

Article

Addition of Industrial Hemp (*Cannabis sativa* L.) Dry Inflorescence in Beer Production

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Featured Application: Featured Application: the utilization of industrial hemp inflorescence as an addition to beer production could result in functional beverages with emphasized and enhanced aromatic notes.

Abstract: Recent research has increasingly focused on the benefits of various plants, including hemp, which has gained prominence for its medical and industrial applications. The incorporation of industrial hemp in beer brewing has further popularized this age-old beverage. In several countries, the cultivation of industrial hemp containing a maximum of 0.2% THC (Δ^9 -tetrahydrocannabinol) has been legalized, leading to a growing recognition of the plant's potential and its derived products. The objective of this study was to produce beer infused with dried industrial hemp inflorescences. Additionally, a sensory analysis was conducted with panelists to evaluate the drinkability of the produced beer, revealing that both beer variants with added hemp inflorescence received higher scores. The addition of hemp inflorescence during boiling resulted with a higher specific gravity (1.01071 mg/L) in regard to control beer (1.01015 mg/L) and beer subjected to dry hemping (1.01018 mg/L). Generally, a significant difference for most physical–chemical parameters was noted in the sample boiled with hemp inflorescence, while the control sample and dry-hemped sample showed no statistically significant difference. The only physical–chemical property that showed no difference between all samples, including control, was bitterness, which exhibited no change in relation to the mode of hemp inflorescence addition.

Keywords: hemp inflorescence; bitterness; climatic changes; functional beer



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1. Introduction

Beer is the most consumed alcoholic beverage in the world and has a long history of use. Due to its composition, it enables many experiments during production. Industrial hemp can be added to change the aroma profile, taste, and even color of the beer. Hemp was used for various purposes in the past, but over time, due to its phytochemical content such as CBD (cannabidiol) and TCH (Δ^9 -tetrahydrocannabinol), it began to be abused, which led to a legal ban on its cultivation and use [1]. The potential benefits of this plant have been recently recognized and thus involved in the legislation of most countries. In the European Union, the levels of THC in industrial hemp are regulated and set to be under 0.2% [2].

Industrial hemp (IH) is grown primarily because of its stems, which have been used since ancient times for fiber production. Hemp seeds are also a high-value dietary prod-

uct for both humans and animals [3]. Industrial hemp inflorescences (IHF) are, on the other hand, considered purely as a crop residue despite them being a potential source of value-added products, essential oil, and beverage flavoring agents [4]. Industrial hemp is well adapted to climatic conditions and shows a robust insensitivity to abrupt climatic changes. It is a close relative to hops (*Humulus lupulus*), which is an important plant for the pharmaceutical, agricultural, and other industries [5–7]. Thus, in recent years, cannabis has found its place in the food and beverages industry. Cannabis-enriched foods and beverages are gaining popularity as a promising alternative utilizing dietary and medicinal benefits for consumers [8]. This is mainly rooted not only in the health benefits that attract the consumers but also in the idea of sustainability and reducing the influence on climatic change, the carbon footprint, and general ecological awareness. These are the facts that push industrial hemp as one of the sustainable solutions [9].

This stimulated scholars to experiment with the addition of hemp inflorescences to beer. There are several studies that investigated the addition of industrial hemp inflorescence or other parts of the plant, or essential oil extracts to the beer [4,7,10,11]. In some studies, they even used hemp classified as marijuana (*C. indica* with higher levels of THC) [12]. In that case, the produced beer cannot contain over 0.5% alcohol [6]. Beers produced with industrial hemp are allowed to have 10 mg of CBD and alcohol concentration between 3.5 and 6% *v/v*. While consuming beer with CBD, consumers reported sensations such as “elevating” and “naturally calming”. Beers with THC reportedly enhance intoxication, euphoria, lethargy, and, cravings [13].

Considering the above, this paper aimed to combine the pleasant effects of CBD and beer. Brewing beer commonly starts with wort production which is the extraction of soluble compounds such as fermentable sugars via the decoction process. Plant materials usually respond to this kind of extraction [14] where most of the water-soluble components transfer to the solution. Thus, the combination of an industrial hemp inflorescence (IHF) during boiling with hops could result in beer with enhanced aromatic notes reminiscing of hemp. Dry hopping is a usual method in brewing that ensures the extraction of aromatic compounds from hops to beer [15]. Adding dry industrial hemp inflorescence into the fermenter after the fermentation is finished could result with pleasant aromatic notes of hemp inflorescence. Also, this beer could contain a higher content of polyphenols. This paper aimed to characterize the physical–chemical properties of beers produced with IHF and IHE and to evaluate the sensory acceptance of both beers in regard to the control beer.

