J. McMillan, M. Zunkel, A. Muñoz Insa, and C. Schönberger

The relevance of Hop Storage Index (HSI) for hop usage

The increased use of aroma hops for dry hopping has become a common technique used by brewers to impart hoppy aromas and flavours into beer. The increased demand for aroma hops has led to hop quality becoming more important. Brewers want to ensure the freshest hops are used, however the quality of hops can decrease during storage. The common hop quality indicator is hop storage index (HSI). In this study, two varieties of hops (Citra® and Galaxy®) with three different HSI classifications: fresh (< 0.32), aged (0.41 – 0.50) and over-aged (> 0.61) were evaluated. A pale ale was brewed and dry hopped with each variety at the different HSI classifications. The key aim was to assess the impact of HSI on total oil content and beer quality in terms of hoppy aroma and flavour quality. The total essential oil content and profile of hop oil components were analysed. Sensory and analytical analysis was conducted on all beer samples. The total essential oil content decreased as the HSI increased. The increase in HSI changed the profile of hop oil components causing significant decreases in myrcene and leading to an increase in oxidation components such as caryophyllene oxide, humulene epoxide I and humulenol II. Increase in the vegetal attribute for both varieties were significant, most likely due to the formation of sulphur compounds. Bitterness intensity and perceived bitterness increased as the HSI increased, whereas the bitterness quality was reduced. Humulinone concentration in beer increased alongside the HSI value. Beers dry hopped with hops which had a HSI value > 0.41 led to aroma, flavour and bitterness deviations in comparison to beers brewed with fresh hops. While HSI doesn't measure total oil content of aged hops, it does have a negative correlation on the total essential oil content, indicating that the hop quality has deteriorated.

Descriptors: Hop Storage Index (HSI), dry hopping, sensory, beer quality, hop quality

1 Introduction

Hops (*Humulus lupulus* L.) are a key raw material in the production of beer by adding bitterness, aroma, and flavour [1, 2]. The quality and brewing value of hops used in brewing is of significance important to both supplier and brewer. The brewing value of hops is determined by the composition of its secondary metabolites: alpha and beta acids and the level and composition of the essential oil. The chemical composition of hops can be impacted by harvest, processing, storage, and transport [3]. Exposure to elevated temperatures and oxygen during these processes will be detrimental to the brewing value. Hops and hop pellets are best stored in foils which are either flushed with an inert gas or vacuum sealed and stored at cold temperatures (0 – 5 °C) to slow the rate of degradation. The material can be stored unopened between 3 – 5 years [4].

Previous studies have examined the changes in alpha and beta acids during hop storage or assessing the impact of HSI on the bitterness profile of beer [5,6]. Multiple processes impact the chemistry of the hop - processes such as volatilisation, oxidation including modification, degradation or polymerisation, and potentially enzymatic changes [7]. For example, humulinones are formed by oxidation of alpha acids, with their concentration increasing over hop storage [8]. However, more recently research has been conducted evaluating the impact of storage conditions on the chemical composition of the hop oil [9]. This has now become an important area of research. As the hop harvest in 2020 and 2021 provided a surplus of hops and with the worst harvest since 2003 in 2022, the supplies for the coming year of varietal shortages will come from previous crop years [10]. This could result in brewers using previous crop year stock, with the aim of achieving the same high quality aroma (orthonasal) and flavour (retronasal) from previous harvests.

Hop freshness has been described as a measure of the degree to which the hop compounds remain unchanged from the time of harvest until they enter the wort in the brewing process [11]. The most common method for determining the freshness and quality of hops is Hop Storage Index (HSI), which is determined spectrophotometrically and measures the ratio of unoxidized hop acids to oxidised hop acids [12]. Results are categorised into five different classes of freshness [13]:

https://doi.org/10.23763/BrSc23-14mcmillan

Authors

Joshua McMillan, BarthHaas UK, Research & Development Department, Paddock Wood, United Kingdom, Mark Zunkel, Dr Alicia Muñoz Insa and Dr Christina Schönberger, BarthHaas GmbH & Co. KG, Brewing Solutions Department, Nuremberg, Germany; corresponding author: joshua.mcmillan@barthhaas.co.uk

Table 1 **Key aroma compounds of hop essential oil, analysed in this study with their aroma & flavour profile and flavour threshold. Table re-drawn from Kemp et al. [15]**

Compounds	Aroma & Flavour Profile	Sensory Threshold (µg/l)*
Monoterpenes		
Myrcene	Green, herbaceous, piney, hoppy	A: 9–1000[a]; F: 450[b]
Limonene	Lemon, citrus, grapefruit	n/a
Monoterpene alcohols		
Linalool	Floral, rose-like, orange	A: 2–80[a]; F: 27–80[a]
α-terpineol	Floral, citrus, lemon	A: 330[a]; F: 2000[c]
Geraniol	Fruity, floral, waxy	A: 4–300[a]; F: 36[b]
Ketone		
2-undecanone	Floral, fruity, waxy	F: 400[c]
Ester		
Methyl geranate	Fruity, floral, waxy	F: 21.5[d]
Sesquiterpenes		
β-caryophyllene	Woody, spicy, herbal	A: 160–420[a]
α-humulene	Woody, spicy, piney, hoppy	A: 120–747[a]
Oxides		
Caryophyllene oxide	Woody, incense	n/a
Humulene Epoxide I	Herbal	A: 10[a]
Humulenol II	Pineapple, mugwort	A: 150–2500[a]; F: 2500[c]

* Aroma (A) and flavour (F)

References for sensory threshold are superscript: [a] [11]; [b] [16]; [c] [17]; [d] [18].

- Fresh – HSI value: < 0.32
- Slightly Aged – HSI value: 0.33 – 0.40
- Aged – HSI value: 0.41 – 0.50
- Strongly aged – HSI value: 0.51 – 0.60
- Overaged – HSI value: > 0.61

HSI examines the oxidation of alpha acids during the processing and storage of hops. If an increase in HSI is observed, this could mean the hops have been stored poorly, which can lead to a decrease in the utilisation [14].

The aim of this study was to determine if there is a correlation between the HSI increasing and an impact on the total oil content, hop oil compounds and the sensory and analytical impact on dry hopped beer. Table 1 shows the hop oil compounds investigated in this study, along with the aroma (orthonasal) and flavour (retronasal) profile and the compounds sensory threshold.

The research was conducted with two of the most popular varieties used for dry hopping: Citra® Brand HBC 394 cv and Galaxy® Brand 94-203-008 cv. The range of HSI classifications used within this trial were fresh, aged, and overaged, which were achieved by using pro-oxidative storage conditions. Brewing trials were conducted where beers were dry-hopped with hop pellets of varying HSI classifications to identify any differences in the sensory profile and hop oil components, which were determined using a trained taste panel and analytically by HS-SPME-GC-MS. Additionally, the humulinone content in beer was measured due to the potential for an increase in perceived bitterness in beer which has been dry hopped with aged hops.

2 Materials and methods

2.1 Chemicals and Standards

HPLC grade methanol, phosphoric acid (85 %), ethylenediaminetetraacetic acid (EDTA), toluene, sodium chloride, sodium hydroxide were purchased from VWR (Lutterworth, United Kingdom). 6-undecanone (97 %) was purchased from Sigma Aldrich (Gillingham, United Kingdom). DCHA-Humulinones, ICS-Hum1 HPLC standard was obtained from Labor Veritas (Zurich, Switzerland).

