BIO Web of Conferences **59**, 01016 (2023) https://doi.org/10.1051/bioconf/20235901016
ICBB 2023

Aging of Hops and Their Effects on India Pale Ale Flavor

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Abstract: In this experiment, two hops varieties Simcoe and Saaz were selected as the research objects to explore the influence of hops on beer flavor after aging treatment under different conditions. The hops obtained under five different aging conditions were subjected to fermentation experiments. The aroma characteristics of hop oil, beer samples at different brewing stages and finished beer were analyzed by gas chromatography-mass spectrometry (GC/MS). The results showed that, except caryophyllene oxide (0.21 μg/L and 0.15 μg/L), the contents of other aroma compounds decreased. The results of expert sensory evaluation and consumer evaluation survey were combined with the experimental data for analysis. We found that moderate aging may make beer more drinkable. Among them, hops represented by Simcoe with more aroma substances will lose their original aroma after aging. However, hops with higher humulus content represented by Saaz became softer after moderate aging treatment.

1. Introduction

Hops (Humulus lupulus) is one of the important raw materials of beer brewing, which plays a role in clarifying wort and increasing the flavor and aroma of beer during brewing. Hop aroma is mainly derived from the volatile compounds in hop essential oil[1]. In the actual brewing process, most of the use of aged hop products rather than fresh hops. So the aromatic compounds in aged hops have been studied extensively. Further chemical and biochemical transformations of these aromatic compounds occur during beer ripening, storage, and aging[2].

In traditional beer production, hops are added during the boiling process to give the beer a unique bitter taste[3]. In recent years, more and more brewers choose to dry hop in the fermentation stage, in order to make the beer produce more aroma. The difference of hop varieties and different adding stages lead to the complex composition of aroma components in beer[4]. These include the composition of the essential oil of hops and the conversion of various terpenes and alcohols during fermentation[5].

In previous studies, based on the analysis of the chemical composition of essential oils contained in different types of hops after aging, it was generally believed that aging hops would have a negative impact on beer. However, some studies have shown that proper aging can enhance the so-called noble aroma of some hop varieties[6]. Daniel et al. in 2017 have studied aroma properties of Lager beer dry-hopped with oxidized hops. Hops treatments were prepared at two different levels: oxidized and superoxidized. Oxidized hops were exposed to pure oxygen a single time and held at 38℃ for two weeks; superoxidized hops were exposed daily to pure oxygen and held at 38℃ for two weeks. They found that the aging of hops can favour the increase in compounds related to " noble-like " aromas such as woody and herbal[7]. Some researchers reported that the oxidation products of humulene contributed to the herbal and spicy notes in beer. The oxygenated sesquiterpenoid fraction of hops in relation to the spicy hop character of beer. Although a number of hop-derived oxygenated compounds have been identified in various different beers, the arguments regarding which one contribute to the noble hop aroma in beer has still not been clarified[8].

In this study, Simcoe and Saaz, were selected to investigate the concentrations of aroma compounds derived from fresh and aged hops and their effects on beer aroma and flavour. The summer normal temperature in China of 30 ℃and the upper limittemperature in China of 40 ℃ were chosen to accelerate the aging of hops to compare with temperatures used in previous studies[9].

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2. Materials and Methods

2.1 Brewing material

2.1.1Malt and Yeast

Australian ZINNBACH malt, provided by Shandong Ideal Brewing Biology Technology Co. Ltd. Dry ale yeast (US-05) was purchased from Barth-haas Trading Co. Ltd, the date of production was March 27, 2022, and The best use period is within three years.

2.1.2Chemicals

Linalool, citronellol, α -caryophyllene, oxide caryophyllene, geraniol, nerol, terpineol, farnesol were purchased from Yuanye Biotechnology Co. Ltd. (Shanghai, China). Citronellal, β -pinene, citronellyl acetate, methyl geranate, 2-Nonanol （international standard） were purchased from Sigma-Aldrich.

2.2 Pretreatment of Hops

Simcoe: A hop cultivar bred in the United States, the percentage of α-acid andβ-acid was 12.6% (w/w) and 3.7% (w/w), respectively. HSI was 0.264, and the total hop oil content was 1.9 mL/100 g;

Saaz: A hop cultivar bred in the Czech Republic, the percentage of α-acid andβ-acid was 2.9% (w/w) and 3.3% (w/w), respectively. HSI was 0.22, and the total hop oil content was 0.8 mL/100g.