2. Materials and Methods

2.1. Materials and Chemicals

2.1.1. Industrial Hemp Analysis and Extraction

Plant Material

Dried inflorescence of industrial hemp (*Cannabis sativa* L.) obtained from a Croatian company (Hemp Agro d.o.o., Selci, Croatia) was studied.

Chemicals

The standards of cannabinoids purchased as solutions were used for HPLC analysis as follows: cannabidiol (CBD) (Dr. Ehrenstorfer GmbH, Augsburg, Germany), 100 µg/mL in methanol; cannabidiolic acid (CBDA) (Dr. Ehrenstorfer GmbH, Augsburg, Germany), 100.0 µg/mL in acetonitrile; cannabidivarinic acid (CBDVA), 100.0 µg/mL in acetonitrile; cannabidiolic acid (CBDA) (Cerilliant Corporation, Round Rock, TX, USA), 500 µg/mL in acetonitrile; cannabigerolic acid (CBGA) (Cerilliant Corporation, Round Rock, TX, USA), 500 µg/mL in acetonitrile; tetrahydrocannabivarinic acid (THCVA) (Cerilliant Corporation, Round Rock, TX, USA), 500 µg/mL in acetonitrile; tetrahydrocannabinolic acid

(THCA-A) (Cerilliant Corporation, Round Rock, TX, USA), 500 µg/mL in acetonitrile; cannabiol (CBN) (Dr. Ehrenstorfer GmbH, Augsburg, Germany), 100 µg/mL in methanol; cannabichromene (CBC) (Dr. Ehrenstorfer GmbH, Augsburg, Germany), 100 µg/mL in methanol; cannabigerol (CBG) (Dr. Ehrenstorfer GmbH, Augsburg, Germany), 100 µg/mL in methanol; and (-)-delta9-THC solution (THC) (Cerilliant Corporation, Round Rock, TX, USA), 1.0 mg/mL in methanol. All solvents and chemicals used were of analytical grade.

HPLC Characterization of Cannabinoids

An HPLC analysis of the crude material (hemp inflorescences) was performed by extracting 1 g of plant material in 20 mL of solvent (acetonitrile/methanol, 55:45% *v/v*) for 1 h at 40 °C on a magnetic stirrer. The mixture was filtered, and 100 L of the obtained extract was diluted to 2 mL with methanol and then filtered through a syringe filter (13 mm) with a pore size of 0.45 µm into a vial.

An HPLC analysis of cannabinoids was performed according to the procedure explained in detail in our previous published paper [16].

2.2. Beer Production

For this purpose, pale ale beer was brewed. An amount of 20 L of wort was prepared containing pale ale malt (5 kg). Pale ale (Simpsons Malt, Northumberland, UK) malt was purchased from the local brewing and malting equipment store (Sve za pivo d.o.o., Slavonski Brod, Croatia). Reverse osmosis water was adjusted for pale ale production by 8.0 g CaCl₂, 5.5 g MgSO₄ and 3.6 g CaSO₄. Mashing was performed at 65 °C for 60 min, and mash out was performed at 75 °C for 10 min. Boiling was performed at 100 °C for 60 min with the addition of 40 g of Smaragd hops (8.1% α-acids) at the beginning of boiling. Industrial hemp inflorescence (30 g) was added 45 min after the boil started. Cooling was conducted using a coil chilled with tap water until the temperature was reduced to 26 °C. Then, the wort was transferred to the fermentation vessel, 18 L, marked as “HF”. Inoculation was performed with Safale US-05 yeast (Fermentis, Marcq en Baroeul, France). One bag (11.5 g) of dried yeast was used for 20 L of wort, as recommended by the producer. Upon inoculation, vessels were placed in a cooling chamber at 21 °C. At the end of fermentation, beer was transferred to kegs, carbonated, and stored in cool place (4 °C).

