d.	n.d.	n.d.	n.d.	n.d.
trans-calamenene	α-humulene	n.d.	18.56	2.98	9.78	n.d.	n.d.	92.95	n.d.	8.57	11.14	3.32
Geraniol	Geraniol	7.60	5.03	11.72	12.32	5.30	12.21	15.49	2.88	8.76	9.98	3.53
Ethyl 6,9,12-hexadecatrienoate	methyl nonanoate	104.44	106.25	76.99	134.39	18.49	59.03	95.72	69.61	101.58	144.97	86.85
2-(Octadec-9-enyloxy) ethanol	linalool	3.34	n.d.	n.d.	4.40	n.d.	1.40	5.84	n.d.	n.d.	1.98	n.d.
caryophyllene oxide	β-caryophyllene	217.71	248.79	153.42	310.34	49.95	134.57	228.02	147.48	221.34	318.61	191.95
unknow	methyl nonanoate	22.29	14.82	42.81	20.48	25.31	29.46	11.53	21.76	27.36	14.70	27.95
unknow	methyl nonanoate	11.00	11.61	11.91	8.01	6.96	17.79	41.34	7.48	8.62	31.94	11.38
2-pentadecanone	methyl nonanoate	5.47	118.31	18.66	22.51	17.49	73.88	460.00	33.52	13.02	142.83	45.25
humulene epoxide II	α-humulene	6.59	36.42	11.19	62.00	n.d.	0.64	8.68	0.35	4.73	39.36	20.55
Gamolenic Acid	3-Methylbutanoic acid	9.68	12.65	7.36	7.73	9.74	7.45	10.38	6.79	8.38	10.56	7.50
Linoleic Acid ethyl ester	methyl nonanoate	31.47	12.65	10.14	41.56	8.86	52.63	24.49	12.09	12.04	43.15	15.95
decanoic acid	3-Methylbutanoic acid	29.03	26.60	13.49	22.26	16.61	29.16	27.83	19.46	9.81	55.20	25.73
1-Monolinoleoylglycerol trimethylsilyl ether	methyl nonanoate	131.80	96.08	79.09	85.40	42.44	99.65	27.12	57.20	118.41	110.57	66.65
unknown	methyl nonanoate	108.86	124.33	119.67	149.57	403.00	76.78	134.29	81.11	119.87	69.02	126.80
(2S,2'S)-2,2'-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	methyl nonanoate	139.41	137.86	98.59	210.27	41.66	89.22	153.26	99.32	139.69	192.40	164.21
Heptaethylene glycol monododecyl ether	methyl nonanoate	578.52	565.64	438.21	908.11	968.41	347.55	563.57	437.75	673.02	714.07	648.28
Octaethylene glycol monododecyl ether	methyl nonanoate	200.35	169.09	240.67	184.90	757.76	244.25	158.12	183.11	211.45	181.08	229.67
2-phenylacetic acid	3-Methylbutanoic acid	43.95	51.90	30.89	72.68	57.64	55.74	98.90	22.33	101.07	71.19	34.36
vanillin	methyl nonanoate	3698.42	1388.56	1908.14	2460.19	1798.19	1065.04	1176.39	1162.43	3399.63	2322.67	1818.91
humulone	3-Methylbutanoic acid	278.09	198.65	190.66	231.92	335.52	105.87	101.31	146.30	281.47	277.88	349.28
Lupulone	3-Methylbutanoic acid	926.90	638.62	1044.92	1107.92	1080.74	407.16	267.17	598.67	753.96	858.12	1928.54
Diisooctyl phthalate	methyl nonanoate	131.47	109.97	100.12	171.04	499.02	108.56	125.49	105.31	126.55	153.78	131.97

[a]Compounds are listed in the order of retention time. [b]Mean of triplicates; standard deviations were <20% . n.d.: not detected

4.3.2. Aroma models for proprietary hops

The quantified aroma profiles of public hops were performed to model the aroma characters of proprietary hops (Citra, Mosaic, and Simcoe). The aroma characters of proprietary hops were modeled as the percentage combination of individual public hops. In comparison with proprietary hops, blended models produced by QP method minimized differences in overall aroma profiles. The differences of aroma characters were compared by the sum of the squared difference between the concentration of individual aroma compound. **Table 4.4** shows the formulas of blended models for three proprietary hops. To model the aroma profiles of Citra and Mosaic, Triple Pearl, Centennial, and Eureka were blended in percentages, while Simcoe was modeled by blending Cascade, Triple Pearl, Us Goldings, and Centennial. Additionally, the total amount of the model mixtures for Citra and Mosaic was less than that of the proprietary hops, in which they were 95.1% and 94.3 %, respectively. Modeling the aroma of Simcoe required 141.5% of its total amount by mixing individual public hops. For Mosaic and Simcoe, Centennial was the most quantity hop variety, accounting for around 90% and 50% of the total quantities. The public hops were accounted more equally in Citra blended model.

Table 4.4. Blended models for proprietary hops by mimicking the concentration of individual aroma compounds.