2.2 Hop Pellet T90 Samples and Treatment:

2.2.1 Hop Pellet T90 Samples

Citra® T90 pellets, crop year 2021 were donated by John I. Haas Inc. (Yakima, WA, USA) and Galaxy® T90 pellets, crop year 2021 were donated by Hop Products Australia (Hobarth, Tasmania, Australia).

2.2.2 Hop Pellet T90 Treatments

The control hop pellets were vacuum packed in aluminium foils and stored in a freezer at –25 °C. The hops were aged to two different states: aged and overaged. Aged hop pellets were flushed with oxygen and then sealed and stored in an incubator at 35 °C for two weeks, to reach a target HSI level between 0.41 – 0.50. Overaged pellets were flushed with oxygen every other day and stored in an incubator for two weeks at 35 °C, to reach a target HSI of > 0.61. Both the aged and overaged pellet samples were flushed with oxygen using a Household Oxygen Bar (HuiZhou, China). After treatment, the aged and overaged pellets, were vacuum sealed and stored in the freezer at –25 °C until used for analysis or brewing trials.

2.3 Hop analysis:

2.3.1 Hop Storage Index (HSI) Analysis

The HSI value was determined using ASBC Methods Hops 6A/12 [19]. For each HSI value, three analytical replicates were prepared and analysed for each variety. Hop T90 pellets were ground using an IKA A11 basic analytical mill (Oxford, United Kingdom). Toluene (100 ml) was added to 5 g of ground hop pellets and placed on a mechanical shaker for 30 minutes. After the extraction, the samples were left for 15 minutes to allow for the hop material and solvent layer to separate. Five millilitres of the toluene extract were pipetted and diluted to 100 ml with methanol (solution A). Solution A was further diluted by pipetting 3 ml and diluting to 50 ml with alkaline methanol (solution B). Solution B's absorbance was measured spectrophotometrically against a blank at 275 nm, 325 nm, and 355 nm. The ratio between the value at wavelengths 275 nm and 325 nm gives the HSI value.

2.3.2 Volatile Oil Analysis

For volatile oil analysis, three analytical replicates of each HSI value were prepared for each variety. Volatile oil analysis was conducted using EBC method 7.10 [20]. 100 g of pellets were weighed into a 5 L round bottom flask. De-ionised water (2 L) was added, before being put on a thermostatically controlled heating mantle from Fisher Scientific (Loughborough, United Kingdom) which was used to maintain a steady, gentle boil for three hours. The cooling system consisted of Liebig condensers which were cooled by a recirculating water bath from VWR (Lutterworth, United Kingdom) set at 5 °C. The oil was collected using a 3 ml graduated oil Clevenger, with the total oil content measured once cooled and reported as ml/100g of hop material.

2.3.3 GC-FID Analysis of Hop Oil

Hop oil samples (150 µl) were pipetted into 300 µl GC vials. The samples were analysed by gas chromatography using Perkin Elmer Clarus 580 with flame ionisation detector (FID) (Waltham, MA, USA). Perkin Elmer TotalChrom Navigator (v6.3.2) was used for data analysis. Operating conditions for GC-FID: GC column - RXi-1ms (30 m x 0.25 mm ID x 0.25 µm); injection volume – 0.5 µl; oven temperature – 60 °C for 2 min, 60 – 22 5 °C at 5.0 °C (5 min hold); split – 151:1; mobile phase – hydrogen; FID temperature – 280 °C; total run time – 40 minutes.

2.4 Brewing Trials

2.4.1 Brewing Process

A pale ale beer was brewed using the BarthHaas Germany Concept Brewery (Nuremberg, Germany). The brewery consisted of a 1 hl brewhouse (mash tun, lauter tun, kettle and whirlpool) and 1 hl open fermenter with a Perspex lid from Kasper Schulz (Bamburg, Germany). The malt bill consisted of 90 % pale ale malt, 5 % wheat malt and 5 % Carapils malt from Weyermann Speciality Malts (Bamberg, Germany). A 90-minute temperature-programmed mashing regime was used to produce a 12 oP wort (53 °C for 5 minutes, 63 °C for 20 minutes, 68 °C for 30 minutes, 72 °C for 20 minutes and 78 °C for 15 minutes). Herkules hop extract from BarthHaas Germany (Nuremberg, Germany) was used for bittering to 20 IBUs during the kettle boil (90 minutes, with an overall evaporation rate of 12.5 %). Dried ale yeast strain SafAle US-05 (Fermentis, Marcq-en-Baroeul, France) was pitched at 62.5 g/hl and fermented for five days. After fermentation the beer was split into three batches (17 L each) and transferred to VOLLI-TANKS (HW Brauerei-Service, Erbshausen, Germany). The VOLLI-TANKS were statically dry hopped at 0.05 g/hl for seven days with three different HSI values of the hops Citra® and Galaxy®, with the vessel contents being agitated on day three. The beer was filtered using Novox 200 ST from Filtrox (St. Gallen, Switzerland), with Filtrox FibraFix AF-31H filter sheet (4 kg per m2 holding capacity). The beer was stored in 20 L stainless steel kegs from Thielmann (Hausach, Germany) for conditioning/maturation and carbonation at 5.49 g/l of CO_2 and stored at 3 °C until bottling. Bottling (250 ml bottles) was conducted to allow for sensory evaluation using a HELBApro1 bottle filler (one-organ filler) (Heinr. Leicht, Bamberg, Germany). The brewing trial was conducted in duplicate for both hop varieties.

2.4.2 Brewing Trial Analysis

pH was measured using a pH 50+DHS pH meter from XS Instruments (Carpi, Italy). Original gravity and alcohol were measured using a DMA 4500 DM + Alcoholyzer Beer ME from Anton Paar (Graz, Austria).

2.5 Sensory Analysis

Triangle tests were performed before the descriptive analysis and ranking sensory analysis. There were 16 participants (eleven males and five females) for the triangle test who all had various sensory training, so the panel was defined as mixed. Data was analysed using a z test.

A panel of 12 participants (eight males and four females) who all had experience with the BarthHaas Hopsessed® tasting scheme carried out the descriptive sensory analysis. Participants were given 50 ml of beer with a randomised three letter code for each beer. The BarthHaas Hopsessed® tasting scheme was used to qualitatively evaluate the beers for 12 flavours, as shown in table 2.

Table 2 BarthHaas Hopsessed® Categories and their specific attributes

Aroma & Flavour Category	Specific Attributes
Floral	Elderflower, camomile blossom, lily of the valley, jasmine, apple blossom, rose, geranium, carnation, lilac, lavender; lily, osmanthus
Citrus	Grapefruit, orange, lime, lemon, bergamot, lemon grass, ginger, tangerine, pomelo
Sweet Fruits	Banana, watermelon, honeydew melon, peach, apricot, passion fruit, lychee, dried fruit, plum, pineapple, cherry, kiwi, mango, guava
Green Fruits	Pear, quince, apple, gooseberry, white wine grapes
Berry & Currant	Cassis (black currant), red currant, blueberry, raspberry, blackberry, strawberry, wild strawberry, cranberry, mulberry
Cream Caramel	Butter, chocolate, yoghurt, honey, cream, caramel, toffee, coffee, vanilla, tonka bean, coconut
Woody Aromatic	Tobacco, cognac, barrique, leather, woodruff, incense, myrrh, resin, earthy, cedar, pine
Menthol	Mint, lemon balm, sage, camphor, menthol, wine yeast, eucalyptus
Herbal	Marjoram, tarragon, dill, parsley, basil, fennel, coriander, rosemary, thyme, green tea, black tea, mate tea, oregano, lovage
Spicy	Pepper, chilli, curry, juniper, aniseed, nutmeg, liquorice, clove, gingerbread, fennel seeds, cinnamon, coriander seeds
Grassy-Hay	Green-grassy, fresh cut grass, hay, tomato leaves, green pepper, nettle, cucumber, bamboo leaves
Vegetal	Celery root, leek, onion, artichoke, celery stick, garlic, wild garlic, radish