The second batch of wort was produced the same way as the previous one. However, no hemp inflorescences were added during boiling. Fermentation vessel was marked as “DH” After the fermentation finished (7 days after inoculation), 50 g of hemp inflorescence was added as dry “hemping” material. Dry “hemping” lasted for 2 days at 20 °C. After dry “hemping”, beer was transported to a cool place and then treated as previously described.

Control beer was produced in the same way, sans the addition of industrial hemp inflorescence.

2.3. Physical–Chemical Analysis of Beer

Before the inoculation of wort, samples were analyzed using an Anton Paar Beer Analyser (Anton Paar GmbH, Graz, Austria). Fermentation was followed via portable EasyDens Anton Paar (Anton Paar GmbH, Graz, Austria). Polyphenols (B-590.41.111) analyses were performed according to a MEBAK analysis [17], while color (9.6) and bitterness (9.8) were evaluated according to the EBC methods [18,19]. The final beers were analyzed using an Anton Paar Beer Analyser (Anton Paar GmbH, Graz, Austria). All analyses were performed in duplicate for all beer samples.

2.4. Sensory Beer Evaluation

2.4.1. Evaluation of Beer Characteristics

A sensory evaluation was conducted by 5 trained consumers (1 female and 4 males). The panel consisted of beer consumers from different age groups (aged 42–59 years old). The materials used in ranking tests for intensity and sensory descriptors were adjusted from the general evaluation sheet for beer [20]. The intensity scales producing the best discrimination between samples and the most reproducible results were chosen. The scoring of each sensory attribute was performed on a five-point intensity scale, where 1 point means 'fault' and 5 points means 'excellent'. Descriptors were used to anchor/explain the points of the scale (7 sensory attributes: grainy, honey-like, caramel-like, smoky, grassy, hay-like, yeasty, fruity, flowery, spicy, hoppy). Foam, color, bitterness, carbonation, mouthfeel, astringency, and smell were also evaluated. Highest possible score was 100. Tasting tests were performed in an appropriate room, and beer samples were kept at room temperature for 10 min before the test. Samples were poured into a clean glass and covered with a watch glass to prevent volatile compounds from escaping the glass. All beer samples were numbered, and every sample was tested in triplicate. Evaluators were offered flat mineral water between the samples, together with plain white bread. All beers were served as draught beer. No personal data were collected from the panelists. Sensory evaluation was approved by the Ethical Council of our Faculty.

2.4.2. Drinkability Test

After the sensory evaluation, a drinkability test was performed. The drinkability test included the produced beers, with one being a control sample. Twenty consumers aged 20–60 evaluated the samples. Panelists were initially offered to try all beers (100 mL in a glass marked with numbers) in a relaxed atmosphere (a bar-like environment with a person waiting on the participants). After the initial tasting of all beers, panelists were left to choose on their own between the samples. The test lasted for 2 h and was conducted in the afternoon (6–8 PM). The consumers were engaged in a conversation and offered snacks (beer pretzels and peanuts), and they only had to note in the report the number of beer glasses consumed for each beer. Each panelist was always given 200 mL of beer in the glass. Leftover volumes were subtracted from the reports for each panelist. The initial 100 mL glass was not included in the result. The final result was obtained by summing up the number of fully drunk glasses for each beer. All beers, including the control sample, were served as draught beer.

2.5. Statistical Analysis

An analysis of variance (ANOVA) and Fisher's least significant difference test (LSD) were conducted, with the least statistical significance set to $p < 0.05$. Statistica 13.1. (TIBCO Software Inc., Palo Alto, CA, USA) was the software of choice for this data set. A PCA (principal component analysis) was also performed using Statistica 13.1. (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

3.1. Hemp Inflorescence Material Analysis

Due to legal requirements, industrial hemp growers must determine the THC levels in their crops. Since this paper dealt with the addition of IHF to beer, a product that contains alcohol, it was essential to verify the levels of THC as well as other cannabinoids in the starting raw material, industrial hemp inflorescence. The industrial hemp inflorescence was subjected to HPLC analysis, and the results can be seen in Table 1.

Table 1. Results of dry industrial hemp inflorescence analysis.