Proprietary hops	**Public hops**					
	Cascade	**Triple Pearl**	**Us Goldings**	**Centennial**	**Eureka**	**Total (%)**
Citra	0	0.367	0	0.332	0.252	0.951
Mosaic	0	0.030	0	0.848	0.065	0.943
Simcoe	0.100	0.104	0.500	0.711	0	1.415

4.3.3 Validating models based on sensory evaluation of hoppy beer

To contribute the intense aromas to beer, the dry hopping treatment was applied due to the minimization of volatilization of hop aromas via lower treating temperature (Wolfe, Qian, & Shellhammer, 2012). Although the early kettle hopped treatment could impact the aroma on beer (Praet, Van Opstaele, Aerts, & De Cooman, 2015; Sharp, Qian, Shellhammer, & Shellhammer, 2017), its processes are hard to control at the same level, causing the complexity of hops aromas. In this study, the amount of hops addition, the temperature of treatment, the extraction time were controlled at the same level for dry hopping treatment to deliver the hop-derived aromas to beer. In this study, two sensory evaluations were conducted to assess the effectiveness and similarity degree of blended models in hoppy beer. Difference test was used to evaluate similarity with proprietary hops. Discrimination test was applied to determine the difference between proprietary hops and their models

4.3.3.1 Difference test of blended models

Difference test of aroma was assessed as the overall differences. A panel of well-trained experts evaluated the hops aroma difference degree of proprietary hops (Citra, Simcoe, or Mosaic), their blended models, and their main component hop varieties from proprietary hops (control). The un-hoppy beer was regarded to distinguish the “hoppy” aroma. In this regard, blended models can be confirmed as successful if they bear no significant difference from their proprietary hops, while their main components are substantially different. To avoid the variation of sensory sensitivity among panelists, the sensory similarity was rated as the ranks as well. The results of evaluations for Citra, Simcoe, and Mosaic were shown in **Figure 4.2**. The blended models for Mosaic and Simcoe obtained the highest similarity scores and ranks. In other words, the blended models were placed in the same similarity group as their proprietary hops, while the main components of models

were significantly different from the proprietary hops. Additionally, the un-hoppy beer was scored and ranked as completely different, indicating that the "hoppy" aroma affected the similarity evaluation of beer aroma, and the similarity was measured by the overall differences in hoppy smell. Blended model for Citra obtained higher similarity scores and ranks shown in **Figure 4.2 (a, d).** However, Eureka has achieved the same similarity evaluation as the model, and both model and Eureka were significantly different from Citra in rank-ordering. There could be a possibility that the blend model's aroma intensity is not sufficient to emulate Citra, since the blend model is substantially less than Citra when it is brewed in beer. Additionally, the linalool content of the blended model was lower than that of Citra (data not included), where linalool concentration was regarded as the good marker for hoppy flavor (Hanke, Herrmann, Rückerl, Schönberger, & Back, 2008) that positively correlated with aroma intensity in odor (Fritsch, & Schieberle, 2003). The less aroma intensity may lead to difficulty and negative effects in capturing aroma characters. Moreover, it might be caused by the less Eureka in the model since Eureka is quite a similar hops variety compared with Citra. Overall, according to the difference test, the blended models for Simcoe and Mosaic were rated as having high sensory similarity, whereas the blended model for Citra was improved, but not enough.