The two hops were treated separately in the following five ways: sealed in the refrigerator at -20℃(Fresh); aged at 30 ℃ in a ventilated environment for 5 days (aged 1); aged at 30 ℃ for 10 days (aged 2); aged at 40 ℃ in a ventilated environment for 5 days (aged 3); and 40 ℃ for 10 days (aged 4). The two varieties of hops both received the same aging treatment.

2.3 Determination of the HSI of Hops

Hop sample (5 g) was crushed and weighed into 250 ml extraction bottle, 100 ml benzene or toluene was added, the cap was tightened and placed on the mechanical oscillator, and shaken vigorously for 30 minutes. Extraction supernatant (5 ml) was diluted to 100 ml with methanol in a conical flask, then 3 ml was transferred into another conical flask and diluted to 50 ml with alkaline methanol. With benzene or toluene (depending on the extract) as a blank, the absorbance of the final diluent was determined in a quartz cuvette at 275 and 325 nanometer wavelengths.

2.4 Beer Fermentation Experiment

Samples of treated hops were used for beer fermentation experiments. Ten single-hopped beers (total 300 L, divided into 30 L batches) were brewed at the Qilu University pilot brewery. The total amount of hops added to each wort was 1.6 g/L, equating to 48 g of hops, during wort boiling. Equal additions were added at three brewing stages (5 minutes after the boiling start, 30 minutes after the boiling start and 5 minutes before the end of boiling). The boiling time was 60 min (timed from the beginning of wort boiling). After the boiling process, the wort was clarified and cooled to 18-22 ℃ for yeast inoculation. the cooled wort was transferred to 2.5 L clean PET bottles, 2 L each, and labelled with the date and type of hops. After bottling, yeast was inoculated at a dose of 0.5 g/L. The fermentation process is treated by avoiding light. After 5-6 days of fermentation, the sugar content dropped to 4 °P. Then dry hopping was completed at a rate of 3 g/L and the fermenting vessels sealed. The beers were tested daily for diacetyl content, and the temperature was reduced to 4 ℃ when the diacetyl content was less than 0.1 mg/L. The temperature was reduced to 0 ℃ and the storage process continued for 7-10 days. Samples of hopped wort, fermenter drop, dry hopping, storage drop, and finished beer were taken and analyzed by GC-MS.

2.5 Enrichment and Solid Phase Micro Extraction of Aroma Compounds

Hop-derived volatiles was extracted via HS-SPME (fiber coating, polydimethysiloxane, 50/30um DVB/Carboxen on PDMS on a StableFlex fiber: sigma-aldrich). HS-SPME was accomplished using a Gerstel MPS multifunctional injector. NaCl (3 g) was weighed into a 20 mL headspace bottle, 5 mL degassed beer and internal standard 2-nonanol solution(the final concentration of the internal standard in the headspace bottle is 20 μg/L) was added. The mixture in the vial was sealed and magnetically stirred by an internal magneton for 45 min at a speed of 550 rpm in a 45 ℃ water bath temperature. The SPME needle penetrated the sample bottle gasket and extended into the bottle, pushing the handle to extend the fiber head out of the needle tube. The fiber head was placed in the upper space about 20 mm above the surface of the sample stirring for. The needle was removed and directly injected into the injector hold for 5 minutes. All samples were analyzed three times in parallel.

2.6 Extraction of Hop Essential Oil

Essential oils were isolated from hop pellets using the method of steam distillation. Accurately weighed 50 g (plus or minus 0.1 g) of hops powder was added to a 2 L boiling flask. At the same time, 1.5 L of simulated wort culture medium was added to the 2 L boiling flask. A polytetrafluoroethylene (PTFE) gasket was added to the flask to ensure gently boiling. Under the maximum heat setting of the temperature-regulating electric heating sleeve, the water was rolled and boiled, and then the heating power of the electric heating sleeve was reduced to give a rotary boiling state. Concurrently, the distillation rate was controlled at 25-35 drops/min (based on the drop of condensed liquid drops from the end of condenser tube), and distillation continued for 4 h. The hop essential oil was separated, and each hop extracted as per HS-SPME. An aliquot of a stock solution containing 500 μg of naphthalene (internal standard) in hexane was added to 100.0 mg of hop oil, to which hexane was added to make a 1.0 mL solution. Samples were by HS-SPME and analyzed by GC-MS[10]. (Field et al., 1996)