Compound:	Unit	Result
Cannabidiol (CBD)	%	0.78
Cannabidiolic acid (CBDA)	%	9.95
Cannabidiol (CBD + CBDA)—total	%	9.51
Cannabigerol (CBG)	%	-
Cannabigerolic acid (CBGA)	%	-
Cannabinol (CBN)	%	-
Δ 9-Tetrahydrocannabinol (Δ 9-THC)	%	0.09
Tetrahydrocannabinolic acid (THCA)	%	0.13
Tetrahydrocannabivarinic acid (THCVA)	%	-
Tetrahydrocannabinol (Δ 9-THC + THCA)—total	%	0.20
Cannabichromenic acid (CBCA)	%	0.30
Cannabichromene (CBC)	%	-
Cannabidivarinic acid (CBDVA)	%	-

-, not detected.

The results in Table 1 verify that the TCH level was under the legally prescribed limit of 0.2% and was quantified as 0.09%. CBDA (cannabidiolic acid) was the dominant compound in industrial hemp (9.95%), while the share of CBD (cannabidiol), its product, was only 0.78%. The dominant presence of CBDA compared to CBD is expected since decarboxylation had not been previously carried out. CBDA, as the main precursor of cannabinoid acids, degrades under high temperatures to its decarboxylated analog, CBD. The content of CBD and its acidic precursor (CBDA) is particularly important for industrial hemp, as they are attributed to analgesic, antibacterial, antidiabetic, antiepileptic, anti-inflammatory, antiproliferative, and antipsychotic properties [21].

When THC oxidizes due to exposure to air and light, it breaks down to form CBN. Therefore, the proportion of CBN increases as more THC degrades, and it is especially present in dried cannabis flowers that have been stored for a long time. Good storage conditions in airtight containers will prevent the formation of CBN, which can cause paranoia in large amounts. Table 1 shows that CBN was not detected in the samples, indicating that fresh samples were suitable for brewing.

The results of the starting raw material analysis exhibited satisfactory levels, and thus, the DHI could be used in further study. Even though these levels of THC and CBD in beers produced with industrial hemp are marked as safe, it should be noted that the Δ 9-THC guidance level does not cover possible adverse effects caused by other cannabinoids, including CBD, as well as the potential combination of effects of the intake of Δ 9-THC- and CBD-containing products [22]. The Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) in Germany has recommended that the daily intake of Δ 9-THC in hemp-containing foods should not exceed 1–2 μ g per kg body weight. For alcoholic and non-alcoholic beverages, this would mean that an amount of 5 μ g/kg is allowed [22].

3.2. Wort and Beer Analysis

Table 2 shows the data regarding the starting and finishing fermentation parameters.

The results from Table 2 indicate that the addition of industrial hemp inflorescence did not affect bitterness. The values of pH, specific gravity, real extract, original extract, apparent extract, alcohol content, polyphenol content, color, CO₂, and O₂ in beer were affected by the addition of different industrial hemp derivatives. The specific gravity was mostly affected by the addition of industrial hemp inflorescence during the boiling phase. This contributed to the specific gravity values and increased it to 1.01071 mg/L in comparison to the control (1.01015 mg/L). The addition of industrial hemp during boiling contributed to

the extract content in the prepared wort. This could be since hemp flowers contain 5–15% proteins [23]. All other extracts were increased in the sample with the addition of industrial hemp inflorescence during boiling. A significant difference was visible in all samples for the original extract, where the addition of inflorescence during boiling showed the highest contribution to extract values, reaching 14.88 °P.

Table 2. Basic physical–chemical indicators of produced beers.

Quality Indicator	Unit	Control	Hemp Inflorescence	Dry Hemping
Specific gravity	mg/L	1.01015 ^a ± 0.00004	1.01071 ^b ± 0.00009	1.01018 ^a ± 0.00010
Real extract	%	4.90 ^a ± 0.04	5.05 ^b ± 0.10	4.85 ^a ± 0.03
Original extract	°P	14.49 ^a ± 0.50	14.88 ^b ± 0.02	14.52 ^a ± 0.07
Apparent extract	%	2.83 ^a ± 0.05	2.67 ^b ± 0.03	2.61 ^a ± 0.04
Alcohol content	%	6.19 ^a ± 0.12	6.59 ^b ± 0.06	6.37 ^a ± 0.05
Polyphenol content	mg/L	180 ^b ± 2	339 ^a ± 3	272 ^b ± 1
Color	EBC	7.0 ^b ± 0.1	7.5 ^a ± 0.1	7.2 ^b ± 0.3
pH		4.06 ^b ± 0.02	4.09 ^a ± 0.06	4.11 ^b ± 0.02
Bitterness	IBU	25 ^a ± 1	25 ^a ± 1	25 ^a ± 2
CO ₂	g/L	1.18 ^a ± 0.03	1.07 ^b ± 0.02	1.05 ^b ± 0.01
O ₂	ppb	4014 ^a ± 2	3807 ^c ± 5	4006 ^b ± 2