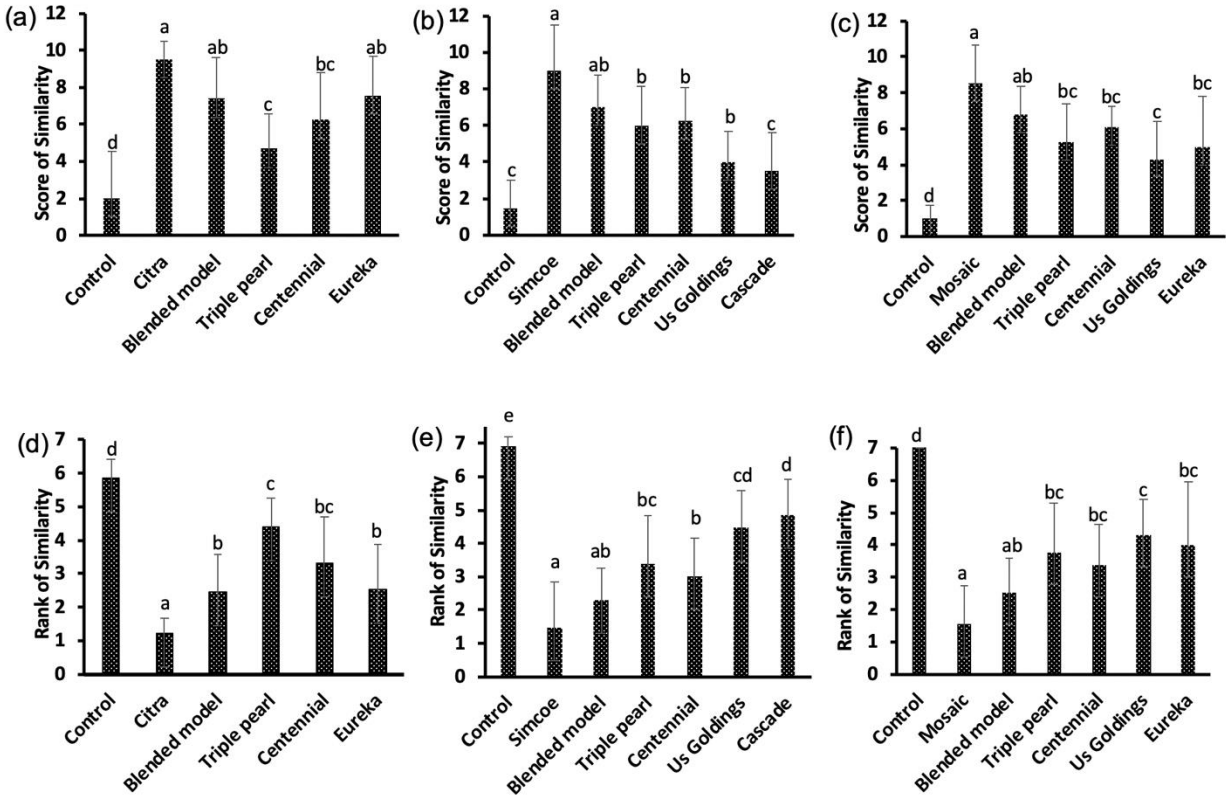

Fig. 4.2. (a-c) Scores of similarity (n= 5) and (d-f) ranks of similarity (n= 5) of individual main component hoppy beer, blended models, proprietary hops and un-hoppy beer for each proprietary hoppy beer (a, d – Citra; b, e – Simcoe; c, f – Mosaic). Data are mean ± SD of triplicate analyses. Different letters represent significant differences among the evaluation group ($p<0.05$).

4.3.3.2 Discrimination test of blended models

To speculate the difference between proprietary hops and their blended models, a discrimination test was followed to determine if the overall difference exists. There was no need to truly quantify the degree or character of difference, therefore the highly valuable discrimination information that increased reliability and accuracy of the study could be obtained. Serving orders satisfied the random arrangements to be presented to panelists. Five panelists who are knowledgeable and well-trained about the samples met the requirement of the minimum number

for triangle test to achieve the statistically significant results ($p \leq 0.05$). The critical value was 15 (**Table 4.5**), which was the minimum number of correct responses required for significant difference of samples, given the total 30 responses collected for each proprietary hop. However, the blended models and their modelled proprietary hops were statistically significant different ($p \leq 0.05$), which meant panelists were able to distinguish different aromas between proprietary hops and their models. The odd sample was correctly identified by 18 to 20 out of the 30 responses. While the blend models were significantly different from the proprietary hops, their aroma similarity was significantly improved, as confirmed by difference test. The size of the hop pool used in this study might not be sufficient; expanding the database would help model the aroma without sensory differences. In addition, understanding the fate of hops during brewing processes, such as the biotransformation by yeasts (Forster, & Gahr, 2013; Haslbeck et al., 2017), additive or masking effects of hop-derived aromas in beer (Schönberger, & Kostelecky, 2011), or transfer rates of specific aroma compounds in different hops varieties (Hanke et al., 2008), may help to supply the insufficiency of modeling aroma profiles.

Table 4.5. The summary of sensory discrimination test between proprietary hops and their blended models.

	Variable					
Proprietary hops	P_0	Critical value	C	N	p ≥ C [d]	**Result**
Citra	1/3	15	18	30	0.00246	Significant different
Mosaic	1/3	15	21	30	0.0000443	Significant different
Simcoe	1/3	15	20	30	0.000194	Significant different

P_0, Probability of guessing the right answer by chance. [C] Number of correct responses [N] Total responses. [D] Probability of obtaining this or a more extreme result if the samples are the same

4.3.4 Validating models based on aroma profile of hop oil and hoppy beer by GC analysis

4.3.4.1 Aroma profile similarity in hop oil

Besides a few uncommon compounds that are detected in the specific hop varieties, almost all hop varieties commonly contain the same components, only varying in the amount of individual aroma compounds (Gros, Nizet, & Collin, 2011). Different combinations of aroma compounds present specific aroma profiles, which reveal the differences between hop aroma characters. Since each hop variety has a typical essential oil pattern, the composition of hop oil is critical to evaluate hop aroma (Inui et al., 2013). Therefore, it is possible to determine the hop aroma characters from the aroma profile of hop oil analyzed by instruments as well as to present the differences among different hops in the aroma profile. The less differences between the aroma profiles of hops, the shorter the distances between hop varieties, the more similar the aroma characters are. The similarity of the aroma characters of different hops varieties were indicated as the difference degree of their aroma profiles, which could be presented as R^2 value. **Table 4.6** shows the comparison of the similarity of aroma profiles at the level of hop oil. Both the predictive models obtained from statistical method and the tested models blended by hop oil, achieved the desired aroma profile similarity ($R^2 > 0.93$).

