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Chemical characterization of the two major hop varieties produced in Patagonia (Argentina) for the brewing industry

The yields and chemical compositions of the resinous and essential oil fractions vary in different hop (*Humulus lupulus* L.) varieties and in different environmental conditions. South American hop crops with most of the production located in North Patagonia (Argentina), have not been thoroughly described. The two main varieties grown in this location are Cascade, widely used for aroma purposes and Nugget, a high alpha variety. To characterize the Patagonian hop products, the essential oils and resinous components of dried hop cones and pellets were analyzed. Both cones and pellets showed low HSI values indicating "freshness" of the samples. Argentinian Cascade alpha-acid levels and the ratio of alpha to beta acids (α/β), were above or in the upper range of Cascade typical values. For Patagonian Nugget, α- and β-acid values, as well as the α/β ratio, were within the range reported for other producing regions. As expected, essential oil yields were higher for the Nugget variety compared to Cascade (1.9 % and 1.0 % respectively); with both varieties high in myrcene (> 50 %). The α-humulene to β-caryophyllene (H/C) ratio values were typical for these varieties as well as the amounts of the main essential oil components, including a high proportion of (E)-β-farnesene in Cascade. Geraniol, nevertheless, was found in low amounts in Cascade; though two of its esters were present. This work indicates that hop varieties Cascade and Nugget appear well adapted to Patagonian growing and production conditions, producing high quality hop products. Future experiments to unveil the impact of the observed differences are of interest; including sensory analysis of beers hopped with the Patagonian terroir varieties compared with those from high producing regions.

Descriptors: hop, *Humulus lupulus* L., patagonia, nugget, cascade

1 Introduction

Hops are used in beer for the bitter taste and hop flavor that they impart. These are derived from the resins and essential oils that are produced in the lupulin glands of the hop cone [1]. The chemistry of these compounds has been summarized [2,3], being α- and β-acids important for bitter taste and essential oils related to aroma and flavor. In addition, hops possess antimicrobial and antioxidant properties and can contribute to beer foam stability [3]. There are a growing number of commercial hop varieties [4], some with unique brewing properties due to the composition of accumulated plant secondary metabolites. The yield and the chemical composition of plant secondary metabolites varies within each variety depending on environmental characteristics like latitude, water irrigation, average temperatures and harvest time, and may be strongly influenced by post-harvest processes and conditions [1].

Plants of hops were introduced in Patagonia (Argentina) during the 19th century [5], with commercial production for the brewing industry initiated in the mid-20th century. After numerous attempts to grow plants in Buenos Aires and Mendoza provinces, only Patagonian lands proved to be suitable for this crop. Since then, hop was successfully grown in Río Negro province, mainly: Fernández Oro and El Bolsón Valley (El Bolsón, Lago Puelo and Epuyén). Varieties introduced in Argentina included Spalter, Cluster, Pride of Rinwood, Semsch, Hallertauer, Tettnanger, Cascade, Nugget, Victoria, Bullion and Magnum, some which are no longer available. Other varieties, like Mapuche and Traful, were developed and commercially released after local breeding programs [6]. Only few new local varieties were released, including Nahuel and Patagonia Red [4].

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El Bolsón Valley is a region located in latitude 42°, within the historical range of latitudes for successful commercial hop production (between 35° and 55° latitude), and reported almost 80 % of the 160 ha of hop in Argentina in 2018 [7]. In this Valley, Cascade (USDA, released in 1972) represents nearly 70 % of the cultivated area whereas Nugget (USDA, released in 1983) represents 20 %. Although cultivation and commercialization of hops in the region date back to more than 70 years, very few published studies on the composition and quality of this highly valued local ingredient are available.

The main purpose of this study was to characterize the chemical composition, in terms of α- and β-acids and volatile compounds, of varieties Cascade and Nugget cultivated in Argentina, particularly in El Bolson Valley. Comparative analyses were conducted on type T90 pellets and dried cones, the two main hop products in Argentina.

2 Materials and Methods

2.1 Hop samples

H. lupulus samples were obtained from an 18 ha commercial hop farm (Lúpulos Patagónicos) in El Bolsón Valley (Rio Negro Province, Argentina) managed with fertilized irrigation. Meteorological data was acquired with a Meteorological station (Davis vantage pro2) located at the hop farm (Lat: -41.9007 Long: -71.5194; Ground Elevation: 451 m). Analyzed crops: 2015, 2017, 2018, 2019 and 2020. Supplementary tables 1 and 2 (see page 101) include details on harvesting and pelletizing periods and available meteorological information (2019/2020). Samples included dried female inflorescences (dried cones) and type T90 pellets. Cones were collected and separated from plant material with special equipment, dried on the same day of harvest, stored at –20 °C in bales; and pelletized by the hop grower. Drying conditions: air temperature 55 °C, bed depth 0.2–1 m, airflow 40 m^3/h, kilning time 11–13 h. Pelletizing conditions: die temperature 35 °C. Dried cones and pellet samples were stored sealed under vacuum and refrigerated at –20 °C before analysis.

