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Hop extracts to provide functional and tunable haze to bright beer and design specialty styles

Many of today's beers are designed to impart a pronounced haziness which is viewed as a highly desirable visual characteristic by the consumer, particularly among the specialty beer categories. In classic beers, haze is normally associated with a lack of filtration and/or chemical stabilization. As such, most of the turbidity lacks standardization over its storage time as seen in commercial hazy beers, which causes technical processing challenges for brewers when formulating new styles. Also, other ingredients used to add haze can be detrimental in terms of processing, final beer organoleptic quality and beer shelf-life. To that end, a hop extract, denominated Hop Haze Fraction (HHF), has been developed, characterized, and further explored for its potential application in bright beer to impart functional haze which could emulate unfiltered styles. HHF was spiked and assessed for stability in representative bright lager beers, as well as in malt test medium, using IPA, Ale and Wheat beers as references. Its impact on the overall beer taste was assessed. Results have shown that most beers displayed a high degree of haze stability over time, when dosed with HHF. It has also contributed towards synergies with beer base boosting the hop aroma, juicy character, and bitterness perception. The amount of haze imparted was tunable, and the degree of haze stability was affected by a combination of multiple factors such as the beer composition and its processing conditions. Beers spiked with HHF have shown increased haze stability and outperformed references. This work shows that HHF has the potential to be used as a post-fermentation and liquid dosing solution to standardize haziness into a bright beer matrix while maintaining the desirable beer taste quality parameters. In addtion showing promissing technical benefits for the brewhouse optimization, such as reproducibility, tunability, and maintaining hops naturalness status.

Descriptors: NEIPA, turbidity, haze, hops, functionality

1 Introduction

Many recent specialty beer styles developed are designed to impart an intentional haziness. This is viewed as a highly desirable visual characteristic among specialty beers by the consumer. Traditionally, hazy beers from the so called old world styles would include the German wheat ales Hefeweizens, or the Belgian-Style Witbier, and nowadays with more popularity, a new range of unfiltered lagers, as well as juicy IPA and New England IPA.

Among the different beer styles, hazy variants have brought a new appeal and acceptance to the consumer with a renewed trust based on the recently added official beer style categories. A significant increase in numerous unfiltered brews have been seen over the past years in EU and US where brewers aim to capture an increasing number of consumers with value-add brands and labels, in addition to known classic beer styles. This has been particularly driven by the expansion and popularity of the craft/artisanal beers and a reflection to what is perceived as more natural beers following the global mega trend of naturalness [1]. The Brewers Association have added the new Juicy and Hazy Juicy and/or Hazy Ale categories to their pale ale style guidelines since 2018 where haze is seen as a key characteristic of specific styles of beer [2]. These styles are popular examples that reflect the acceptance from the wider industry/consumer towards turbid beers and a style that is likely to see continued growth and further diversification/experimentation. In early 2018, Hazy IPAs rolled out from the US craft segment and reached a combined sale of $ 5.2 million. Furthermore, NEIPAs has since been a top emerging beer style [3]. Beer styles such as NEIPA, have been designed as an alternative to the existing West Coast-style IPA as a highly aromatic, juicy, and soft bitter beer. Hops and yeast are the two key drivers dictating its taste character, without bracing bitterness. The ingredient selection, and its control over biotransformation, as well as functional turbidity, makes this a complex style to master and a good case to show what hazy beers can offer combining aromatic hops, a smooth body and a controlled bitterness which is much appreciated globally [4]. This beer style is detailed in table 1. One of the main advantages of designing

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unfiltered hazy beers is that the haze can act as a functional carrier for added flavor and aroma active molecules [2, 5], thus boosting its taste impact due to increased non-polar hop components [5]. Furthermore, avoiding the use of stabilization agents and filtration stages, brewers would often limit the number of aromatics and taste components filtered out [1]. Nevertheless, several beer recipe parameters will also influence the haze formation and consequently, the flavor release, such as alcohol content, pH, temperature, oxygen, light, type of carbohydrates, lipids, proteins, polyphenols, and metal ions [6, 7, 8, 9, 10, 11].

Table 1 **NEIPA beer style profile description from the Brewers Association Guidelines**

NEIPA beer style Characteristics	
Key Parameters	Original Gravity (°Plato) 1.044 – 1.050 (11 – 12.4 °Plato) Apparent Extract/ Final Gravity (°Plato) 1.008 – 1.014 (2.1 – 3.6 °Plato) Alcohol by Weight (Volume) 3.5 % – 4.3 % (4.4 % – 5.4 %) Bitterness (IBU) 30 – 50 Color SRM (EBC) 4 – 7(8 – 14 EBC).
Color	Straw to light amber.
Clarity	Low to very high degree of cloudiness is typical of these beers.
Perceived Malt Aroma & Flavor	Low to medium-low malt aroma and flavor may be present.
Perceived Hop Aroma & Flavor	Medium-high to very high hop aroma and flavor are present, exhibiting a very wide range of attributes, especially fruity, tropical, and juicy.
Perceived Bitterness	Low to medium. The impression of bitterness is soft and well-integrated into overall balance and may differ significantly from measured or calculated IBU levels.
Fermentation Characteristics	Medium-low to medium-high fruity esters are present and can contribute to the perception of sweetness and be complementary to the hop profile. Diacetyl should not be present.
Body	Medium-low to medium-high. Perceived silky or full mouthfeel may contribute to overall flavor profile.
Additional notes	Grist may include oats, wheat, or other adjuncts to promote haziness. Taste and aroma attributes often from late additions of hops. Other hop-derived attributes such as citrus, pine, spice, floral or others may be present with or without the presence of juicy attributes. also, non-alcoholic offers have become available in the market.

One of the most important quality features in beer is its colloidal stability which is still of concern to brewers as it can compromise the overall beer taste stability and requires consideration to formulate novel hazy styles. The composition of haze varies widely due to different solids in solution. These include unfermented sugars, proteins, yeast, β-glucans, soluble fibers, polyphenols, hop resin, sediments etc. [6, 13, 14]. Turbidity in beer will depend on the number of particles, its size and composition (Table 2) [13, 7]. Its degree of absence is known as clarity and may range from bright, to clear, to slight haze, to hazy, to opaque. Traditionally, the presence of haze in regular bright beer styles is associated with physico-chemical instability in beer and thus a decrease in shelf-storage acceptability [7, 8].