The tested model for Citra, however, did not achieve the same similarity as the predictive model; the similarity decreased from predictive 0.9842 to tested 0.9349. The insufficiency of the similarity of model for Citra might lead to reason why the sensory evaluation of model and Eureka achieved the same degree of similarity. Overall, the blended models achieved the higher similarity than their main components, which meant that the similarity was improved by mixing the public hops. Additionally, the similarity assessments of the predictive and tested models were quite closer for all proprietary hops, which indicated our mathematical optimization method for modeling aroma of hops is feasible.

Table 4.6. R squared values for aroma profile similarity of hop oil detected by GC-FID between proprietary hops and blended models.

Proprietary hops	Predictive model	Cascade	Triple pearl	Us Goldings	Centennial	Eureka	Tested model
Citra	0.9842		0.8340		0.8952	0.8751	0.9349
Mosaic	0.9883		0.8650	0.7798	0.9076	0.9163	0.9800
Simcoe	0.9772	0.5405	0.8272	0.7382	0.9088		0.9585

4.3.4.2 Aroma profile similarity in hoppy beer

Not only were the hop oil aroma profiles compared, but also the hop-infused beer. The complex components of hop oil contribute the "hoppy" aroma to beer, providing the strong aroma impacts on the quality of beer. Therefore, it is also necessary to analyze the aroma compositions of beer to evaluate the "hoppy" aroma, not only through sensory evaluation but through instrumental analysis. The aroma profiles of beer were extracted and analyzed by HS-SPME-GC method, which is the headspace-based sampling technique for volatile detection. At the level of hoppy beer, the higher similarities of the blended models were achieved ($R^2 > 0.96$) and found that the aroma similarity was also higher than the main components (**Table 4.7**). The aroma profile of un-hoppy beer (control) was quite different from that of hoppy beer, showing the lower similarity ($R^2 < 0.50$) for all proprietary hops. The hoppy aroma had a strong impact on the sensory evaluation and aroma characters of beer. Generally, the beer with a "hoppy" aroma was more closely similar than that without a "hoppy" aroma. Despite the aroma profile similarity of models was validated, the similarities of models and public hops were not presented the same trends in hop oil and hoppy beer. That is because the aroma contributions of aroma compounds from hops are not in the same way as in the beer (Vollmer, Lafontaine, & Shellhammer, 2018), showing different impacts due to the brewing process.

Table 4.7. R squared values for aroma profile similarity of hoppy beer detected by HS-SPME/GC-FID between proprietary hops and blended models.

Proprietary hops	Control (Un-hoppy beer)	Cascade	Triple pearl	Us Goldings	Centennial	Eureka	Tested model
Citra	0.485		0.662		0.787	0.693	0.966
Mosaic	0.470		0.726	0.826	0.897	0.805	0.981
Simcoe	0.463	0.657	0.548	0.736	0.796		0.962

4.3.5 Linear relationship between sensory evaluation and aroma profile analysis

To speculate whether the aroma profile similarity of either hop oil or hoppy beer were associated positively with the sensory evaluation for overall aroma similarity, ANOVA analysis for linear regression were additionally carried out for each proprietary hop. **Table 4.8** clearly shows that, for all of the proprietary hop varieties, the sensory evaluation for aroma similarity shows a positive relationship with similarity of aroma profiles in hop oil and hoppy beer. The similarity of hop oil aroma profiles explained more than 70% of the variability in sensory evaluation. However, the linear regression test of how well the similarity of the aroma profiles of hoppy beer explains the sensory evaluation did not demonstrate the same level of stability as the testing of hop oil. The aroma profile similarity of hoppy beer could explain 86.6 % of the sensory evaluation for Mosaic, while only explain 61.4 % of that for Simcoe. It might be caused by the masking and synthetic influences among volatile and nonvolatile components in beer (Praet, Van Opstaele, Jaskula-Goiris, Aerts, & De Cooman, 2012), which increase the complexity of the sensory perception of beer. In addition, hop oil and hoppy beer were not related to sensory evaluation in the same way, since the aroma characters of hop oil were not balanced the level as the contribution of aroma in the beer (Inui et al., 2013). Overall, with higher aroma profile similarity of hops the according sensory similarity increased (**Figure 4.3**), which confirmed the aroma patterns detected by instruments could indicate the sensory evaluation and speculated the

sensory similarity. In the future, it might shorten the time and ensure the reliability for the sensory evaluation, if we substitute part of sensory evaluation with instrumental analysis.

Table 4.8. The summary of positive linear relationship between sensory similarity for hoppy beer and aroma-profiles similarity.