2.2 UV spectrophotometric analysis of hops α- and β-acids

The Hops-6 method of the ASBC was followed with some modifications [8]. HSI (Hops Storage Index) values were determined following Hops 12-A method [9]. Dried cones were ground in a meat grinder (Ken Brown), and pellets were milled with a coffee grinder (Moulinex). Grounded material (2.5 g) was extracted with 50 ml of toluene. Dilutions in methanol and alkaline methanol were adjusted to a final volume of 2 ml (0.1 ml extract). Samples were analyzed in a UV spectrophotometer (Shimadzu UV-1800) at 275, 325 and 355 nm. The α- and β-acids contents (% w/w) were calculated with ternary equations [10].

2.3 HPLC analysis of α- and β-acids

Quantification of α- and β-acids was done according to protocol Hops-14 (ASBC) with modifications [11]. Quantitation was conducted with reference to an external standard (ICE-3, ASBC), prepared by dissolving 0.15 g in methanol up to a 50 ml final volume. Extraction was carried out using 2.5 g hop material with 10 ml methanol, 50 ml diethyl ether and 20 ml of 0.1 M hydrochloric acid solution. Ether phase (5 ml) was further diluted in methanol (final volume 50 ml). After preparation, samples and standard were centrifuged. A volume of 10 µl of sample and standard were injected into a C18 column (Phenomenex Luna 5 µm C18, 25 cm x 4.6 mm). Isocratic runs included a mobile phase of 90% methanol and 10 % acidified water (17:0.25, water: phosphoric acid) with a constant flow of 0.8 ml/min. Detector was set at 314 nm (HPLC Waters Delta 600, detector Waters PDA 2898). For identification and quantification, chromatographic peak areas of standard and samples were compared (Software Empower 2). The four identified and resolved peaks included 1. cohumulone, 2. adhumulone + n-humulone, 3. colupulone and 4. adlupulone + n-lupulone.

2.4 Essential oils extraction and compound identification

Hops essential oils were extracted from dried hop cones, using a Clevenger type trap hydro distillation system [12]. The yield was calculated in ml of extracted oil per 100 g of dry material. Relative percentage of the main components and identification of compounds were analyzed using a Gas Chromatograph coupled to a flame Ionization Detector and a Mass Spectrometer (GC-FID-MS), with a GC Clarus 500 (Perkin Elmer) with a special configuration [13]. The detection of the minor constituents was performed by a GC–FID–MS (Agilent Technologies, Santa Clara, CA, USA) 7890A/5975C equipped with an injector (split ratio 1:100) connected by a flow splitter to two capillary columns (HPWAX and DB-1, both 60 m × 0.25 mm with 0.25 µ of fixed phase). The polar column was connected to a FID, whereas the non-polar column was connected to the quadrupolar mass detector (HP 5975C) (70 eV). Helium was used as gas carrier, at 1.8 ml/min. The injector temperature was set at 250 °C. Injection volume was 0.3 µL. The column temperature was programmed according to the following gradient: 100 °C, increasing at 2 °C/min to 240 °C and kept constant for 15 min. FID temperature was 260 °C, and temperatures for the transference line and the ionic source were set at 280 and 230 °C, respectively. Mass range (m/z) was 40–500 Da. Data acquisition, processing and instrument control was performed using the Agilent Chem Station (Agilent Technologies, Santa Clara, CA, USA) software. The identification of the compounds was achieved by analysing the linear retention indexes (LRI, relative to C8–C24 n-alkanes) obtained in both columns and compared with those of reference compounds, compounds identified in chemically well-known essential oils, our own database and from bibliography [14,15,16]. Additionally, each mass spectra obtained was compared to those from the literature libraries [14,15,17,18] and mass spectra obtained from reference compounds, our own database or previously published spectra in chemically well-known essential oils. Relative percentage contribution of the compounds was always calculated from the FID responses using the method of area percentage, assuming all the responses factors were 1.