2.7 Sensory Evaluation of IPA

Descriptive sensory analysis was conducted by nine trained experts in a Blind Evaluation. To conveniently describe the aroma of each beer, samples with hops added to Simcoe were named beer Nos. 1-5, and those with hops added to Saaz were named Beer Nos. 6-10. Each panellist was presented with 60-80 mL samples in 150 mL clear glasses capped with clear plastic, odourless lids. Samples were kept cold on ice for no more than 2 h prior to evaluation. Ambient temperature during evaluation was 18-20 ℃.

With the approval of the school and the health department, the product evaluation meeting was organized, and the personnel who attended the meeting were randomly selected to participate in the evaluation and give scores on the evaluation form. Due to the limited location of the tasting meeting, the survey objects have certain limitations, and the consumers who are interested in beer and college students account for a large proportion in the survey group. Check and delete invalid score tables. Finally, a total of 374 valid scores tables were obtained (with no omission and no obvious perversion or logical conflict as the screening criteria). Ordered Logistic model is a multivariate analysis method of probabilistic nonlinear regression. The variables in this study were classified and ordered, so ordered Logistic regression analysis was used.

3. Result and discussion

3.1 Hop Oil Composition

Analyzes of the hop storage index (HSI), oil content, α- and β-acids content are summarized in Tables 1 and 2. As expected, the HSI increased as the oil contents of α- and β-acids significantly decreased with the aging of these two hops varieties. The content of hop essential oil in fresh Saaz hops was much lower than that in Simcoe hops, and it could be found that the aging degree of hops at 40℃ for 5 days was much lower than that at 30℃ for 10 days.

Table 1. Chemical Information of Simcoe Hops

Level	HSI[a]	Oil	α Acids[b]	β Acids[b] β
Fresh	0.28±0.01	1.6±0.02	12.2±0.01	3.6±0.02
Aged 1	0.33±0.02	1.2±0.01	10.4±0.01	3.2±0.01
Aged 2	0.37±0.01	0.8±0.03	8.6±0.02	2.9±0.02
Aged 3	0.41±0.01	0.5±0.02	7.4±0.01	2.4±0.01
Aged 4	0.56±0.01	0.2±0.01	5.2±0.01	1.3±0.03

Table 2. Chemical Information of Saaz hops

Level	HSI[a]	Oil	α Acids[b]	β Acids[b]
Fresh	0.27±0.01	0.8±0.01	2.8±0.02	3.1±0.01
Aged 1	0.31±0.01	0.6±0.01	2.3±0.01	2.7±0.01
Aged 2	0.39±0.02	0.5±0.02	1.9±0.01	2.2±0.02
Aged 3	0.48±0.01	0.3±0.02	1.5±0.02	1.8±0.01
Aged 4	0.61±0.02	0.1±0.01	1.0±0.01	1.1±0.02

a. Hop Storage Index.
b. Hop bitterness components: reported by the UV spectrometric method of the American Society of Brewing Chemists (1976).

3.2 Aroma Compounds derived from Hops during Beer Brewing

3.2.1 Process of Fermentation

After the saccharification wort itself already contains a variety of flavor substances. Later in the fermentation process, the sugar is utilized by the yeast to produce carbon dioxide and ethanol. When the sugar content dropped to 4° P, dry hopping was carried out. Geraniol could be transformed to β-citronellol and linalool; nerol could be converted to linalool and α-terpineol, the biotransformation of geraniol to β-citronellol was observed to be fast within the first 2-4 days of the fermentation. [11] Noticeably, geranyl acetate was not present in wort, but existed in the fermenter drop. An initial increase in the level of linalool was observed both in Simcoe and in Saaz, and the amount of linalool was higher among Simcoe than Saaz samples. In the Simcoe fermenter drops, caryophyllene oxide was presented in all drops except aged 4, while it was only present in fresh Saaz fermenter drop[12]. (Yang et al., 1993)

3.2.2 Dry-hopping

Dry hopping is a technique whereby hops are added to beer to leverage the maximum aroma potential of the hop oil[11](Schnaitter et al., 2016). It is noteworthy that there was no linalool and citronellol in Simcoe samples after dry hopping. β-pinene only existed in fresh hopped fermenter drops. For Saaz samples, nerol, geraniol, methyl geranate, citronellyl acetate and geranyl acetate were detected in all samples, however citronellal was not found in aged 3.