Proprietary hops	Hoppy beer			Hop oil		
	R^2_{adj} (%)	P value	Sensory Similarity	R^2_{adj} (%)	P value	Sensory Similarity
Citra	72.8	0.0096 **	significant positive-related	75.3	0.0180 *	significant positive-related
Mosaic	86.6	0.0007 ***	significant positive-related	70.7	0.0113 *	significant positive-related
Simcoe	61.4	0.0114 *	significant positive-related	79.2	0.0055 **	significant positive-related

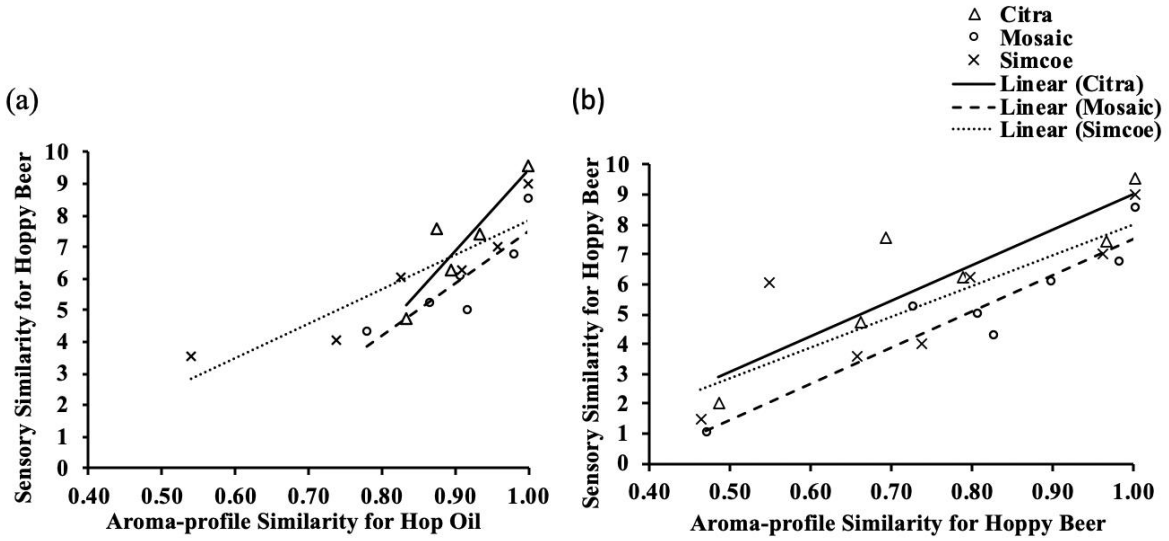

Fig. 4.3(a-b). The linear relationship between sensory similarity and aroma-profile similarity of hoppy beer detected by HS-GC. (b) The linear relationship between sensory similarity and aroma-profile similarity of hop oil detected by GC. (The results for aroma-profile similarity for hop oil and hoppy beer were presented in Table 4.5 and 4.6, respectively; the sensory similarity for hoppy beer were presented in Figure 4.2 (a-c))

4.3.6 Key components influencing the distinctive aroma characters of proprietary hops

The similarity of blended models for proprietary hops was improved greatly, which were confirmed by both sensory difference test and the analysis of aroma profiles. However, they could be discriminated. The aroma components of hops were complex in which include a variety of compounds; thus, the same profiles were impossible to model. Moreover, since the combination of aroma components was quite similar, the balance of the aroma components of hops was supposed to differentiate the hop varieties (Inui et al., 2013). To improve the similarity of blended models in the future, the aroma characters that highly contribute to the specific hops variety were detected by multivariate analysis of components combination of hop oil. The components of the proprietary hops, the blended models, and their main components were selected to visualize the separation and direction of aroma compounds. **Figure 4.4** shows the biplot scores graph of PCA for each proprietary hop with the first two PCs. As for Citra, PC1, responsible for 42.9% of the explained variance, clustered the Citra and blended model as the most relevant group with slightly negative scores. PC2 was responsible for 28.1% of the explained variable, where Citra and Eureka were grouped in positive scores. Separation and direction between Citra and Eureka, or Citra and Model, confirmed the results of sensory evaluation that model and eureka were more similar with Citra at the same level. In addition, from the biplot, β-ocimene, methyl heptanoate, linalool, β-chamigrene, and gamolenic acid were the aroma compounds that highly contribute to the Citra. Simcoe and its model were in negative scores of PC1 and close to zero of PC2, accounting for 43.1% and 28% of the explained variance, respectively. They could be clustered in the same group, supporting the results of sensory evaluation and aroma profiles analysis. Among the aroma compounds, β-pinene, methyl 6-methylheptanoate, 3-methylbutanoic acid, trans-calamenene, geraniol, 2-(octadec-9-enyloxy) ethanol, and humulene epoxide II had the strong impacts on the

aroma of Simcoe. Regarding Mosaic, it and its model were found to have similar negative scores on PC1 that was responsible for 48.9% of the explained variable, while it had a slightly negative score on PC2, and the model was found to have a positive score. The blended model and Centennial were located closer in both the negative scores of PC1 and positive scores of PC2. The location of hops varieties confirmed that the blended model was like Mosaic evaluated by panelists. In addition, Centennial was the public hop variety that had assessment results that were close to blended model. B-pinene, β-myrcene, methyl octanoate, copaene, α-humulene, trans-calamenene, caryophyllene oxide, humulene epoxide II, lupulone, and linoleic acid ethyl ester were the highly impact aroma compounds for Mosaic. We can clearly see that specific hops varieties have different high-impact aroma compounds that contribute to their unique aroma. And also, these aroma compounds contribute to the more similar aroma of different hops varieties, which are supported by sensory evaluation. Moreover, the closely related aroma compounds are not only limited to the reported key aroma compounds, including linalool, geraniol, β-myrcene, α-humulene, and β-caryophyllene.