Table 2 **Different types of solids in beer, its particle size and examples (adapted from literature [6, 13, 7, 8]**

Solids	Particle size [µm]	Turbidity	Examples
Coarse particles	≥ 0.1	Macroscopic and visible	Yeast
Colloidal dispersed	0.001 to 0.1	Cause via light scattering	Protein, polyphenols
Molecular dispersed	< 0.001	Dissolved and no turbidity	Combine with medium

Table 3 **Overview of associated causes with turbidity types in beer. Adapted from literature [6, 13, 8]**

Cause/trait	Turbidity	Origin	Kind
Reversible if heated	Chill Haze	Protein-Polyphenol complex	Organic, non-biological
Irreversible	Permanent Haze	Protein-Polyphenol complex	Organic, non-biological
Caused by malt or yeast; possibly filtration issues; increase in viscosity	Turbidity due to carbohydrates	Carbohydrates	Organic, non-biological
Small insoluble particles/crystals	Calcium oxalate	Calcium; oxalic acid	Organic, non-biological
Taste and aroma changes	Cultured yeast; wild yeast; bacteria	Microorganism	Organic, biological
Label remnants; filter aids	Dirt particles	Residues in containers; process error	Inorganic
Flocculation	Mineral turbidity	Residues from pipes, flocculation	Inorganic
Indigestible 150 µm particles	Microplastics	Decomposition processes	Organic, non-biological

Excluding microbial origin, turbidity is typically inorganic, carbohydrate-based or formed by polyphenols and haze-active proteins, held together by hydrogen bonding [7, 8, 9, 10]. The latter represents most of the haze in beer, and a frequent challenge due to a complex aggregation because of polyphenols oxidation and polymerization [7, 8, 15, 16]. Hop derived resins can also contribute to haze [6, 7]. The major phenolic precursors of protein-polyphenols haze found in hop materials are catechins, epicatechins and ferulic acid [17, 18]. Most haze-active polyphenols are dimeric proacthocyanidins, and higher polymers of gallocatechin, epicatechin, and catechin [8]. Turbidity in beer can be further categorized as chill haze or permanent haze where chill haze is to be avoided due to its reversibility. Particles with a diameter of 0.1 – 1.0 µm in size, formed between – 8 °C to 5 °C will dissolve when exposed to temperature variations (at 20 °C) [13, 15]. This leads to inconsistencies during beer shelf-life as polymerization tends to increase over time, increasing turbidity levels in beer [19]. Table 3 shows associated causes for different types of haze.

Brewers utilize several techniques to achieve an even haze, from heavily dry hopping to the addition of unconventional ingredients. However, a solution to provide the required consistent standardized cloudiness to beer still requires further investigation. Beers, such as NEIPA, are typically dry-hopped and double dry-hopped to intensify taste release and deliver haze. Hop pellets/powders are typically added to beers during/or post-fermentation stage. However, excessive dry hopping adds insoluble hop material, as well as infusing other hop components which may further impact beer taste [20]. Polyphenols have seen to impart astringency, mouthfeel, reducing power, as well as affecting the flavor stability of beers and ultimately the sensorially ageing of beer [1, 21, 22]. Spent hop extracts (0.3 – 0.5 % v/v) added in pale ale beer post-fermentation have reduced chilled haze formation without impacting beer haze stability [23]. Furthermore, HPLC analysis revealed the presence of haze active polyphenols procyanidin B2, epicatechin and sinapic acid. Another study using purified lupulin powder composed of resins and oils was used in NEIPA recipes to decrease the dry hop matter added and thus lower polyphenol and humulinones content intake [4]. Brewing trials have shown a positive impact on flavour release, astringency and beer mouthfeel; whereas a decrease in turbidity was reported. The main challenges were powder solubility, its addition time, and potential oxidation. Inversely, hop extract fractions have also been explored as potential finings indicating their versatility [5]. Compounds responsible for the fining activity appeared to be large (30 – 100 kDa, or more) polyphenols, and no increase in haze was observed [5]. Yeast haze of medium to low flocculating strains is another commonly used option to introduce haze in beer. Challenges may rise at industrial production due to the increased volume demands with addition of secondary yeast strains. Other concerns are the risk of microbial cross contamination and off-flavours as by-products from yeast biomass. The use of several other unconventional ingredients like flour, fruit pectin and wheatgerm would be disadvantageous due to non "beer clean label" and can reveal other opportunities to replace with innate beer ingredients. The use of high-protein grist using alternative grains, malt and adjuncts can also impart haziness [15]. High-protein grains, like oats, spelt and wheat, can create a distinctively soft, smooth body and contribute to the beer's cloudiness [2]. High-protein adjuncts such as wheat might raise allergen concerns. This opens opportunities for possible simpler alternative solutions. Furthermore, processing and storage conditionings play a role in haze quality and require monitoring to control any undesirable intensified ageing. Like pasteurization or any prolonged shaking might increase unfavorable haziness. Furthermore, malt bill and mashing as well as hot-side conditions may have a significant effect in haze [24, 25]. Certain inorganic constituents can also favour chill or permanent haze, such as calcium oxalate, magnesium, and manganese [26, 27, 28], however these were deemed out of scope for this project as naturalness is key for sustainable green technology solutions and thus our focus on natural minimally processed hop resins. Hop cones will typically contain up to 25% waxy components which are to explore as precursors to provide haze functionality as well as 100% utilization of hops to secure future sustainable solutions.

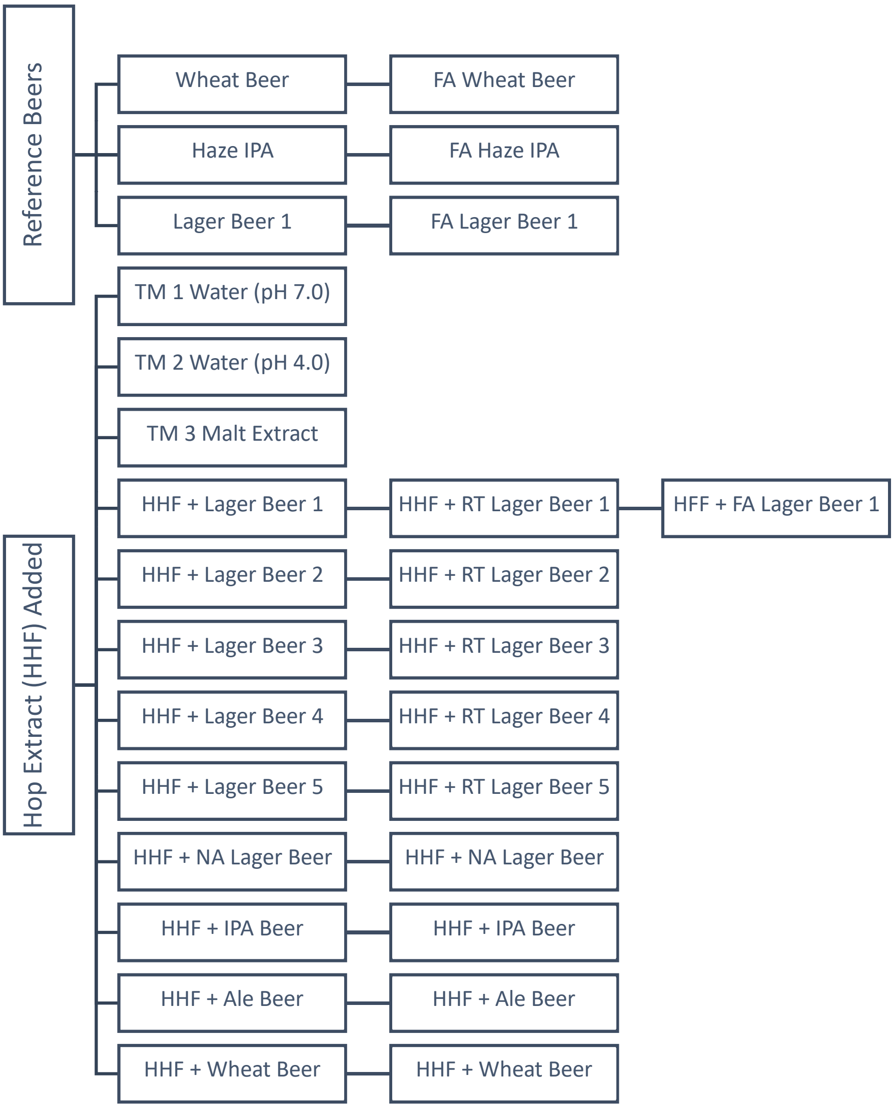

Fig. 1 **Experimental design outline. TM (test medium); HHF (Hop Haze Fraction); RT (Real Time); FA (Forced Ageing)**