3.2.3 Storage of Beers

The storage is a process to promote beer maturation and make the aroma more harmonious. For Simcoe and Saaz, citronellol was absent in the storage drops and finished beers. For Simcoe samples, the number of aroma compounds in the finished beers was higher than that in storage drop, and only the amount of geranyl acetate in the finished beers was lower than that in the storage drops for Saaz. Citronellal, nerol, geraniol, methyl geranate, citronellyl acetate, terpineol and farnesol survived the whole brewing process and were found in all beers. The level of citronellyl acetate was higher in the Saaz beers, and the level of other aroma compounds was lower than that in the Saaz beers.

3.3 Sensory evaluation results

As shown in Figures 1 and 2, the beer with Simcoe had stronger fruit and floral aromas than the beer with Saaz added. However, the beer with Saaz was more spicy and herbaceous. First, five IPA beers with Simcoe hops were analyzed and compared. Beers No. 1 and No. 2 have strong floral and fruity aromas. On tasting, the palate is well balanced, with mild herbaceous notes and weak resinous and bitter notes. Beer No.3 had a lower fresh hoppy aroma, stronger bitterness, weaker mouthcoating and mellow taste than Beers No. 1 and No.2. No. 4 and 5 beers had a weak fruit aroma and the strongest bitterness, but it was notable that No. 4 beer had a cucumber aroma and No. 5 beer had a baking aroma, neither of which was pleasurable. In summary, with the increase of aging, simcoe hops bring less flavor and mellow depth to beer brewing, and the floral and fruity flavors are replaced by dry and thin bitterness. Next, the Beers No.6-10 made with Saaz hops were analyzed and compared. No. 6 beer has a clear hoppy aroma with moderate bitterness, but also a resinous and grassy aroma that can be unpleasant. No. 7 beer has a strong hoppy aroma, as well as a heavy resinous and astringent taste. No. 8 is a pleasant surprise, with a strong hoppy flavor but a decidedly less resinous and herbal flavor than No. 6 and 7, which makes the beer more balanced and easy to drink. Compared to the previous beers, No. 9 and No. 10 will appear less hoppy, and the reduction in hoppy aroma will result in a more pronounced bitterness. By evaluating beers brewed with Saaz hops at five different levels of aging, we found that green and resinous flavors decrease with aging, and beers brewed with properly aged hops have a more pure and balanced taste than beers brewed with fresh hops. However, excessive aging of hops can still lead to bad taste and high bitterness value.

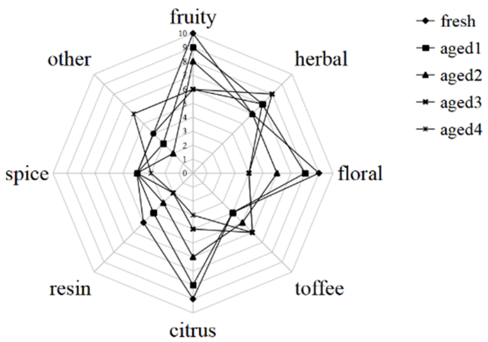

Fig 1. Radar map of sensory evaluation of sample beers No. 1-5 (IPA beers brewed-with Simcoe hops of different aging degrees) by expert group

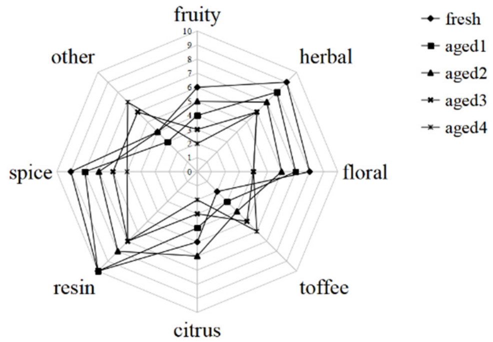

Fig 2.Radar map of sensory evaluation of sample beers No. 1-5 (IPA beers brewed-with Saaz hops of different aging degrees) by expert group