In the future, we might need to focus not only on the overall aroma profiles but also on these highly-impact aroma compounds that closely related specific hops varieties to improve the similarity for modeling the overall aroma characters. Additionally, other than having knowledges of the location and clusters of exact aroma compounds, we might combine the sensory aroma character analysis to group the compounds via aroma attributes (Inui et al., 2013; Kishimoto, Wanikawa, Kono, & Shibata, 2006) to improve the typical aroma contribution.

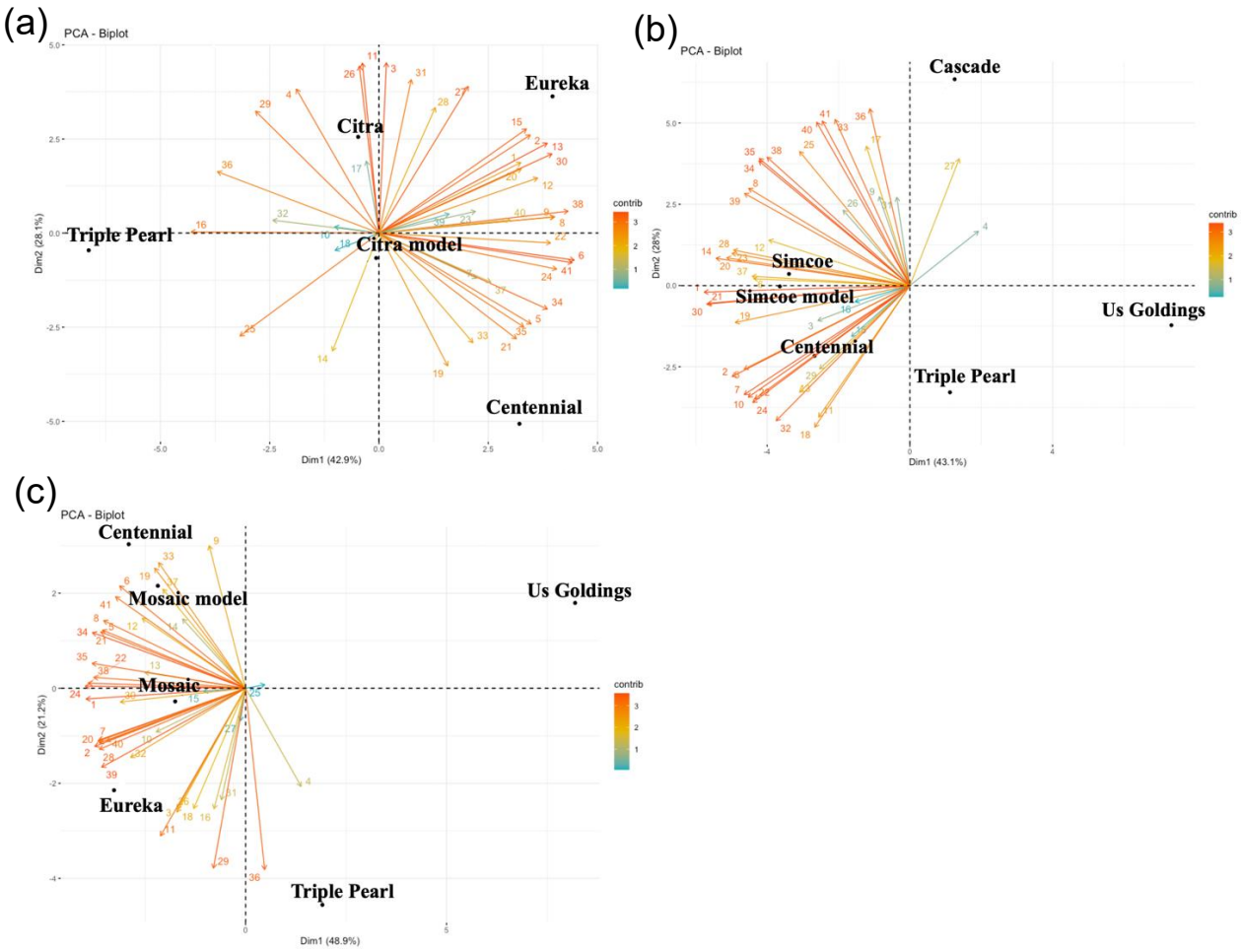

Fig. 4.4. Principal component biplot for each proprietary hop oil (a-Citra; b-Simcoe; c-Mosaic), the individual main component hop oil for blended models (shown in Table 4.3) and blended models, with forty-one (compounds refer to Table 4.2, the order listed refer to retention time) aroma compounds