Table 3 **Concentrations of alpha and beta acids and HSI value for Citra® and Galaxy® T90 hop pellets pre and post hop aging treatment. The values are means from triplicate analysis with standard deviations**

Variety	HSI Classification	Alpha Acids (%)	Beta Acids (%)	Hop Storage Index
Citra®	Fresh	12.26 ± 0.07	3.41 ± 0.04	0.32 ± 0.005
	Aged	10.39 ± 0.21	3.17 ± 0.08	0.43 ± 0.007
	Overaged	7.62 ± 0.05	2.45 ± 0.06	0.61 ± 0.01
Galaxy®	Fresh	16.11 ± 0.1	9.75 ± 0.07	0.31 ± 0.004
	Aged	13.62 ± 0.18	9.62 ± 0.05	0.42 ± 0.02
	Overaged	8.53 ± 0.16	7.03 ± 0.15	0.63 ± 0.01

Additionally, tasters scored the hop aroma intensity, hop aroma quality, bitterness intensity and bitterness quality on a 9-point scale (1-9). The panel scored the perceived bitterness on a sliding scale 0–100. The data was collected and analysed using CompuSense (Guelph, ON, Canada).

2.6 Beer Analysis

2.6.1 HS-SPME-GC-MS Analysis

An internally developed method for the analysis of beer by HS-SPME-GC-MS was used. Beer samples (5 ml) were pipetted into 20 ml vials with 2 g of sodium chloride and 100 µl of the internal standard (6-undecanone) was added. The samples were analysed by gas chromatography Perkin Elmer Clarus 500 (Waltham, MA, USA) coupled with mass spectrometry (Clarus 500; Perkin Elmer, USA) equipped with an autosampler (PAL RSI 120, CTC Analytics AG, Zwingen, Switzerland) using headspace solid-phase microextraction (HS-SPME). Perkin Elmer TurboMass (v6.1.2) with NIST MS Search (v2.3) was used for data analysis. Operating conditions for HS-SPME-GC-MS were: SPME Fibre: 50/30 µm DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA); incubation time – 5 minutes; extraction time – 30 minutes; agitation rate – 500 rpm; pre-desorption time – 5 minutes; desorption time – 2.75 minutes; liner – Ultra inert SPME liner 0.75 mm; split ratio – 31:1; GC column - RXi-1ms (30m x 0.25 mm ID x 0.25 µm); mobile phase - hydrogen; temperature program - 35–60 °C at 45 °C/min (one minute hold), 60–200 °C at 2.0 °C/min (five minute hold), 200–300 °C at 30.0 °C/min (five minute hold); ion source temperature – 250 °C; MS quad temperature – 150 °C; gain – 1.0; acquisition mode – SCAN.

2.6.2 HPLC Analysis of Humulinones

Humulinones were analysed in the beer by HPLC using a modified internal method based off EBC 9.47 and 9.50. HPLC analysis was conducted on Perkin Elmer HPLC Flexar FX-15 (Perkin Elmer, Waltham, MA, USA). The components were separated on a Kinetex 2.6 µm C18, 100 x 4.6 mm HPLC column (Phenomenex, Macclesfield, United Kingdom) and measuring absorbance at 270 nm. Methanol, de-ionised water and phosphoric acid in a ratio of 660:340:2 (v/v/v) was used for the mobile phase at a flow rate of 0.75 ml/min through a column heated at 40 °C. Each sample was eluted for 32 minutes with an injection volume of 10 µl. The International Calibration Standard (ICS-Hum-1) was used to quantify the humulinones concentration (Labor Veritas, Zurich, Switzerland).

2.7 Statistical Analysis

Descriptive analysis was evaluated using one-way analysis of variance (ANOVA). Post hoc analysis using Tukey's honest significant difference (HSD) with a significance level of $P < 0.05$ was carried out. Tukey's Honest Significant Difference (HSD) test was used for pairwise multiple comparisons at 95 % confidence interval to determine significant differences between samples at $p = 0.05$ for all sensory attributes (Compusense, ON, Canada). Data analyses of GC-FID data was conducted using Minitab® (v21.1.0) (Minitab®, PA, USA). Analysis of Variance (ANOVA) was conducted GC-FID datasets to identify significant differences. Dunnett's Test was used to create intervals for differences between the mean of the control and the mean of each factor at $p = 0.05$.

3 Results and Discussion

3.1 Alpha & Beta Acids and HSI Analysis

It must be highlighted that different varieties all have different storage stability [21]. The hop pellet samples were analysed for alpha & beta acids and HSI values fresh and after the hop aging treatment described in section 2.2.2. Table 3 shows the results of these hop aging treatments.

As expected, and reported in previous studies, the alpha acid content dropped significantly as the HSI value increased during the hop aging treatment in the presence of oxygen (Table 3). In the Citra® hop pellets the aged HSI condition showed a 15 % loss of alpha acids and the overaged showed a 38 % increase. The Galaxy® hop pellets showed a larger decline in alpha acids than the Citra® hops. The Galaxy® aged HSI condition showed a 16 % loss and the overaged showed a 47 % loss in alpha acids. It has been observed previously by Hartley [22] that higher levels of alpha acid oxidation have been associated with a higher essential oil content. The essential oil content in both Citra® and Galaxy® are relatively high at 2.0 ml/100 g and 2.3 ml/100 g respectively, and possibly a contributory factor for significant decrease in the alpha acids, especially in Galaxy®.

As this research is looking at the impact of HSI on the essential oil and aroma and flavour, humulinones were not measured but it would be expected the concentration would have increased as the alpha acids decreased due to the hop treatments.

3.2 Total Essential Oil Content

The total essential oil content in both Citra® and Galaxy® varieties

measured at the different HSI levels, showed a significant decrease, as demonstrated in figure 1 and 2.

Citra® fresh started with an oil content of 2.0 ml/100 g and showed a 15 % decrease in the aged treatment (1.70 ml/100 g) and a 40 % decrease in the overaged storage condition (1.20 ml/100 g).

Galaxy® showed a more substantial decrease in the essential oil than Citra® after hop aging. Galaxy® fresh started with 2.3 ml/100 g and lost 24 % from the aged treatment (1.75 ml/100 g) and 50 % from the overaged storage treatment (1.15 ml/100 g). The decrease in Galaxy® essential oil content found in this study is similar to that observed by Tedone [23], even with different hop aging treatments.

This data suggests that as the HSI increases due to mainly oxidation the oil content decreases. Tedone et al. [23] and Rutnik et al. [8] also reported significant decreases in the essential oil as the HSI increases. This study indicates there is a negative correlation between HSI and the overall essential oil content hops.