The main objective of this study was to characterize the Hop Haze Fraction (HHF) and assess its potential to impart stable haze into different beverage applications. Following its characterization, its potential application in bright beer to impart functional haze was further explored. A methodic design experiment approach was taken to provide stability data when applied into different beverage matrixes. HHF was spiked and assessed for stability in a malt extract test medium, and pre-selected bright lager beers, IPA, Ale and Wheat beer styles. Time zero references were used for direct comparison, as well as real aged and forced aged samples. Furthermore, HHF impact on overall beer taste/attributes quality was assessed.

2 Materials and methods

2.1 Samples

To assist in the HPLC analysis performed the following chemicals were sourced from Fischer Scientific: Methanol >= 99.8 % (HPLC grade, Trinidad & Tobago), Ortho-phosphoric acid (H3PO4) (HPLC grade, China), Water (HPLC Grade, UK). Ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA, Great Britain) 99 +% was sourced from Alfa Aesar. To assist in the quantification of hop acids, alpha acid, beta acid, and iso alpha acid international calibration standards ICE-4 (Germany) and ICS-14 (USA) were sourced from Labor Veritas AG. A CB30/65 Malt extract was kindly provided by PureMalt (UK) with a known protein content of 3.1 g/100 g protein, and 62 – 68 ° Brix. Hop Haze Fraction (HHF) was produced from Herkules hops, cropped in 2017/18. HHF is a fractionated alkaline aqueous resin extract, 100 % hop derived, prepared from hop cones, and hop pellets. A selection of 9 commercially available beers were selected to perform suitable assessments upon dosing in HHF. The beers selected have been chosen due to their different beer formulations. These have been previously selected as representatives of a larger number of beers tested, in total 20. Their malt and carbohydrate composition, protein content, and ABV % were taken from the original beer description. Additional parameters were analyzed as described in this chapter. Beers were selected with approximate production dates and stored at 5 °C.

2.1.1 Experimental Design

The experimental design was planned as shown in figure 1 to assess the functionality of HHF. A variety of test mediums were dosed with HFF and to be further screened. These included neutral and acidic demineralized water, and a diluted malt extract. Water medium at two different pH values has provided an indication for acidity impact as well as possible applications into beverages such as waters, sodas, RTD's and seltzers. By including a malt extract medium, it is possible to observe the additional impact of increased protein content present in solution and the respective interaction with the hop extract and haze expression. Secondly, to assess an application closer to a final product, HHF was dosed in finished beer, including six bright lager beers and additional IPA, Ale and Wheat style beers as references. To understand the haze stability of HHF additions, real time and forced ageing tests were performed and compared to references.

2.1.2 Physico-Chemical Analysis

pH – All pH measurements were performed using a Mettler Toledo FiveGo™ pH probe calibrated with 3 buffer solutions, namely a pH 4 phthalate buffer, a pH 7 phosphate buffer, and a pH 10 carbonate buffer fully traceable to NIST, supplied by Fisher Scientific (UK).

Density – Measurements were performed using a Mettler Toledo DA-100M density meter. Total Polyphenols - Analyzed and quantified using standard spectrophotometric Analytica-EBC 9.11 method [29].

Turbidity – Measurements were performed using the Hach turbidity meter 2100QISO. An adapted ISO7027 – 1:2016 method was performed, and the meter calibrated using the Hach supplied 0, 100 and 800 NTU StablCal® calibration standards. Haze scales: The relationship between the different beer haze scales is: 1 EBC formazin unit = 40 Helm units = 69 ASBC units. Other turbidity units based on formazin as TEF (Trübungseinheiten Formazin), FTU (Formazin Turbidity Units), FNU (Formazin Nephelometric Units), FAU (Formazin Attenuation Units) and NTU (Nephelometric Turbidity Units) and have a conversion factor of 1 EBC to 4 TEF/FTU/FNU/FAU/NTU. Calculation: Chill Haze = Total Haze (TH) – Permanent Haze (PH). Where, Total Haze (TH) = final turbidity of the beer and Permanente Haze (PH) = initial turbidity of the beer.

Forced Ageing Testing – A Challenge test adapted from EBC (1963 method) was devised in which commercially available beers and beers spiked with HHF were analyzed for their haze content. Beer bottles were placed in a water bath for two days at 60 °C and immediately chill conditioned at 4 °C for 4 days. Samples were then brought to ambient temperature, degassed and a permanent haze reading was taken. A visual inspection on the beer to inspect for any precipitation/flocculation was noted prior to turbidity readings.

Real Time Testing – Multiple bottles of 9 commercially available beers were sourced to determine what extent the beer composition (cereals, protein content, ABV % etc..) affects the resulting haze upon HHF addition. Firstly the beers were degassed and an initial haze reading taken. A second set of bottles were then spiked with 80 g/hl HHF, shaken, degassed and their haze reading taken. This marked T = 0. One last set of bottles were spiked with 80 g/hL HHF, shaken, recapped and left at ambient conditions for 14 days. Once the 14 days had elapsed, 100mL was carefully poured into a beaker, as to minimise shaking (this was important to assess the haze reading of the undisturbed spiked beer), degassed and the haze reading assessed. This represented the haze at T = 14. A haze stability percentage value could then be detemined mathematically taking the haze reading at T = 0 / the haze reading at T = 14 * 100. All application recipes were prepared under control laboratory conditions and further preliminary bench trials for specific formulations and processing conditions are recommended.

Microbiological Analysis – A screen was carried out on HHF using traditional spread plate methods (Wallersteins Laboratory Nutrient; Yeast and Mould; de Man, Rogosa and Sharpe agar; Lysine) for the recovery of yeast, wild yeasts, moulds, aerobic and anaerobic bacteria incubated at 27 °C. The limit of detection (LOD) for this analysis was set to less than 100 CFU/mL. A microbial challenge test was performed using yeast and lactic acid bacteria prepared between 100 and 500 CFU/mL. Inoculated samples were incubated statically at 25 °C for 2 weeks and the number of cells determined after one and two weeks.

Organic acids – The organic acids (acetic, citric, oxalic, lactic, succinic, tartaric acid, pyruvic acid) were determined by UV spectroscopy.