4.4 Conclusions

By combining mathematical optimization method with flavor-detection technique, a simple and straightforward method for modeling proprietary aromas has been developed. As shown by the aroma profile and sensory analyses in hop oil and hoppy beer, there was a significant improvement in the similarity of the models for Citra, Mosaic, and Simcoe. However, they were

not yet very successful as indicated by discrimination test, in which panelists assessed them as significant differences. A possible reason might be the inability to capture the overall aroma characteristics. In addition, the limitation of the detection threshold of GC system has strong impacts on results (Vollmer et al., 2018), leading to the incomplete aroma profiles of hops. They challenged the process of modeling aroma and blocked the accuracy of statistical results. Overall, by comparing the existing aroma profiles of hop oil and hoppy beer with sensory evaluation, we may conclude that there existed the positive linear relationship between them. The finding of the relationship confirmed the feasibility of modeling aroma profiles to improve similarity. Moreover, the PCA biplots of the hop oil components for each proprietary hop aid in confirming the sensory results and visualizing the separation of aroma compounds to identify the highly-impact aroma compounds for specific hops variety. It may be possible in the future to model the more similar aromas by developing efficient methods for extracting and maintaining hop aromas, understanding the balance and transformation of aroma attributes in hops and hoppy beer, and focusing on the highly-impact aroma compounds.

4.5 References

Compusense.com. (2020). *Quality Assurance with Difference from Control Testing.* [online] Available at: http://compusense.com/wp-content/uploads/2020/03/Difference_from_Control_Testing_White_Paper.pdf Accessed 12 September, 2021.

Duarte, L. M., Amorim, T. L., Grazul, R. M., & de Oliveira, M. A. L. (2020). Differentiation of aromatic, bittering and dual-purpose commercial hops from their terpenic profiles: An approach involving batch extraction, GC–MS and multivariate analysis. *Food Research International*, *138*, 109768.

Dong, L., Hou, Y., Li, F., Piao, Y., Zhang, X., Zhang, X., ... & Zhao, C. (2015). Characterization of volatile aroma compounds in different brewing barley cultivars. *Journal of the Science of Food and Agriculture*, *95*(5), 915-921.

Forster, A., & Gahr, A. (2013). On the fate of certain hop substances during dry hopping. *Brew Sci*, *66*, 94-103.

Forster, A., & Gahr, A. (2014). A comparison of the analytical and brewing characteristics of Cascade and Comet hop varieties as grown in Yakima (USA) and Hallertau (Germany). *Brew. Sci*, *67*, 137-148.

Fritsch, H. T., & Schieberle, P. (2003). Changes in key aroma compounds during boiling of unhoped and hopped wort. In *Proc. 29th EBC Congress*.

Gros, J., Nizet, S., & Collin, S. (2011). Occurrence of odorant polyfunctional thiols in the super alpha Tomahawk hop cultivar. Comparison with the thiol-rich Nelson Sauvin bitter variety. *Journal of agricultural and food chemistry*, *59*(16), 8853-8865.

Gonçalves, J., Figueira, J., Rodrigues, F., & Camara, J. S. (2012). Headspace solid-phase microextraction combined with mass spectrometry as a powerful analytical tool for profiling the terpenoid metabolomic pattern of hop-essential oil derived from Saaz variety. *Journal of separation science*, *35*(17), 2282-2296.

Haslbeck, K., Bub, S., Schönberger, C., Zarnkow, M., Jacob, F., & Coelhan, M. (2017). On the fate of β-myrcene during fermentation–The role of stripping and uptake of hop oil components by brewer̀s yeast in dry-hopped wort and beer. *Brew Sci*, *70*(11-12), 159-169.

Hanke, S., Herrmann, M., Rückerl, J., Schönberger, C., & Back, W. (2008). Hop volatile compounds (Part II): Transfer rates of hop compounds from hop pellets to wort and beer. *Brew. Sci*, *61*, 140-147.

Inui, T., Tsuchiya, F., Ishimaru, M., Oka, K., & Komura, H. (2013). Different beers with different hops. Relevant compounds for their aroma characteristics. *Journal of agricultural and food chemistry, 61*(20), 4758-4764.

Kovačevič, M., & Kač, M. (2002). Determination and verification of hop varieties by analysis of essential oils. *Food Chemistry, 77*(4), 489-494.

Kishimoto, T., Wanikawa, A., Kono, K., & Shibata, K. (2006). Comparison of the odor-active compounds in unhoped beer and beers hopped with different hop varieties. *Journal of agricultural and food chemistry, 54*(23), 8855-8861.

Mikyška, A., Olšovská, J., Slabý, M., Štěrba, K., Čerenak, A., Košir, I. J., ... & Krofta, K. (2018). Analytical and sensory profiles of Slovenian and Czech hop genotypes in single hopped beers. *Journal of the Institute of Brewing*, *124*(3), 209-221.

Pinho, O., Ferreira, I. M., & Santos, L. H. (2006). Method optimization by solid-phase microextraction in combination with gas chromatography with mass spectrometry for analysis of beer volatile fraction. *Journal of chromatography A*, *1121*(2), 145-153.