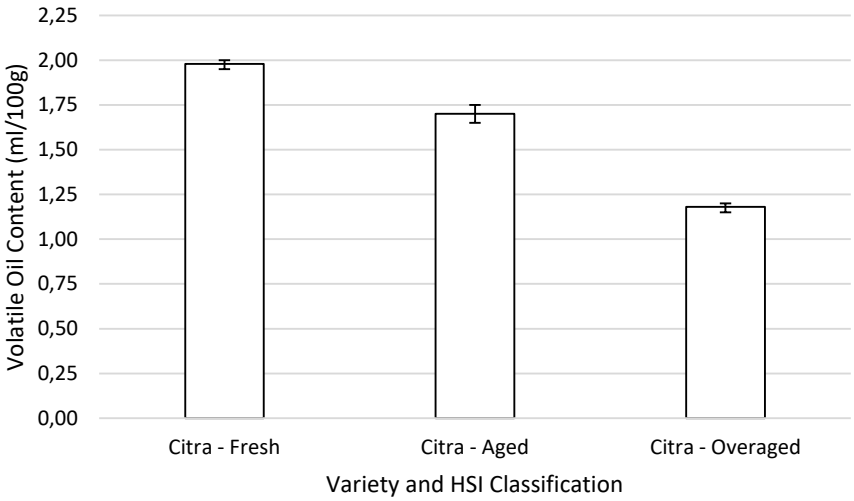

Fig. 1 Volatile oil content (ml/100 g) of Citra® with the three different HSI classifications (fresh, aged, and overaged). Values are from triplicate analysis. Error bars represent the range

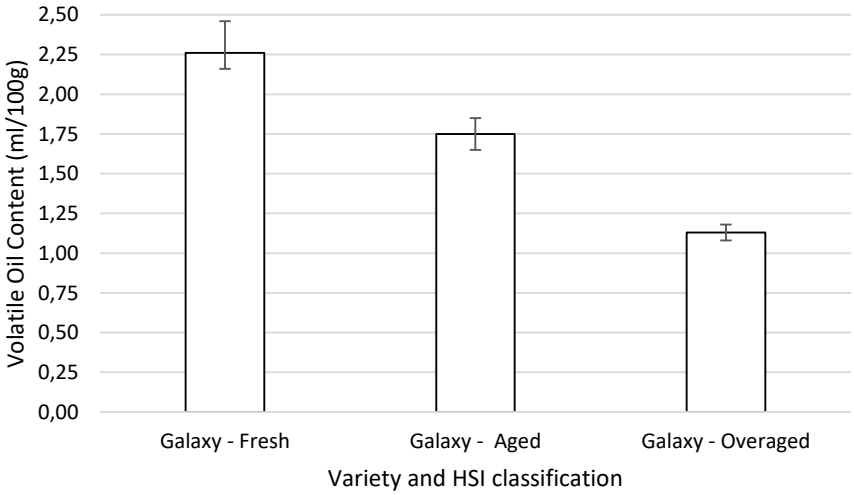

Fig. 2 Volatile oil content (ml/100 g) of Galaxy® with the three different HSI classifications (fresh, aged, and overaged). Values are from triplicate analysis. Error bars represent the range

3.3 Profiles of Hop Oil Components

Citra® is a hop variety which has aroma and flavour characteristics of sweet fruits (mango), citrus (grapefruit) and floral (lilac). The aroma and flavour characteristics of Galaxy® are described as sweet fruits (passion fruit and peach) and citrus. Hop varieties have different aroma and flavour characteristics dependent on various factors, however the profile of hop oil components is the main contributing factor.

The essential oil fraction in hops contains over 1000 different compounds, with around 440 compounds being identified [24, 25], and with most of the compounds having effects on aroma and flavour at subthreshold concentrations [26]. Hop varieties with higher essential oil contents are commonly correlated with increased levels of monoterpenes, mostly myrcene relative to sesquiterpenes (caryophyllene and humulene) [27]. An increase or decrease in specific hop volatile component levels within the essential oil and the identification of reaction compounds can be used to evaluate the freshness and quality of hops [25].

The hop oil samples collected from the total volatile oil measurement were analysed by GC-FID to identify the impact of increased HSI on the concentration of hop oil components included in this study (Table 1). Table 1 shows the aroma and flavour characteristics and the sensory threshold for each component analysed in this study. A summary of the hop oil component results is shown in table 4 for Citra® and table 5 for Galaxy®.

ANOVA analysis with Dunnett's analysis test used for the comparison of the key aroma compounds in the hop between the fresh, aged, and overaged hop treatments. Asterisks indicate which samples are significantly different to the fresh hop pellets ($p < 0.05$).

The largest decrease in all the Citra® hop oil components was observed in myrcene. The calculated absolute concentration of myrcene started at 9.91 g/kg and decreased by 18 % in the aged oil (Table 4). The overaged myrcene content deteriorated by 58 % from the fresh HSI classification. Limonene and linalool remained the same in the fresh and aged samples, but decreased in the overaged sample. Linalool and limonene decreased by 22 % and 40 % respectively, in the overaged sample. α-terpineol remained stable during the hop treatment process. Geraniol increased by 14 % in both the aged and overaged samples. Methyl geranate decreased by 12 % and 44 % in the aged and overaged samples respectively. 2-undecanonce decreased by 22 % in the aged and 48 % in the overaged. The sesquiterpene components caryophyllene and humulene also reduced in calculated absolute concentration. Caryophyllene decreased by 19 % in the overaged sample compared to the fresh. Humulene decreased by 13 % from 2.48 g/kg

Table 4 **Calculated absolute concentration of key aroma compounds of Citra® hop oil analysed by GC-FID. Results are mean values of triplicate analysis with the standard deviation**

Hop Oil Component	Citra® - Fresh (g/kg)	Citra® - Aged (g/kg)	Citra® - Overaged (g/kg)
Myrcene	9.91 ± 0.40	8.15* ± 0.28	4.12* ± 0.29
Limonene	0.10 ± 0.01	0.10 ± 0.002	0.06* ± 0.003
Linalool	0.18 ± 0.02	0.18 ± 0.03	0.14* ± 0.008
α-terpineol	0.02 ± 0.001	0.02 ± 0.004	0.01 ± 0.0008
Geraniol	0.07 ± 0.002	0.08 ± 0.002	0.08 ± 0.007
2-undecanone	0.27 ± 0.008	0.21* ± 0.008	0.14* ± 0.005
Methyl Geranate	0.25 ± 0.007	0.22 ± 0.006	0.14* ± 0.003
β-caryophyllene	1.52 ± 0.07	1.38 ± 0.06	1.23* ± 0.04
α-humulene	2.48 ± 0.11	2.32 ± 0.09	2.16* ± 0.07
Caryophyllene Oxide	0.13 ± 0.005	0.13 ± 0.004	0.13 ± 0.01
Humulene Epoxide I	0.06 ± 0.002	0.05 ± 0.003	0.07 ± 0.005
Humulenol II	0.07 ± 0.002	0.07 ± 0.003	0.09* ± 0.007

Table 5 **Calculated absolute concentration of key aroma compounds of Galaxy® hop oil analysed by GC-FID. Results are mean values of triplicate analysis with the standard deviation**

Hop Oil Component	Galaxy® - Fresh (g/kg)	Galaxy® - Aged (g/kg)	Galaxy® - Overaged (g/kg)
Myrcene	9.17 ± 0.52	6.39* ± 0.21	2.70* ± 0.17
Limonene	0.08 ± 0.003	0.06 ± 0.001	0.04* ± 0.001
Linalool	0.12 ± 0.003	0.12 ± 0.001	0.09* ± 0.01
α-terpineol	0.03 ± 0.002	0.02 ± 0.001	0.03 ± 0.002
Geraniol	0.03 ± 0.002	0.01 ± 0.001	0.03 ± 0.003
2-undecanone	0.14 ± 0.004	0.14 ± 0.002	0.08* ± 0.003
Methyl Geranate	0.66 ± 0.02	0.52* ± 0.005	0.34* ± 0.006
β-caryophyllene	1.98 ± 0.1	1.62* ± 0.05	1.37* ± 0.05
α-humulene	0.88 ± 0.06	0.58* ± 0.03	0.34* ± 0.01
Caryophyllene Oxide	0.06 ± 0.004	0.05 ± 0.007	0.13* ± 0.006
Humulene Epoxide I	0.38 ± 0.07	0.32 ± 0.007	0.49* ± 0.006
Humulenol II	0.05 ± 0.002	0.04 ± 0.003	0.06 ± 0.008

in the fresh to 2.16 g/kg in the overaged. Oxidation of humulene to other compounds are the most likely cause for the decrease. Interestingly, caryophyllene oxide did not increase even though caryophyllene decreased. Humulene epoxide I increased by 17 % from the fresh to overaged sample. Humulenol II increased by 29 % from the fresh to overaged sample. The increase in both humulene epoxide I and humulenol II would explain the decrease in humulene.