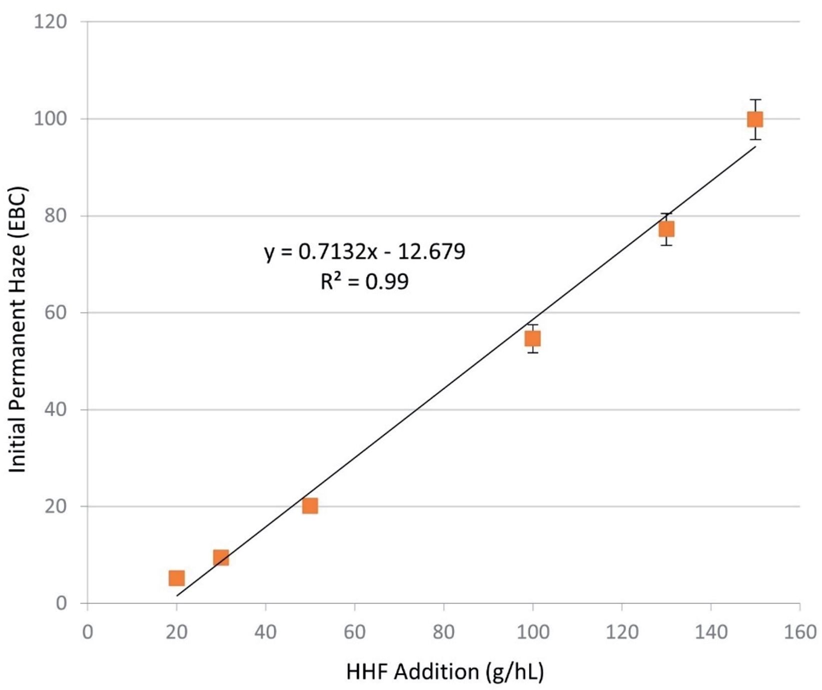

Fig. 2 **Calibration Curve: HHF dosed into lager beer 1 and corresponding initial permanent haze (EBC)**

Hop Acids – The Hop acid content of HHF was determined using an adapted EBC method 7.7 (alpha and Beta acids in hops and hop products by HPLC) and performed on an Agilent 1260 DAD HPLC machine. The alpha (cohumulone, n+ad humulone) and beta (colupulone, n+adlupulone) content was then determined using an international calibration standard was sourced from Labor Veritas AG. Similarly, the determination of the iso alpha acid content of HHF was screened following an adapted EBC 7.8 method.

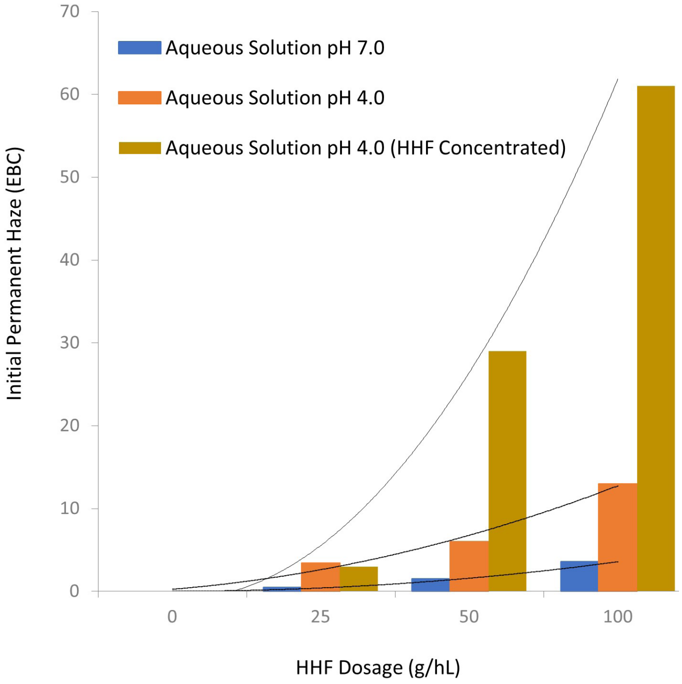

Fig. 3 **Two aqueous solutions at pH 7.0 and 4.0 dosed with HHF and the resulting initial permanent haze assessed**

Foam Analysis – Beer foam was visually assessed with a bench top pouring method where 330 mL of beer was poured into a beer glass. Furthermore, head retention of beer was analysed (NIBEM 10, 20, and 30) as per EBC method 9.42.

Sensory Analysis – The sensory impact upon dosing HHF into a reference lager has been characterized via an internal TNS panel of eight experienced tasting panelists. Descriptive profiling has been used to characterize the aroma and taste of HHF addition.

3 Results and Discussion

3.1 Hop Haze Fraction (HHF) Characterization

HHF was firstly characterized to assess its potential to be used as a functional ingredient by the brewing industry. HHF is visually characterized as an amber to brown alkaline aqueous hop extract with slightly hoppy to neutral odour. Upon dosing into solution, HHF was fully soluble in water medium and there was a slight visible colouration to the beer up to concentrations tested at maximum 2 g/L (v/v). Specific gravity at 20 °C was measured as 0.900 – 1.100. A calibration curve with various HHF concentrations was prepared. As seen from figure 2, the calibration curves show linearity ($R^2 = 0.99$) when assessing the haze imparted upon varying dosages of HHF into a reference lager. This indicates that HHF can provide an initial tunable haze. The HHF product was seen to be microbiologically stable with absence of microbial growth over the storage time. Results for all incubations have shown to be below 100 CFU/mL (aerobic and anaerobic bacteria, total, Saccharomyces and wild yeast). Trace amounts of beta acids were detected (< 0.15 %) and the content of alpha acids detected was negligible. The content of iso-alpha acids present was not quantifiable. The organic acid content of HHF determined via UV spectroscopy has shown that all organic acids were below 20 mg/L in the concentrated HHF solution. The exception was the presence of oxalic acid at 160 (± 15) mg/L. Oxalic acid is known to be present in hops, malts, and plants generally and its content in crops appears to depend on the growth region rather than on the variety [25, 26]. It has been reported to be present in different beer styles, including lagers and ales, with amounts from 2 to 28 mg/L in beer [29, 30, 31, 32]. The amount present in HHF is within this average concentration considering that is the concentrated solution, however, still of relevance as it is known that the formation of calcium oxalate may contribute to the appearance of hazes and sediments in beer and requires to be in suspension to avoid any beer stone [29, 31, 33, 34].

3.2 Hop Haze Application in Water Solutions

Firstly, to anticipate HHF's behaviour in different solution mediums and its potential for multiple applications, HHF was dosed into demineralized water at pH 7.0 and acidified water at pH 4.0, as shown in figure 3. The haze imparted was shown to increase significantly when dosed at the lower pH. At neutral pH, there was only a minimal expression of haze. Afterwards, both test solutions were monitored for another two weeks and HHF appeared to stay as a homogenous dispersion upon dosing into acidic water. Results indicate that the HHF haze expressed is dependent of the pH and that it maintains an initial homogeneous and stable suspension in acidic waters. The concentration of HHF via a rotary evaporator allowed to express significantly higher turbidity.