Praet, T., Van Opstaele, F., Jaskula-Goiris, B., Aerts, G., & De Cooman, L. (2012). Biotransformations of hop-derived aroma compounds by Saccharomyces cerevisiae upon fermentation. *Cerevisia*, *36*(4), 125-132.

Praet, T., Van Opstaele, F., Aerts, G., & De Cooman, L. (2015). Sesquiterpene oxidation products as key impact compounds for "kettle hop" aroma. In *ASBC Annual Meeting, La Quinta, CA*.

Sharp, D. C., Qian, Y., Shellhammer, G., & Shellhammer, T. H. (2017). Contributions of select hopping regimes to the terpenoid content and hop aroma profile of ale and lager beers. *Journal of the American Society of Brewing Chemists*, *75*(2), 93-100.

Schönberger, C., & Kostelecky, T. (2011). 125th anniversary review: The role of hops in brewing. *Journal of the Institute of Brewing*, *117*(3), 259-267.

Sharp, D. C., Townsend, M. S., Qian, Y., & Shellhammer, T. H. (2014). Effect of harvest maturity on the chemical composition of Cascade and Willamette hops. *Journal of the American Society of Brewing Chemists*, *72*(4), 231-238.

Vollmer, D. M., Lafontaine, S. R., & Shellhammer, T. H. (2018). Aroma extract dilution analysis of beers dry-hopped with Cascade, Chinook, and Centennial. *Journal of the American Society of Brewing Chemists*, *76*(3), 190-198.

Vázquez-Araújo, L., Rodríguez-Solana, R., Cortés-Diéguez, S. M., & Domínguez, J. M. (2013). Use of hydrodistillation and headspace solid-phase microextraction to characterize the volatile composition of different hop cultivars. *Journal of the Science of Food and Agriculture*, *93*(10), 2568-2574.

Van Opstaele, F., De Rouck, G., De Clippeleer, J., Aerts, G., & De Cooman, L. (2010). Analytical and sensory assessment of hoppy aroma and bitterness of conventionally hopped and advanced hopped pilsner beers. *Journal of the Institute of Brewing, 116*(4), 445-458.

Wolfe, P., Qian, M. C., & Shellhammer, T. H. (2012). The effect of pellet processing and exposure time on dry hop aroma extraction. In *Flavor chemistry of wine and other alcoholic beverages* (pp. 203-215). American Chemical Society.

Chapter 5 Summary, Conclusions and Recommendations

5.1 Summary and conclusions

This thesis reports that modeling the aroma of proprietary hops by blending several public hops was feasible. Moreover, the aroma similarity of blended models with their proprietary hops was improved at the level of hops and hoppy beer by aroma profiles and sensory evaluation.

In Chapter 3, it has been found modeling the full-profile compounds detected by GC of proprietary hops obtained higher similarity and reliability than that of volatile-compounds by HS-SPME-GC. The higher similarity was validated in aroma profiles analysis ($R^2 > 0.95$) and sensory evaluation. In addition, weighing the reported highly-impact aroma compounds of hops (β-myrcene, α-humulene, β-caryophyllene, linalool, and geraniol) when modeling overall aroma profiles was not validated in hoppy beer, although was proved in hops. Therefore, there was no significant similarity improvement for the weighted blended models.

In Chapter 4, the similarity of the models for Citra, Mosaic, and Simcoe was improved greatly, as shown by the aroma profile and sensory analyses in both hop oil and hoppy beer. A positive linear relationship between aroma profiles similarity and sensory evaluation was identified, which means the more similar an aroma profile, the closer the sensory similarity. Also, the PCA biplots of the hop oil components for each proprietary hop aid in confirming the sensory results and visualizing the separation of aroma compounds to identify part of aroma compounds that are closely related to the specific hops variety. Although we validated that there was no significant improvement by weighing the reported highly-impact aroma compound in Chapter 3, it is also meaningful to explore the importance of PCA-supported compounds in the future due to specific hops variety has unique related compounds and their contributions to sensory closer varieties.

The study suggests that it is plausible to model the aroma profiles to match the flavor through the combination of mathematical optimization and flavor-detection techniques.

5.2 Recommendations

Recent studies have suggested that the combination of mathematical optimization – QP and flavor-detection techniques to model the aroma of target profiles, offering a novel approach in flavor developments. In this thesis, the aroma similarity of blended models was improved significantly in aroma profiles and sensory evaluation, however, they were not yet very successful as indicated by discrimination test. Therefore, in future planning, the relationship between hop aroma chemical composition and human sensory should be investigated to group the aroma attributes for more detailed modeling. Also, understanding the fates of hops during brewing processes, such as the biotransformation by yeasts, additive or masking effects of hop-derived aromas in beer, or transfer rates of specific aroma compounds in different hops varieties, may help to supplement the insufficiency of modeling aroma profiles. The combined different extraction methods are suggested to offer the most comprehensive coverage of volatiles. Moreover, expanding the pool of public hops is also recommended to model the aroma characters of proprietary hops more accurately.