^{a–b}: Means within rows with different superscripts are significantly different ($p < 0.05$); IBU—international bitterness unit. EBC—European brewing convention.

In that light, the ethanol content was also increased in the same sample, amounting to 6.59% *v/v*. The control sample had the lowest ethanol content, 6.19% *v/v*, while other samples had similar values. The sample subjected to dry “hemping” had 6.37% *v/v* ethanol.

Polyphenol levels were highest in the sample with the addition of inflorescences during boiling, 339 mg/L, while the control contained 180 mg/L. Dry “hemping” resulted in higher levels of polyphenols, 272 mg/L, which is still significantly lower than the sample with the addition of inflorescences during boiling. Although polyphenols are desirable in higher concentrations in many foods, beer quality generally exhibits a negative correlation with high amounts of polyphenolic compounds. Namely, a high concentration of polyphenols in beer could cause the occurrence of haze, which could impair its sensory properties and affect storage time. They tend to react with proteins in packaged beers and form undesirable haze before and after the expiration date [24,25]. On that note, this could be a potential problem in commercial production.

The color was slightly increased with the addition of inflorescence during boiling, reaching 7.5 EBC, while the control sample showed no changes in color, reaching 7.0 EBC. Dry “hemping” slightly attributed to color increase, reaching 7.2 EBC. It can be concluded that the addition of the inflorescence during boiling impacted the color of the produced beer, probably due to the increased availability of colored substances due to hot water extraction, and softening of plant material. Since the addition of inflorescence increased the color during boiling, Maillard reactions are probable a cause as well.

The pH values were slightly influenced in all samples. Namely, the control sample had a pH of 4.06. The sample with the highest shift was the dry “hemped” one. Its pH reached a value of 4.11. The sample with the inflorescence added during boiling showed a pH value of 4.09. The addition of hemp inflorescence acts to increase pH.

Bitterness was not affected at all with the addition of hemp inflorescence at any stage of brewing. This is interesting since industrial hemp belongs to the same family as hops and contains bitter substances. However, as reported by André [26], they are mostly variety-dependent.

The levels of CO₂ were lowest in the dry “hemped” sample, 1.05 g/L, while the control contained 1.18 g/L. In the sample with the addition of inflorescence the level of CO₂ was 1.07 g/L. The discrepancies are probably related to the forced carbonation process; however, this should be further conducted.

Oxygen levels also varied among samples. The sample with the highest levels of O₂ (4014 ppb) was the control sample. The sample which underwent dry “hemping” had 4006 ppb. This can potentially be related to the fact that dry hemping entails the opening of the fermenter to insert the dry hemp inflorescences and thus allows fresh air to enter the container. Lowest levels of oxygen were detected in the sample with the addition of inflorescences during boiling, at 3807 ppb. This could be due to the higher levels of polyphenolic substances that have antioxidative activity.

3.3. Sensory Analysis of Beer

The sensory analysis results are presented in Table 3 and the drinkability results are in Table 4. Even though the addition of industrial hemp inflorescence always arouses interest among consumers, it is important to check the acceptance of a new product. A beer with the addition of hemp was available on the market but alas, the taste was not rated well by the consumers. The evaluation was conducted by five trained panelists, and it gave an insight into the major errors and suggested some improvements. The results are presented in Table 3. To follow up on the sensory stability of beer, the evaluation was repeated after one month of storage at 4 °C in the refrigerator.

Table 3. Results of sensory evaluation.

Sample	Score/100	
	Fresh Beer	Beer After 1-Month Storage
Control	92	91
Hemp inflorescence	93	95
Dry hemping	90	95

Table 4. Results of drinkability test.