The influence of the individual buffer was investigated to determine whether the pH shock upon dosing into a finished beer would have an influence on turbidity. As such, 1g/L of an alkaline demineralized water (pH10) solution was dosed into a reference lager. Results have shown that no impact on haziness because of the pH shock into beer.

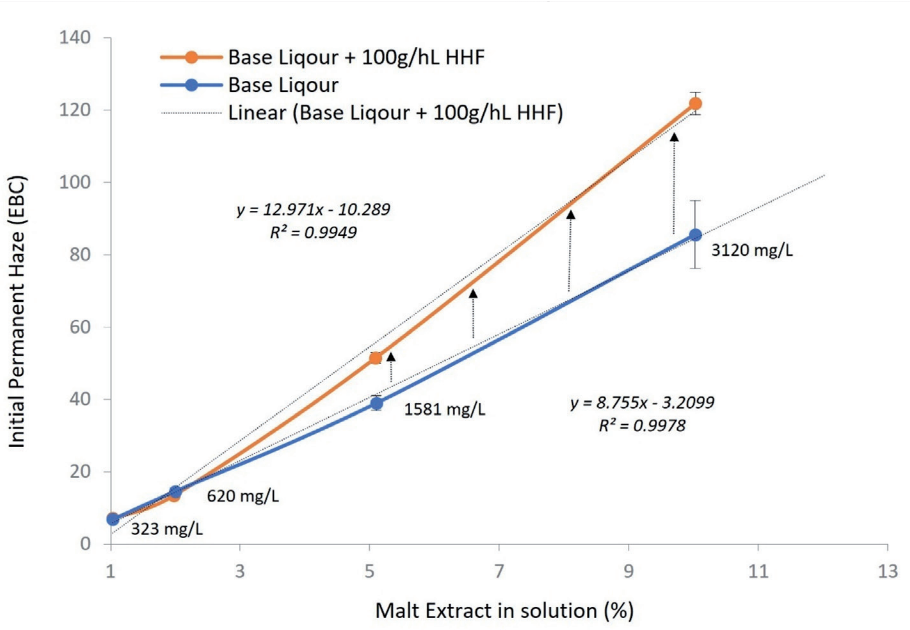

Fig. 4 **Solutions with increasing malt extract % and subsequent protein content assessed for initial permanent haze (EBC). Solutions dosed with and without HHF as shown by the orange and blue lines respectively. Protein concentration is expressed as mg/L**

Upon dosing HHF into solution the impact upon the solution's pH was tested. A change of + 0.1 pH units was determined upon the addition of 100g/hL HHF into a reference lager. The pH impact will be dependent of the dosage level of HHF.

3.3 Hop Haze Application in Malt Extracts

Beer typically contains approximately 500 mg/L of proteins with 2 mg/L sufficient to cause turbidity [35]. It was of interest to determine whether the protein content affects the resulting haze upon HHF dosing. To that end, four x 100 g solutions of increasing percentages of malt extract were prepared. A malt extract was used with a known protein content of 3.10 g/100 g and thus a constant increase in the protein content was determined mathematically as shown in figure 4. Results have shown that dosing 100g/hL of HHF into a 5 % malt solution, containing a protein content of 1581 mg/L, it was possible to obtain a haze expressed in excess of 39 % (51.5 EBC). When doubling the amount of malt extract to 10 %, a respective increase of 41 % in haze expressed was observed (122 EBC). As such, it was observed that the haze expressed in dependent of the protein content and that an increase in protein content results in an increased haze imparted upon the same HHF concentration (Fig. 4).

It is known that the quantity of haze formed when oxidised polyphenols are added to medium containing proteinaceous matter is a lot smaller than when polyphenols oxidise in this same medium. Similar results for immediate haze formation in beer were reported previously. [18, 35].

3.4 Hop Haze Application in Beer

It is well established that the catechins and proanthocyanidins from malt and hops are responsible for the formation of colloidal beer hazes. One of the easiest ways to demonstrate this phenomenon is to add these compounds to beer and to study the rate of haze formation [18, 36].

Impact of beer matrix in hop haze. The tests in this study have predominately been carried out in clear pilsners and lagers, which had very low initial turbidity levels of below 1.0 EBC, reflecting most available bright lagers in the market. This way, avoiding any interference from other possible haze sources and, as well, providing a suitable control beer for the intended studies/purposes.

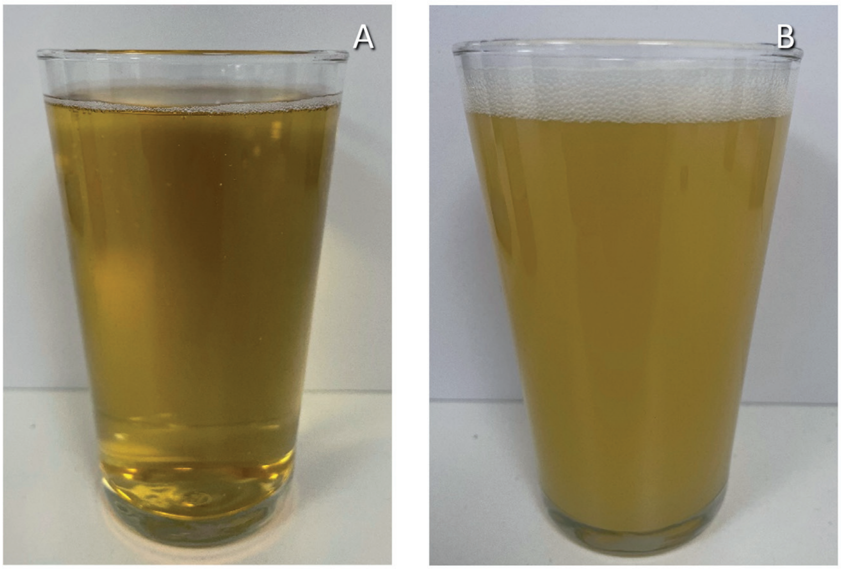

Fig. 5 **[A] – Lager beer – Reference (0.5 EBC); [B] Lager beer + 100g/hL HHF (50 EBC)**