ANOVA analysis with Dunnett's analysis test used for the comparison of the key aroma compounds in the hop between the fresh, aged, and overaged hop treatments. Asterisks indicate which samples are significantly different to the fresh hop pellets ($p < 0.05$).

The Galaxy® fresh HSI classification had 9.17 g/kg myrcene absolute concentration, however as the hop treatment process took place the myrcene content decreases (Table 5), as was seen in the Citra® hop oil. The aged myrcene decreased by 30 % and the overaged decreased by 71 %. Limonene decreased by 50 % in the overaged sample. Linalool also decreased in the overaged sample by 25 % from the fresh. Geraniol and α-terpineol remained stable throughout the hop treatment process. Methyl geranate decreased by 21 % and 48 % in the aged and overaged samples respectively. 2-undecanone decreased by 43 % in the overaged sample. Caryophyllene concentration decreased by 18 % and 31 % in the aged and overaged samples respectively. Humulene concentration also decreased with a 34 % and 61 % reduction in the aged and overaged samples respectively. The decrease in caryophyllene and humulene concentration as the HSI increases, most likely due to oxidation and the formation of oxidation products from these compounds. Caryophyllene oxide increased significantly by 117 % in the overaged sample. Humulene epoxide I increased by 29 % in the overaged sample compared to the fresh. Humulenol II increased by 20 % from the fresh to overaged sample. The increases in caryophyllene oxide, humulene epoxide I and humulenol II explain the decrease in both caryophyllene and humulene.

The terpene hydrocarbon component myrcene deteriorated significantly over the course of the hop treatment process for both varieties. Myrcene is one of the least stable compounds in the essential oil fraction. It's been identified by Lermusieau and Collin [28] that components in the essential oil containing conjugated dienes such as myrcene are less stable during storage than those not containing conjugated dienes. The myrcene content in hops during storage undergoes both oxidation and polymerisation. The oxidation of myrcene can form various products which include terpenoids such as linalool and geraniol [29]. Furthermore, oxidation influences the biogenesis of myrcene resulting in significant losses during storage [30].

Linalool and geraniol are key hop oil components much like myrcene for hop aroma, which impart citrussy and floral aroma and flavours [29]. Within this study linalool decreased in the overaged samples, whereas geraniol had a different trend. Linalool was at its highest in the fresh and aged samples and lowest in the overaged samples. Geraniol remained stable in the Galaxy® trials but increased in the Citra® trials. As previously reported, this could be due to myrcene degradation forming additional components such as linalool and geraniol. Geraniol content was found to be an important key analytical factor for the quality of hops [31].

Table 6 Physiochemical analysis of the four brewing trials before dry hopping. The results are the mean data from triplicate analysis

	Citra® Brewing Trial Number 1	Galaxy® Brewing Trial Number 2	Citra® Brewing Trial Number 3	Galaxy® Brewing Trial Number 4
Original Gravity (oP)	11.97	12.35	11.89	11.91
Present Gravity (oP)	1.83	2.02	2	2.11
ABV (%)	5.27	5.12	4.97	5.11
pH	4.15	4.15	4.17	4.11
Colour (EBC)	7.01	7.73	6.84	6.65

ABV = Alcohol by Volume

Methyl geranate and 2-undecanone both decreased in both varieties during this trial. This is different to what was observed by Lermusieau and Collin [28] and Tedone et al. [23] trials where methyl geranate and ketones showed no real variation during the storage of hop pellets. The difference in this study compared to the other two studies could be a result of using different processes to age the hop pellets.

Caryophyllene showed a decrease in concentration in both varieties as the HSI increases. Caryophyllene content has previously shown decreases during storage due to oxidation producing caryophyllene oxide and other oxidation products [29].

Table 7 Results from Citra® triangle tests with p-value and significance result

Triangle Test	Number of panelists	Correct	Incorrect	*p*-value	Significantly Different
Citra® - Fresh vs Aged	16	8	8	0.13	No
Citra® - Fresh vs Over-aged	16	11	5	0.01	Yes
Citra® - Aged vs Overaged	16	9	7	0.05	Yes

Table 8 Results from Galaxy® triangle tests with p-value and significance result

Triangle Test	Number of panelists	Correct	Incorrect	*p*-value	Significantly Different
Galaxy® - Fresh vs Aged	16	9	7	0.05	Yes
Galaxy® - Fresh vs Over-aged	16	14	2	0.01	Yes
Galaxy® - Aged vs Over-aged	16	11	5	0.01	Yes

Humulene oxidation products increased during storage, especially in Galaxy® hop oil. Peacock and Deinzer [32] reported an increase in oxidation products of humulene such as humulenol II during storage.

3.4 Beer Analysis

The four different brewing trials were analysed for some key physiochemical characteristics to ensure that the beers had a similar base profile prior to dry hopping and would not be a factor in the outcome of the trial (Table 6). The original gravity and final gravity show an efficient fermentation, with the target alcohol by volume (ABV) of 5 % being achieved for the four trials (4.97 to 5.27 % ABV).

The results in table 6 indicate there are no major differences in the replicate brewing trials, which would impact the sensory outcome of the brewing trial. This ensures that if there are sensory differences between the trials can more reliably be traced back to the dry hop addition.

3.5 Sensory Analysis

3.5.1 Triangle Tests

Triangle tests were conducted comparing the HSI classifications to each other to identify if there was a significant difference in sensory profiles. The fresh hops were compared to aged and overaged, as well as aged directly against overaged, for both hop varieties. The triangle tests were conducted before quantitative descriptive analysis to ensure the panellists had no prior knowledge of the beer samples. The triangle test results are summarised in table 7 for Citra® beers and table 8 for Galaxy® beers.

The results in table 7 indicate that there was no significant difference between the Citra® – fresh beer versus the aged HSI classification. However, when comparing Citra® – fresh against overaged, the panellists identified a significant difference between the samples. Interestingly the panel also identified a significant difference between the Citra® – aged versus overaged. This is consistent with the analytical data pointing to bigger differences between aged and overaged, than between fresh and aged.

The *p*-value of the Galaxy® triangle tests in table 8 show that all the comparisons (fresh versus aged, fresh versus overaged and aged versus overaged) were significantly different. Unlike in the Citra® triangle tests, the fresh versus aged beer for the Galaxy® beers indicates the panel detected evidence of a moderate difference.