3.4 Sensory Evaluation of IPA by Consumers

SPSS 25.0 was used to test the validity level of the whole sample, and the results showed that the KMO value of the questionnaire as a whole was 0.716, and the significance probability P value of Bartlett's spherical test was 0. The results show that the validity of the questionnaire is good, and the subsequent data analysis and conclusion have certain credibility.According to Figures 3 and 4, among the beers brewed with Simcoe hops, people generally prefer fresh Simcoe hops, or Simcoe hops of sizes 1 and 2. Few participants preferred beers made with over-aged Simcoe hops. But when it comes to beer brewed with Saaz hops, most people prefer beer brewed with aged Saaz hops, saying that it has a strong aroma and less resinous and grassy flavor. It also suggests that moderate aging of some hops can make beer taste and aroma more suitable for the public.

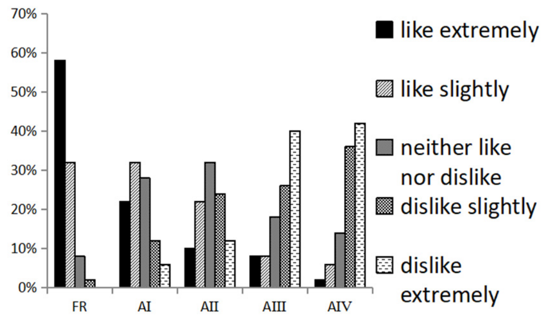

Figure 3: Consumer acceptance test of beer brewed with Simcoe hops.

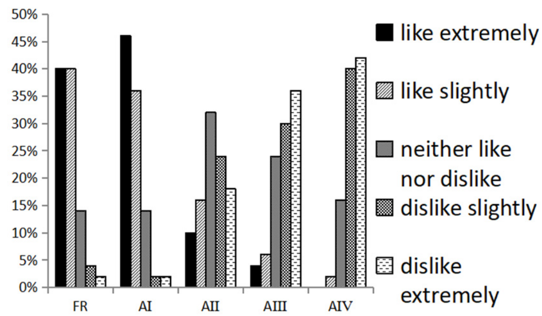

Figure 4: Consumer acceptance test of beer brewed with Saaz hops.

Figure 3 and Figure 4 reflect the useful information obtained by randomly recruiting volunteers to fill in questionnaires after blind testing of sample beers 1 to 10 (IPA beers brewed-with Simcoe and Saaz hops of different aging degrees).

4. Conclusions

In this experiment, the aroma spectrum of hops with different aging degrees in each brewing stage was analyzed to clarify the changes of various aroma substances. The results showed that the hydrocarbon content in the wort was very low. Citronellol, β-citronellol, and hop-related esters are not present in malt juice but are produced by yeast during fermentation. All epoxides were soluble in the wort, but most disappeared during fermentation.

Comparing the aroma composition between the samples, we found that temperature had the greatest influence on the aroma composition of hops. According to the sensory evaluation results, we believe that for hops with rich aroma components and suitable for IPA brewing, the floral and fruity aroma of beer brewed with these hops becomes weaker and the bitterness increases with the aging of hops. However, for hops with high humulus content and green orange flavor, the green orange flavor will decrease with the aging of hops, and the old hops with a long storage time will add a unique flavor. But the use of aging hops to add flavor to beer can add bitterness value and other bad flavors, so it needs to be used with caution.

Acknowledgements

We gratefully acknowledge New Henderson Biotechnology Co. Ltd. for sponsoring this research. The authors also wish to thank Dr. Patrick Ting (former MillerCoors, Milwaukee, WI, USA) for his technical guidance and Yakima Chief-Hopunion LLC for supplying the Simcoe and Saaz hops. Besides, the authors would like to thank Dr Cryn Russell (EIT, NZ) for her painstaking guidance on this paper. The Project was supported by the Foundation (No. 202011) of Qilu University of Technology of Cultivating Subject for Biology and Biochemistry.

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