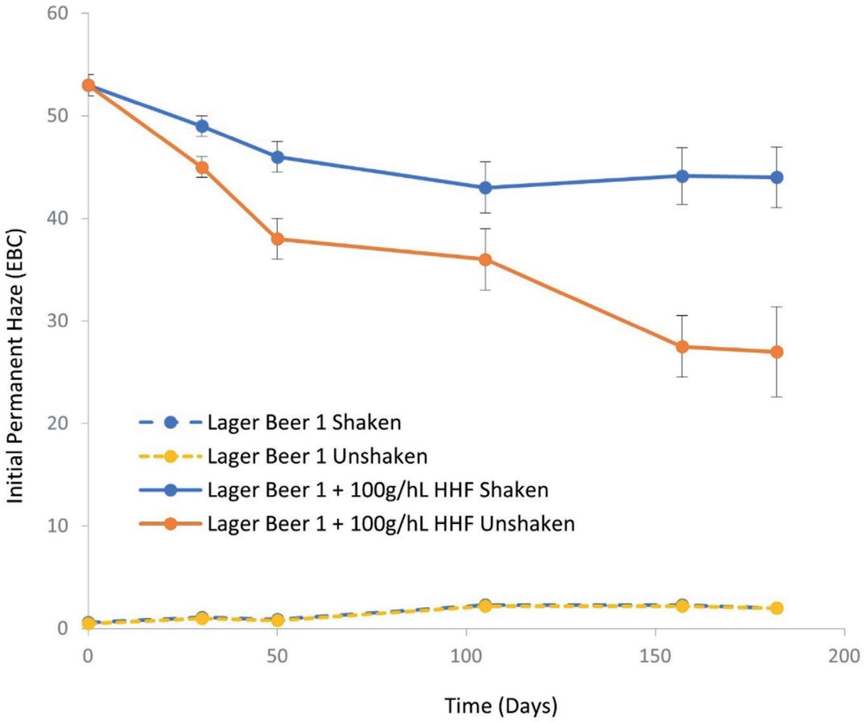

Fig. 6 **Real Time haze stability test; number of days since 100 g/hL HHF spiked into a reference lager beer 1 vs corresponding initial permanent haze**

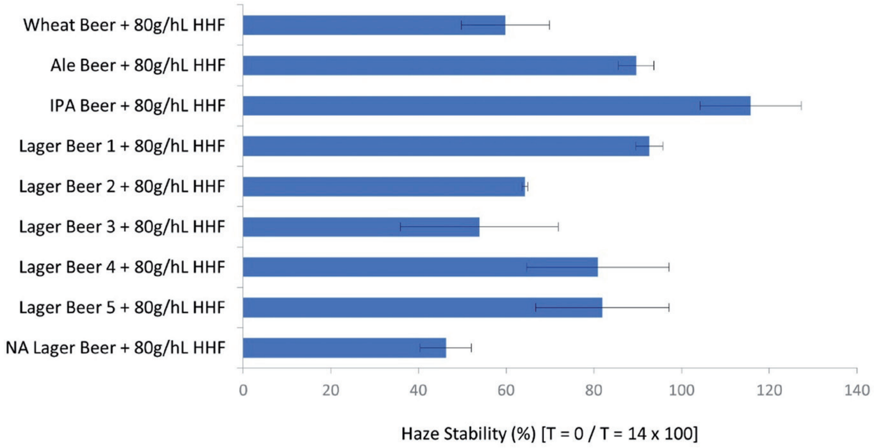

Fig. 7 **Real time haze stability test; selection of commercially available beers spiked with 80 g/hL HHF and the haze stability assessed**

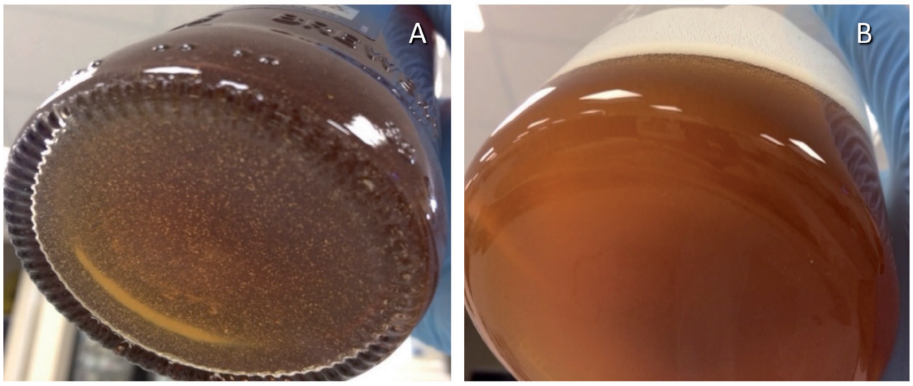

Fig. 8 **A) Dosed direct into bottle and left unmixed for 5 minutes – precipitate was formed. B) Dosed whilst mixing forming a homogeneous haze**

Figure 5 illustrates the visual impact upon dosing in 100g/hL HHF.

Upon dosing HHF into a reference lager, this was shown to produce a largely stable haze over the course of 160 days. Reference lager beers were spiked with 100g/hL HHF, shaken, recapped, and stored in the fridge at 5 °C. Over the course of the 160-day trial, bottles were removed from the fridge and left to equilibrate to room temperature. Two haze readings were then taken in which the dosed bottles were shaken or unshaken to assess the impact of natural sedimentation. It was noted that over the course of time small amounts of a fine white powdery looking sediment formed, and upon shaking this re-suspends into solution causing an increase in the haze valuable. This can be seen comparing the blue and orange lines displayed in figure 6.

A second set of trials were conducted using a range of commercially available beers, see table 4. A variety of beers were selected with differing cereal composition, pH's, protein contents etc. as to screen the influence of these parameters on the resulting haze upon HHF addition. Upon assessing the resulting haze delivered by HHF it was shown that most of the beers selected displayed a high degree of haze stability over time, as shown in figure 8. The wheat beer and non-alcoholic lager beer displayed a reduced haze stability over the two-week trial, and it has been hypothesized that this is due to the wheat beer's initial high degree of haziness and the non-alcoholic's lack of alcohol and the different processing and matrix composition. The content of polyphenols in the beers tested ranged from 83 to 237 mg/L. There was a limited correlation on the impact off turbidity and total polyphenol concentration. Suggested next steps are to assess the individual polyphenol and protein content, for example the content of haze active proteins present in the beer and the resulting haze imparted. Typical impact of composition and hop substances on the colloidal stability was demonstrated previously [37, 38, 39], components such as proanthocyanidins survive into the finished beer and do not tend to result in significant precipitation losses [22, 40]. Correlations between turbidity and protein fractions content in beers is well known and are significantly positively correlated with the turbidity of barley haze-active protein and turbidity in commercial barley and wheat beers [37, 40, 41].

Table 4 **Composition of commercially available beers tested**

Commercial Beers	Malt bill				Protein (g/100ml)	Total poly-phenols (mg/L)	pH	ABV (%v/v)	Carbo-hydrates (g/100ml)	Initial Haze Reading (EBC)
	Malt	Maize	Wheat	Rice						
Wheat Beer	×		×		0.8	112	4.35	5	3.3	113.50
Ale Beer	×				–	162	4.30	4.3	–	0.45
IPA Beer	×				0.5	237	4.37	5.5	3.4	1.2
Lager Beer 1	×			×	0.3	83	4.44	4.5	3.1	0.30
Lager Beer 2	×	×			0.3	67	4.13	4.5	4.0	0.26
Lager Beer 3	×	×			0.4	117	4.23	4.6	3.2	0.25
Lager Beer 4	×				0.35	127	4.55	5	3.2	0.45
Lager Beer 5	×	×		×	0.3	96	4.10	4.5	2.2	0.34
NA Lager Beer	×				0.3	122	4.51	0.03	3.1	2.83

Impact of addition method into beer. The formation of permanent opacification usually requires oxidation to form covalent bounds, which involves the formation of larger particles. Permanent turbidity normally particle size is of 1 – 10 µm [13, 7]. To understand the optimized dosing of HHF into finished beer, two addition method were assessed. Firstly, HHF was directly dosed into a reference ale and then left untouched for 5 minutes. Secondly HHF was dosed under constant agitation. The outcome, as shown in figure 8, indicated that it is important to ensure dosing HHF under significant mixing/agitation to ensure complete dispersibility, providing a clear homogenous haze and avoiding particulate formation.