These results clearly show that as the HSI increases, the sensory profile changes and there is a significant difference between the beers. They also suggest that the effect is more or less pronounced for different varieties. Even between the fresh and the aged category a HSI increase of 0.1 – a mixed panel were able to detect the sample, which was different, for Galaxy®. This could mean that as the HSI increases, its impact on the sensory

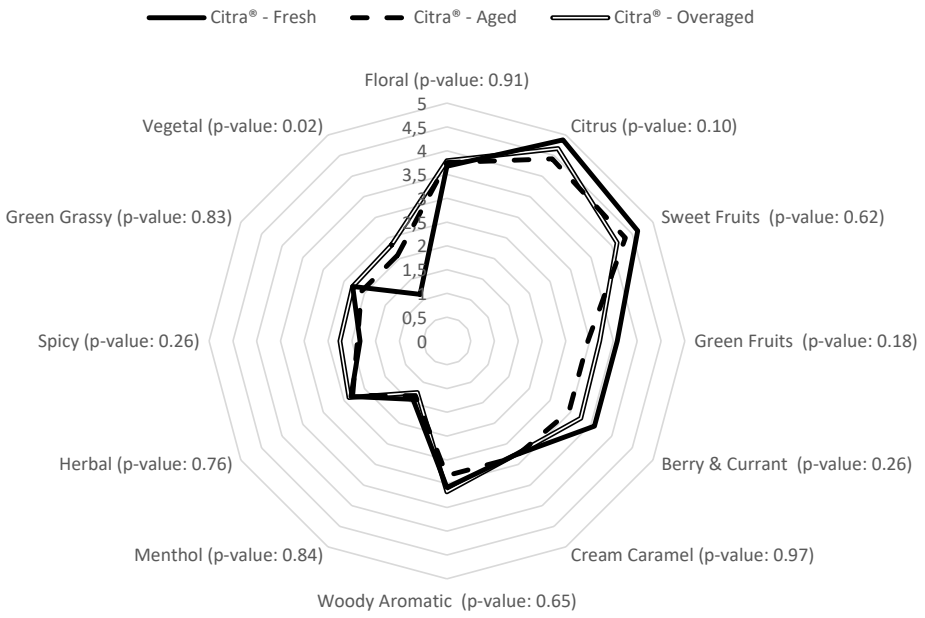

Fig. 3 BarthHaas Hopsessed® attributes quantitative descriptive analysis of Citra® beers with their p-values. Results are mean values of duplicate trials

profile might be based on variety, as the triangle test for Citra® indicated no statistical difference between the fresh and aged HSI classifications.

Triangle tests are also sensitive to panel composition and training. Vollmer et al. [6] found that during their triangle test trials, a consumer panel could not tell the difference between two beers which had been dry hopped with fresh and oxidised pellets. However, a trained panel noticed a significant difference with 80 % of the panellists selecting the different beer. It should be noted that the consumer testing was conducted with a lower dry hopping rate than the trained panellists, which could account for the reason why the trained panel noticed a difference.

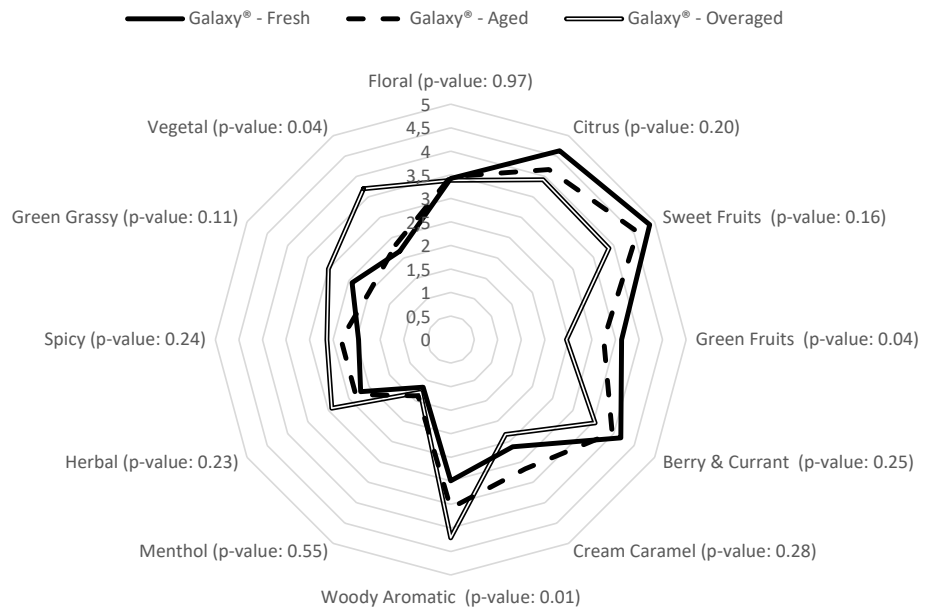

Fig. 4 BarthHaas Hopsessed® attributes quantitative descriptive analysis of Galaxy® beers with their p-values. Results are mean values of duplicate trials

3.5.2 Quantitative Descriptive Analysis

Quantitative descriptive analysis was conducted on the replicate brewing trials for both varieties. The panel were asked to score the various attributes between 1–9, with 9 being the highest intensity. The analysis was split into two categories, initially the aroma and flavour of the beer was scored using the 12 flavour attributes from the BarthHaas Hopsessed® wheel. In the second part of the sensory analysis, the panellists scored taste attributes, such as bitterness and overall hop aroma.

3.5.2.1 Hopsessed® Flavour Wheel Attributes

The 12 flavours from the Hopsessed® attributes were ranked by the 12 people who have all been trained in how to evaluate beers using the BarthHaas Hopsessed® ballot. The results are shown in figure 3 for Citra® and figure 4 for Galaxy®.

Although there are variations in the average means of the Hopsessed® flavour attributes for the Citra® beers (Fig. 3), there was only one statistically different attribute which was vegetal (*p*-value 0.02). As figure 3 shows, the vegetal score increased as the HSI increased, although the difference was more significant between the fresh and the aged and overaged hops than between the aged and overaged. This would indicate that the aging of hops impacts the freshness and gives a more vegetal characteristic as the hops age. The increase in the vegetal characteristic could be caused by the formation of extremely low levels of sulphur compounds, which aren't part of this study. Lermusieau and Collin [33] reported that methyl sulphides can be enhanced in hop oil by heat treatment. The hop pellets in this study were subject to 35 °C for two weeks, which may be the reason for an increase in vegetal intensity. Peppard [34] described methyl sulphides as vegetable, cooked vegetable, onion-like and garlic-like, as well as the compounds having a low flavour threshold. Seaton et al. [35] reported that methyl thioesters with concentrations near their sensory threshold impart unpleasant cooked vegetable and sulphur aroma and flavour characteristics.

For the Galaxy® beers there are slight variations in all of the attributes but only three showed a significant difference (Fig. 4): green fruits, woody aromatic and vegetal. The green fruits mean values drop as the HSI values increase.

Woody aromatic (*p*-value 0.01) scores increased as the HSI values increased. For both attributes, the fresh and aged samples were grouped together but the overaged was significantly different from the fresh and grouped with aged. Similarly, to the Citra® beers, the vegetal attribute showed a significant difference between the fresh and aged compared to overaged. This attribute increases as the HSI increases, reducing hop freshness. As stated earlier, this could be due to the formation of sulphur compounds.

3.5.2.2 Bitterness and Overall Hop Aroma (Intensity and Quality)

The bitterness and overall hop aroma of the beers were assessed, as previous studies by Vollmer et al. [6] and Rutnik et al. [8] showed an increase in the bitterness profile and decrease in hop aroma quality. Bitterness intensity is the relative bitterness specific to the beer style and perceived bitterness is the estimated bitterness units (BU) according to the panellist. Table 9 shows the summary of results for Citra® and table 10 shows the summary of results for Galaxy®.