Sample	V (L) of Totally Consumed Beer	V (L) of Beer Consumed by 20–25 Year Olds	V (L) of Beer Consumed by 40–60 Year Olds
Control	2.0	1.5	0.5
Hemp inflorescence	5.1	4.0	1.1
Dry hemping	5.0	3.8	1.2

From these results, it can be seen that all samples received very high scores, 90 and up. It is notable that in the first round of evaluation, the highest score was attributed to the sample with the addition of hemp inflorescence during boiling (93/100). In this sample, the evaluators noted the flowery aroma related to hemp, which they highly favored. After 1 month of storage, this beer received an even better score (95/100), and the recognizable flowery notes were even more pronounced, which the panel acknowledged as very pleasant and enjoyable. A similar situation occurred with the dry “hemped” sample which received a higher score after 1-month storage as well (95). In the first evaluation, this sample was scored 90. All this indicates that maturation is very important in this case and that aromatic compounds need time to unify in a symphony. The control sample was rated 92 in the first round of scoring, and in the second, it received a slightly lower grade, 91. This is not a

significant difference, but it can indicate that the addition of any kind of industrial hemp form can help improve the storage time of beer.

Foam was rated very high for all samples and in both evaluations. The foam was most stable in the sample with the addition of hemp inflorescence during boiling.

The color was a bit higher in the sample with hemp addition, especially in the sample with the addition of inflorescence during boiling, which the evaluators did notice. This did not influence the score. Bitterness was noted as pleasant.

Even though all beers attracted very high scores, it was important to check the drinkability. The drinkability test is important as it gives insight into the real acceptance of a product. It quantifies as the consumer's wish to have another glass of a tested beverage, in this case beer. Drinkability can be described as the beer's characteristic which urges the consumer to continue taking liquid and to initiate thirst despite the satisfactory level of water in the body [27]. This test is not recognized by many scientists, but several scholars work continuously to upgrade and develop it [27–31]. This test is conducted in a relaxed, bar-like atmosphere because such scenery can activate the subconscious of the consumer and the tester chooses the more appealing beer without even realizing they are in a state of testing. A more appealing beer or a beer with a higher drinkability will be drunk faster and in higher volumes [27]. This test is very useful and common when introducing novel raw materials in brewing (hops, barley variety intended for brewing, yeast) due to its practical feedback. This usually turns marketing strategies in the right direction.

The results of the drinkability test applied for the two produced beers are shown in Table 4. The results indicate that the control sample was consumed in a lower volume, suggesting that it was probably less interesting to consumers.

The results for all hemped samples are very close and indicate a significant liking of such beer. However, there are differences between samples, and the most drunk beer was the one with the addition of inflorescence during the boiling stage, with 5.1 L being drunk. The second most drunk beer was the one subjected to dry "hemping", at 5.0 L. The general conclusion is that this kind of beer could be well received among consumers, but further research should be conducted regarding the amounts of desired cannabinoids in all beer samples.

When viewed from an angle of age distribution of 20 consumers and the amount of beer drunk between ages, it can be noticed that younger population drank more beer with the addition of hemp during boiling, while the older population drank slightly more of the dry-hemped beer. In any case, the addition of dry hemp inflorescences aroused curiosity and was well accepted among consumers.

4. Conclusions

Based on the analysis conducted in this study, several key conclusions can be drawn. Both beers infused with industrial hemp inflorescence exhibited a distinct floral aroma characteristic of industrial hemp. Notably, the beer with hemp added during the boiling process demonstrated a more pronounced aroma compared to the sample with hemp inflorescence added later. Additionally, both hemp-infused beers exhibited a higher alcohol content than the control sample, probably due to the additional extract infused with the inflorescences. The inclusion of industrial hemp inflorescence may enhance the functional value of the beer due to its elevated polyphenolic content and other beneficial compounds derived from hemp. However, this could cause problems in the later production (filtration) and storage time. Sensory analysis of both beers yielded highly positive feedback, indicating that the addition of industrial hemp was well-received by both trained panelists and untrained consumers (drinkability test). However, when adding the hemp inflorescence into the brew, regardless of whether it was added while boiling or during dry hemping, one should

be careful not to overdo it. Namely, the aromas are very strong and could take over the whole beer.

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Conflicts of Interest: The authors declare no conflicts of interest.

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