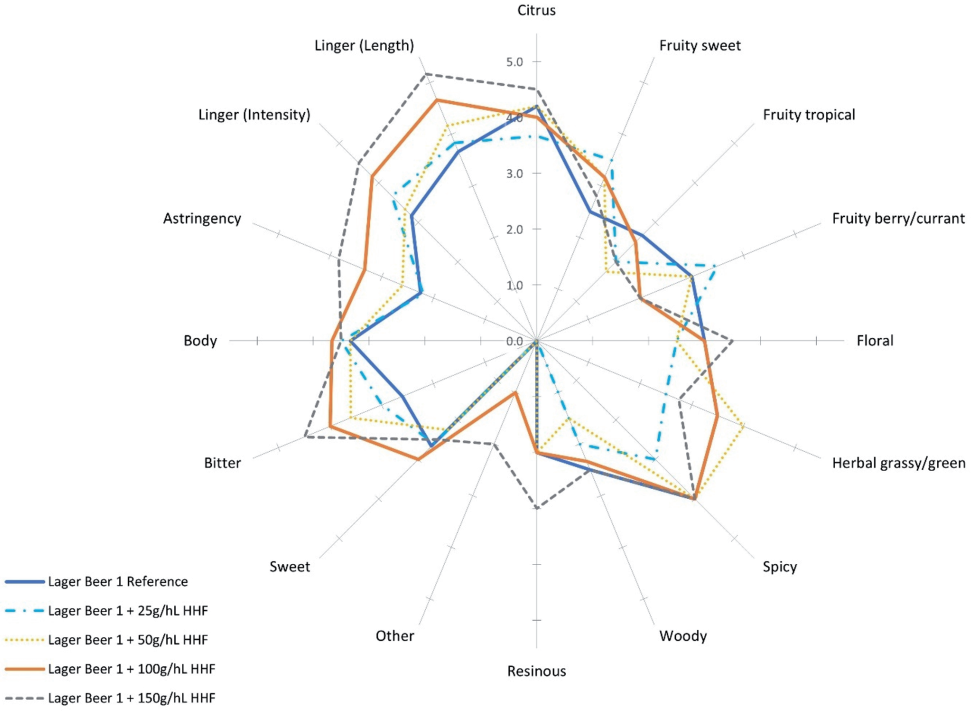

Fig. 9 **Descriptive profiling to characterize the aroma and taste upon HHF addition**

Impact of hop haze into beer sensory attributes. The sensory impact upon dosing HHF into a reference lager beer has been characterized via an internal TNS panel. The taste attributes were previously agreed and locked among panelists to allow sensory assessment of the beer aroma, flavour, mouthfeel, and aftertaste of the beer. Flavour attributes used were overall bitterness and sweetness impact. Aroma top note and retronasal olfaction impact was differentiated further into the clusters of citrus, fruity (tropical, sweet, berry), floral, herbal, spicy, woody, resinous, and overall presence of off-notes (notes absent from the reference). Mouthfeel was defined as a texture measure sensation in the mouth ranking from light to medium to full, when compared to the reference. The attributes included were overall beer body, astringency, and linger intensity/length as an indication of aftertaste. Temperature and carbonation were kept stable to guarantee no impact.

Lager beer 1 (Reference) was selected and then dosed with increasing concentrations of HHF to determine the sensory implications on the organoleptic quality. As seen from figure 9, upon increasing the dosage of HHF there will be an increase in the addition, astringency, bitterness, linger Intensity, linger Length and fruity sweetness. HHF adds significant bitterness perception above at higher dosages, which might be promising as an ingredient to add to lager beer recipes to emulate Hazy IPA and NEIPA's as these beer styles tend to show moderate to strong bitterness.

It was noted that upon the addition of HHF the sensory impact depends on the degree of flavour and aroma originally present in the base beer to which it has been dosed into. This was noticeable upon dosing HHF into Ale and IPA beers, where more aroma intensity and complexity are found in the base beer matrix which will consequently result in a masking and smoothing effect of additional sensory changes caused by the hop extract, and in most of the descriptors below.

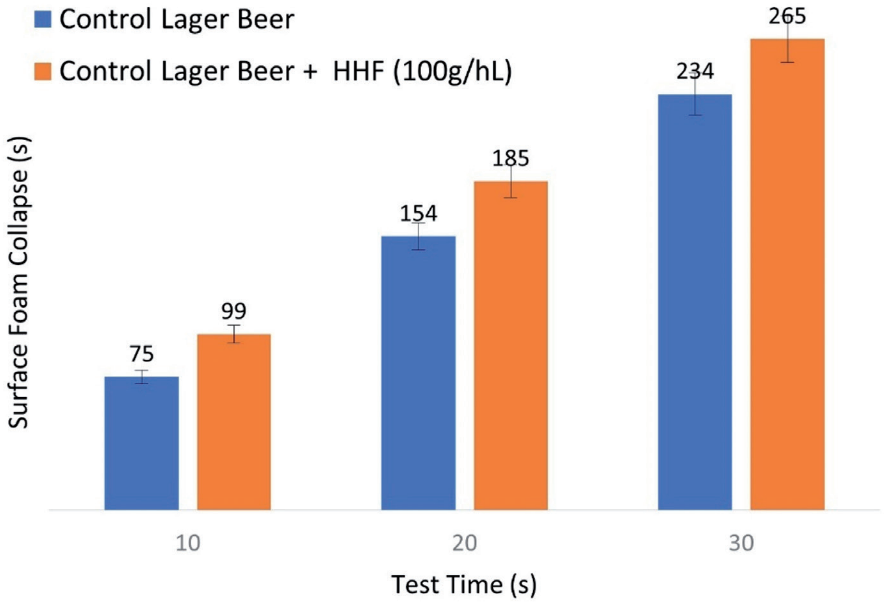

Fig. 10 **Foam retention of lager beer analysed using a NIBEM-T Meter**

Table 5 **Forced Ageing Test on different beer styles**

Sample	Initial Haze Reading (EBC)	Post Challenge Test Haze Reading (EBC)	Haze Stability %
Reference Wheat Beer	51.5 (± 6.5)	45.0	87.38 (± 8.0)
Reference Hazy IPA	56.6 (± 13.6)	43.0	75.97 (± 10.0)
Reference Lager + 80 g/hl HHF	41.0 (± 1.5)	40.1	97.80 (± 4.0)