Table 9 Bitterness and hop aroma sensory analysis of Citra® beers

	p-value	Citra® - Fresh	Citra® - Aged	Citra® - Over-aged
Aroma Intensity	0.29	6.38	5.88	5.79
Hop Aroma Quality	0.007	6.54^{a}	5.75^{a}	4.79^{b}
Bitterness Intensity	0.02	5.50^{a}	5.67^{a}	6.70^{b}
Bitterness Quality	0.03	6.17^{a}	5.38^{ac}	4.67^{bc}
Perceived Bitterness	0.01	28.33^{a}	29.33^{a}	31.75^{b}

Superscript letters indicate Tukey's HSD post-hoc groupings of beers with a *p*-value < 0.05.

Table 10 Bitterness and hop aroma sensory analysis of Galaxy® beers

	p-value	Galaxy® - Fresh	Galaxy® - Aged	Galaxy® - Over-aged
Aroma Intensity	0.02	6.54^{a}	6.33^{a}	5.23^{b}
Hop Aroma Quality	0.03	6.42^{a}	5.50^{ac}	4.21^{bc}
Bitterness Intensity	0.0006	5.54^{a}	6.42^{b}	7.21^{c}
Bitterness Quality	0.02	6.17^{a}	4.71^{b}	3.17^{c}
Perceived Bitterness	0.005	28.46^{a}	31.71^{b}	35.38^{c}

Superscript letters indicate Tukey's HSD post-hoc groupings of beers with a *p*-value < 0.05.

The Citra® beers' (Table 9) hop aroma quality shows a significant difference between the fresh and overaged beers in the Citra® brewing trials. As the HSI increases the hop aroma quality decreases. This could be a result of the loss of myrcene which is a key compound in hop aroma. In addition, the aroma intensity does decrease although not significantly, this could be due to lower oil being dosed into the beer as the total oil content in the aged and overaged pellets decreased, especially in the overaged samples. Furthermore, the bitterness intensity and perceived bitterness increase as the HSI increases. The bitterness quality decreases as the HSI increases and leads to a more harsh and astringent bitterness, as characterised by the panellists.

For the Galaxy® beers (Table 10), again, the hop aroma quality significantly decreases along with the aroma intensity. In addition, as observed in the Citra® beers, the bitterness intensity and perceived bitterness increase significantly as the HSI increases. The bitterness quality also decreases as the HSI increases. The decrease in hop aroma quality corresponds with Rutnik et al. [8] study, which identified a decrease in this attribute as the HSI increases. Vollmer et al. [6] and Rutnik et al. [8] both noted increases in the bitterness intensity as the HSI value increases in hops. It was also reported that the bitterness quality decreases when beers are dry hopped with higher HSI value hop pellets.

3.5.3 Overall Liking

The panellists were asked to give their overall liking for each beer and score the beers between 1 and 10, with 1 being the least liked and 10 being the most liked. The results are summarised in table 11.

The results in table 11 show that a clear favourite from both varieties were the fresh HSI classifications. Citra® and Galaxy® scored 6.63 and 6.33 respectively in the fresh HSI classification. The Galaxy® aged and overaged had a bigger decrease in their likeability than the Citra® versions. This could potentially be due to the larger decrease in the alpha acids and essential oil. All the samples were statistically significantly different to each other according to Tukey's HSD, as the post-hoc groupings indicate. One of the causes for the drop in overall likeability is probably due to the increase in the bitterness intensity and perceived bitterness. These results also correlate with Rutnik et al. [8] who also observed a significant decrease in their sensorial analysis evaluating the overall impression. The observations in their study were that as the HSI value increases the overall impression decreases significantly. It was also observed that only two groupings out of thirty were not significantly different. Beers dry hopped with overaged hops (HSI > 0.61) do not give a well-rounded and likeable perception of beer flavour and taste.

3.6 HS-SPME-GC-MS Analysis of Finished Beer

The beers were all analysed analytically by HS-SPME-GC-MS to investigate the hop oil components and their concentrations for each HSI classification. The HS-SPME-GC-MS results are semi-

Table 11 Results of the overall liking from the sensory analysis, presented as mean values from duplicate trials and Tukey's Honestly Significant Difference (HSD)

	HSI Classification		
Variety	**Fresh**	**Slightly Aged**	**Overaged**
Citra®	6.63^{c}	5.46^{b}	4.54^{c}
Galaxy®	6.33^{c}	4.92^{b}	3.25^{c}

Superscript letters indicate Tukey's HSD post-hoc groupings. Citra® and Galaxy® Tukey post-hoc groupings are different.

Table 12 **Semi-quantitative HS-SPME-GC-MS of key aroma compounds in Citra® beers. Mean values of triplicate sample analysis from duplicate brewing trials**

	Citra® - Fresh	Citra® - Aged	Citra® - Overaged
Myrcene	0.14 ± 0.08	0.12 ± 0.07	0.12 ± 0.09
Linalool	0.42 ± 0.2	0.60 ± 0.26	0.64 ± 0.19
α-terpineol	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.01
Geraniol	0.22 ± 0.11	0.39 ± 0.17	0.41 ± 0.08
2-undecanone	0.10 ± 0.004	0.10 ± 0.005	0.10 ± 0.004
Methyl geranate	0.07 ± 0.02	0.05 ± 0.02	0.06 ± 0.02
β-caryophyllene	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.03
α-humulene	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.007
Caryophyllene oxide	0.06 ± 0.04	0.11 ± 0.1	0.18 ± 0.1
Humulene epoxide I	0.05 ± 0.02	0.12 ± 0.1	0.50 ± 0.1
Humulenol II	0.06 ± 0.04	0.10 ± 0.04	0.70 ± 0.1

Table 13 **Semi-quantitative HS-SPME-GC-MS of key aroma compounds in Galaxy® beers. Mean values of triplicate sample analysis from duplicate brewing trials**

	Galaxy® - Fresh	Galaxy® - Aged	Galaxy® - Overaged
Myrcene	0.23 ± 0.1	0.16 ± 0.04	0.16 ± 0.08
Linalool	0.23 ± 0.1	0.28 ± 0.05	0.37 ± 0.1
α-terpineol	0.02 ± 0.002	0.01 ± 0.004	0.02 ± 0.001
Geraniol	0.23 ± 0.09	0.25 ± 0.07	0.26 ± 0.03
2-undecanone	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.02
methyl geranate	0.10 ± 0.009	0.09 ± 0.008	0.09 ± 0.007
β-caryophyllene	0.04 ± 0.01	0.06 ± 0.03	0.06 ± 0.03
α-humulene	0.03 ± 0.02	0.03 ± 0.01	0.03 ± 0.008
Caryophyllene oxide	0.07 ± 0.03	0.22 ± 0.07	0.46 ± 0.06
Humulene Epoxide	0.10 ± 0.06	0.30 ± 0.1	0.58 ± 0.2
Humulenol II	0.04 + 0.02	0.17 ± 0.02	0.86 ± 0.2

quantitative (integrated peak area/internal standard peak area). The Citra® and Galaxy® beer results are summarised in table 12 and 13 respectively.

The major changes in the hop aroma components in the Citra® beer (Table 12) were the increases in linalool and geraniol content. These contents potentially increased as they represented a larger proportion of the total essential oil, leading to more diffusion into beer. In addition, caryophyllene oxide, humulene epoxide I and humulenol II all increased. This would also relate to the increase in these components in the hop oil being transferred into the beer.

The Galaxy® beers followed a similar pattern to the Citra® beers in terms of key hop aroma compounds in beer (Table 13), with linalool and geraniol increasing in concentration as the HSI increased. This could potentially be due to the compounds making up a higher percentage of the total oil and more diffusing into the beer. Furthermore, the oxidation compounds (caryophyllene oxide, humulene epoxide I and humulenol II) increased in concentration as the HSI increased. The increase in these compounds matches the sensory data as the woody aromatic attribute increased significantly.

Myrcene, caryophyllene and humulene all have low solubility levels in water, and this showed as the semi-quantitative results show very low concentrations in the finished beer. Furthermore, as the myrcene content decreased significantly in the hop oil (Tables 4 and 5), it could result in less myrcene solubilising into the beer. The decrease in myrcene content as the HSI increased could relate to the decrease in the hop aroma quality. In addition, due to the use of a GC coupled with a single quadrupole MS and its limit of detection for sulphur compounds (thiols), these could not be analysed. The aged and overaged beers may have lower concentrations of these thiol compounds which would result in an impact on the hop aroma quality [36]. The low levels of myrcene in the beers could be due to uptake of myrcene by yeast cells [24]. Additionally, myrcene and other compounds can be absorbed to the liner of a crown cap which could be another possible reason for the low concentration in beer [15].

This study showed that some compounds related to hop aroma degraded very slowly (linalool and geraniol). Compounds related to oxidation (caryophyllene oxide, humulene epoxide I and humulenol II) increased as the HSI increased in both brewing trials. Interestingly Rutnik et al. [8] observed decreases in linalool and geraniol in beer as the HSI increased, contrary to the results reported here. The findings with respect to changes in oxidation are consistent with those of Rutnik et al. [8].

3.7 Humulinones in Finished Beer

Humulinones were analysed in all beers as an increase in humulinones concentration was inferred as the most likely cause of the measured increase in perceived and analytical bitterness. Humulinones are primarily introduced into beer via dry hopping process. This is due to the oxidation of alpha acids influencing the polarity and creating a more soluble compound [6, 37]. It has been reported that, around 87 % of humulinones will dissolve into beer within 2–3 days of dry hopping [38]. Tables 14 and 15 show the humulinone concentration in the replicate Citra® and Galaxy® brewing trials, respectively.

As shown in table 14, the humulinone concentration increased in the Citra® beers as the HSI classification changed. In brewing trial number 1, the fresh beer sample has 4.35 mg/l compared to 6.49 and 10.79 mg/l in the aged and overaged beers, respectively. This signifies an increase of 148 % in the overaged beer. In brewing trial number 3, the humulinone concentration also increased as the HSI increased confirming the results from brewing trial number 1. However, the increase was only 60 %.

Table 15 shows a similar pattern for the Galaxy® beers. However, there is a more substantial increase in the humulinone concentration in the Galaxy® beers than in the Citra® beers. This is likely due to higher alpha acid oxidation in Galaxy®. In brewing trial number 2, the humulinone concentration increased by 88 % and 270 % in the aged and overaged respectively. Brewing trial number four saw an increase of 26 % and 150 % in the aged and overaged beers respectively, compared to the fresh hops.

The increase in the humulinone concentration as the HSI increases correlates with the increase in bitterness intensity and perceived bitterness in tables 9 and 10. The panel noticed a significant difference in the bitterness intensity and perceived bitterness, which was most likely caused by the increased concentration of humulinones. However, Rutnik et al. [8] observed a fluctuation in the humulinone concentration, as some beers dry hopped with higher HSI values had lower concentrations. This would indicate that the humulinones are not the only compound responsible for the increase in bitterness intensity and perceived bitterness of beers produced oxidised/aged hops. Other compounds such as hulupones formed from oxidised beta acids could also be present leading to the perception of an unpleasant bitterness [39].

Oladokun et al. [40] identified that different hop varieties and their hop aroma characteristics can have an impact on the perceived bitterness parameter of single-hopped beer. This is dependent and relative to the bitterness profile in the beer and implies the influence of hop aroma on perceived bitterness is relevant for the bitterness quality in beer. The hop aroma characteristics of Galaxy® could potentially be a factor in why the overaged Galaxy® beers had a significantly higher perceived bitterness than the other two HSI classifications, while the difference was not significant for Citra®.

4 Conclusion

To conclude, this study investigated hops and beers brewed with fresh hops and hop ageing treatments with heat and oxygen to a specific HSI. The results show that with a higher HSI value, this appears to have an impact on the total essential oil, hop oil components and the sensory quality of beer. In this research, the total essential oil content decreased as the HSI increased, which impacted the hop oil components. Myrcene content was negatively impacted in the overaged hops and the oxidation compounds of caryophyllene oxide, humulene epoxide I and humulenol II increased, especially in Galaxy® hop pellets. As the myrcene content decreased and oxidation products increased, the hop aroma quality and intensity decreased, especially in the Galaxy® trials. The oxidation compounds increased in concentration in the beer, as well as linalool. To investigate this further, full quantifiable analysis should be conducted. Furthermore, as the HSI value increased the bitterness profile of the beer was negatively impacted. The biggest change in the sensory profile of the beers was in the bitterness intensity, bitterness quality, and perceived bitterness. This study shows similar results to previous studies that the higher the HSI value, the higher the concentration of humulinones present in dry hopped beer. It also matches studies that reported that oxidised and aged hops tend to lead to an increase in perceived bitterness.

Table 14 **Humulinone concentration in beer samples from the duplicate Citra® brewing trials. The values are means from triplicate analysis with the standard deviation**

Variety	HSI Classification	Brewing Trial Number	Humulinones (mg/l)
Citra®	Fresh	1	4.35 ± 0.46
	Aged	1	6.49 ± 0.08
	Overaged	1	10.79 ± 0.26
Citra®	Fresh	3	6.65 ± 0.13
	Aged	3	8.67 ± 0.57
	Overaged	3	10.61 ± 0.24

Table 15 **Humulinone concentration in beer samples from the duplicate Galaxy® brewing trials. The values are means from triplicate analysis with the standard deviation**

Variety	HSI Classification	Brewing Trial Number	Humulinones (mg/l)
Galaxy®	Fresh	2	6.0 ± 0.44
	Slight Aged	2	11.3 ± 0.25
	Overaged	2	22.2 ± 0.06
Galaxy®	Fresh	4	7.4 ± 0.38
	Slight Aged	4	9.3 ± 0.31
	Overaged	4	18.5 ± 0.83

Storing hops correctly is vitally important as the data in this study suggests. Hops which have aged due to poor storage conditions show a deterioration of alpha and beta acids as expected and reported previously. It also shows that poor storage impacts the total essential oil content and the hop oil components, which in turn can impact the hop aroma quality of beer. The humulinone content of the hops is the main source for the significant impact on the beer bitterness due to the oxidation of alpha acids. Although it should be reported that different HSI values impact hop varieties differently, as shown here. Galaxy® was impacted more by the HSI increase than Citra®, indicating that Galaxy® could be a poor storer compared to Citra®.

The key aim was to determine how increasing HSI values impact the total oil content and if measuring the HSI is a suitable tool for assessing hops destined for dry hopping. Although HSI does not directly measure the impact of aging on the total oil content and its components, it does show a negative correlation with oil content, indicating a deterioration in hop quality. This suggests that HSI could be used as a tool for determining the quality of hops which are to be used for dry hopping.

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Received 31 August 2023, accepted 25 October 2023