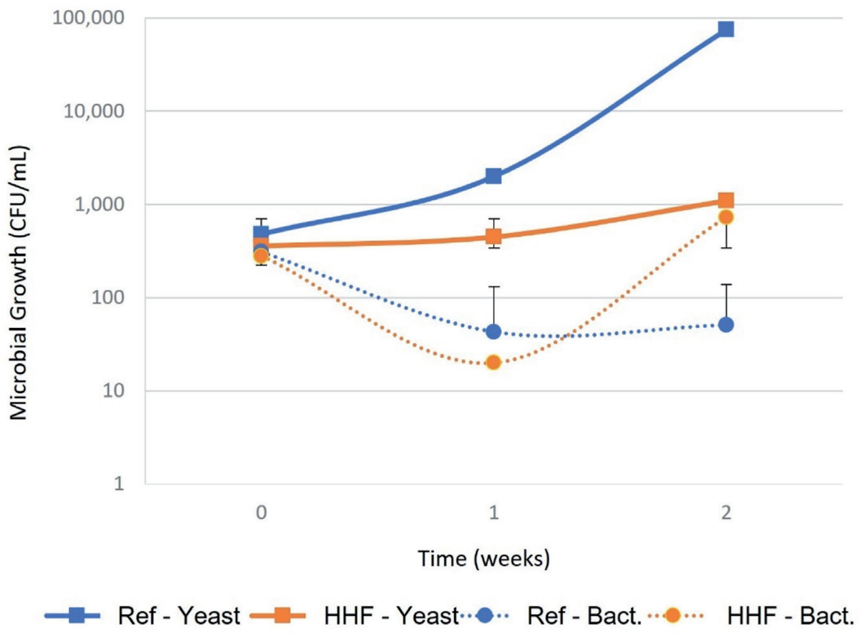

Fig. 11 **Microbial challenge test comparing inoculated lager beer samples (Reference) versus lager beer spiked with HHF (1 g/L)**

Impact on Beer Foam. As detailed in section 2, head retention of beer was analysed using a NIMBEM-T meter, to gauge the surface collapse over the course of 10, 20, and 30 seconds. Upon the addition of 100g/hL HHF to a reference lager beer, this was found to have a slightly positive foam impact when compared to the control, as displayed in figure 10.

3.4.1 Impact of Forced Ageing

The ability to predict and measure the haze in beer is key to anticipate earlier potential colloidal instability challenges and quality deviations over the intended storage/shelf time. Therefore, forced ageing tests are a good prediction to anticipate beverage instability [7, 15, 43]. As mentioned in section 2, a challenge test was devised and carried out to compare a clear reference beer spiked with 80g/hL HHF to a reference wheat beer and hazy IPA. As detailed in table 5, the clear lager beer dosed with 80g/hL HHF displayed a greater degree of haze stability than the reference wheat and hazy IPA. From previous studies, it was observed that haze normally drop below 300 NTU within the first two months storage which reveals a general limitation with these beers' haze consistency [5]. This is a good prediction for a stable turbidity as typically heating haze-containing beer to 70 °C for 10 min, the haze dissolves. Independently of being "chill-haze" and "oxidation haze" these results indicate that this is a more stable complex formed, when compared to previous literature reports where have would not be permanent and the majority tends to reappear overtime [44].

Microbiological Impact. Hops are known to impart antimicrobial potential mainly through its soft resins which should be explored as a functional benefit to formulate final beers. Hop components will act as antibacterial agents, along with other hurdles such as ethanol, CO_2, and a low pH value [45].

The results from figure 11 show that none of the beers were shown to be microbiologically stable as yeast growth was positive on both samples. Nonetheless, a significant decrease in yeast growth over the two weeks incubation on samples spiked with HHF was observed. Lactobacillus was recovered in both samples after two weeks. Growth was considered to have occurred if there was an increase of greater than 0.5 log CFU/mL in the levels of microorganisms observed at any time point when compared to the level present at time zero. Death was considered to have occurred if there was a decrease of greater than 0.5 log CFU/mL in levels of microorganisms at any of the time points when compared to the levels present at time zero.

4 Conclusions/Summary

Beer styles continue to evolve in line with customer trends and more technologies and ingredients are being developed to explore the beer sensorial experience as well as providing natural sustainable solutions for the future. Hops and hop components have been the focus of numerous studies as they are one of the main ingredients contributing to the overall beer taste, such as bitterness character, mouthfeel, astringency, color, stability, antimicrobial protection, as well as beverage functionality [47, 48]. Recently, hops have also been studied for its potential health-promoting effects [48, 49]. The increased demand for sustainable flavouring preparations from the brewing industry as well as advancements in technology have resulted in a wide range of hop extract-based products as potential alternatives to hop pellets, aiming to reduce further production waste material, to simplify supply chains, and lastly contributing to the organoleptic and functional quality increase in beer via brewing process optimizations [50–55].

The results shown in this study confirmed that the resulting haze from hop extract HHF produces a high degree of solid opaque haze which shows stability over its storage time. The amount of haze imparted was tunable and suitable to a wide range of water chemistry profiles. Beers with the addition of hop extract HHF have provided turbidity over 180 days outperforming several commercially available hazy beers. HHF has contributed towards boasting the hop aroma, the juicy character and bitterness of the final beer. Most effective application in solution was seen when dosed downstream with constant agitation to enable high dispersibility upon contact time at variable dosages from a range of 20 g/hL to 100 g/hL beer to, corresponding turbidity of 5.2 (+- 0.2) to 54.6 (+- 3.6) EBC respectively. Furthermore, at recommended dosage rates HHF was seen to contribute to the increased mouthfeel and smoothness which indicates a positive impact acting as a functional carrier in beer. A positive impact on microbial stability inhibiting yeast growth noted might be an intersting topic to investigate further. This study also indicates that the degree of haze stability was shown to be affected by a combination of different factors inherent to the beer matrix composition and processing conditions such as the malt bill, presence of adjuncts, the addition point and mixing method. It also indicates to impart synergistic effects with beer matrix and other ingredients by modulating sweetness, bitterness, and mouthfeel.

The consistency and beer colloidal stability over its shelf-life time is critical for consumer acceptability and HHF shows the potential to be used as a valuable natural ingredient to deliver stability and standardized solutions. A post-fermentation liquid dosing addition will simplify the brewing process, avoid addition of insoluble material while enhancing desirable technical characteristics such as potentially being light-stable, multipurpose, and naturally sourced from hops. HHF addition can be a vehicle for downstream differentiation of clear beers into specialty beer styles with no need to add stabilizers or inorganic constituents.

Advanced hop products derived from pure hop resin extract are increasingly used to obtain a more consistent hoppy character in new beer styles and brands. Consumers are looking for diversity and taste experience which justifies the general interest in hazy beers. Value add comes from designing sustainable engineered ingredients with functional benefits to beer recipes and brewing process optimization. Advanced hop products such as the current HHF have the potential to deliver these benefits and act synergistically with additional ingredients within specific beer styles. Several solutions have been presented due to retail requirements, however, to date, the challenge for haze and overall colloidal stability persists, and more research is required to develop new sustainable solutions. Further studies should focus on longer term real test stability to consider increased storage timing exposure into the matrix and corresponding haze performance over time. Another valuable aspect to monitor will be the possible extension of shelf-life as it is known that aromatic hop varieties have the potential to deceleration sensory aging [56]. As seen, the point of addition and agitation into brewing tank requires optimization and should be tested in next stages.

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