2.5 Statistical Analysis

One way ANOVA was used for comparison of spectrophotometric data, followed by Tukey´s test (Sigma Plot v11.0). Unpaired t test was applied to HPLC data analysis (Sigma Plot v11.0). The

Table 1 Spectrophotometric analyses of resins from Patagonian hop varieties Cascade and Nugget (cones and pellets)

	Cascade cones (n = 52)	Cascade T90 pellets (n = 33)	Nugget cones (n = 26)	Nugget T90 pellets (n = 13)
α-acids (% w/w)	9.4 ± 0.9[a]	9.0 ± 0.7[a]	14.3 ± 0.9[b]	13.6 ± 0.9[b]
β-acids (% w/w)	6.2 ± 0.5[a]	6.2 ± 0.8[a]	5.2 ± 0.4[b]	5.1 ± 0.5[b]
HSI	0.22 ± 0.01[a]	0.24 ± 0.02[b]	0.24 ± 0.01[b]	0.25 ± 0.01[b]
α/β ratio	1.5 ± 0.1[a]	1.5 ± 0.2[a]	2.8 ± 0.2[b]	2.7 ± 0.1[b]

Values are expressed as mean ± standard deviation. Cone samples represent different harvest days/kiln floors (crops 2015, 2017, 2018, 2019 and 2020). Pellet samples represent different pelletizing days (crops 2017, 2018, 2019 and 2020). In rows, different letters indicate statistically significant differences at 0.95 confidences by Tukey's test. Water content was 10.0 ± 1.9 % w/w in dried cones (n = 20) and 8.6 ± 1.4 % w/w in pellets (n = 13)

statistical tests were performed at the significance level α = 0.05 and data are presented as mean values with the standard error.

3 Results and discussion

3.1 α- and β-acid content of Patagonian Cascade and Nugget varieties

Resins were characterized using both spectrophotometric and HPLC analysis. In both methods, the percentages of total α- and β-acids (% w/w) and the α/β ratio were obtained. Additionally, spectrophotometric evaluation allowed estimation of the Hop Storage Index (HSI) (Table 1). HPLC analysis was used for accurate estimation of α- and β-acids, and for cohumulone content determination (Table 2).

In all cases, α- and β-acid content was lower when determined by HPLC method compared to UV spectrophotometry ($p < 0.05$). This was expected since the latter method can overestimate, possibly due to its low specificity, since many compounds absorb in the UV region, also making it susceptible to interference [19]. Nevertheless, the spectrophotometric α- and β-acids analysis is very robust and useful for routine analysis [20]. The method also allows the direct calculation of the HSI parameter, a good estimator of the level of oxidation of hop acids, which can be related to "freshness" of the sample. On the other hand, HPLC analysis is more accurate, specific, and allows identification and quantification of each peak separately, including cohumulone content. In this study, both methods showed each variety has a characteristic and different α- and β-acid content and also α/β ratio ($p < 0.05$); with Patagonian Nugget almost doubling this ratio when compared to Patagonian Cascade. Also, both methods showed that the levels of α- and β-acids for the Cascade and Nugget variety grown in Patagonia are comparable to those of other producing regions [21, 22, 23, 24]. Notably, when spectrophotometric data is compared, Patagonian Cascade samples are above or near the highest α-acid values (9 %) and α/β ratio (1.4), considering reports for USA, Germany and Australia samples [25]. Nevertheless, comparative studies should be ideally performed with all samples analyzed under the same platform. On the other hand, α/β ratio as well as the percentage of α- and β-acids for the Nugget variety from Patagonia were in the same range as those reported from other regions (11–16 % w/w α-acids content) [21,22,23,24].

Table 2 HPLC analysis of resins from Patagonian hop varieties Cascade and Nugget (cones)†

	Cascade (n = 5)	Nugget (n = 3)
α-acids (% w/w)	8.3 ± 0.4[a]	12.5 ± 0.8[b]
β-acids (% w/w)	5.4 ± 0.3[a]	4.3 ± 0.1[b]
α/β ratio	1.5 ± 0.1[a]	2.9 ± 0.1[b]
cohumulone (% w/w)	35.2 ± 0.6[a]	26.8 ± 0.7[b]

† Values are expressed as the mean ± standard deviation. In rows, different letters indicate statistically significant differences by Student's t-test ($p = < 0.02$). Cone samples represent different harvest days/kiln floors (crops 2015 and 2017)

Pelletizing and storage conditions can be expected to influence both acids and essential oil content in pellets [26]. In Patagonian samples, Hop Storage Index (HSI) values were all within "fresh" accepted ranges (all = < 0.25 ± 0.01). As a general reference, values above 0.32 are indicative of lower quality for fresh cones and above 0.38 for pellets, though the initial HSI and behavior during subsequent storage are variety specific [27]. Cascade pellets showed marginally higher HSI values than Cascade dried cones. This is consistent with its reported poor-storage capacity [21,28], also observed under our own storage stability tests for Patagonian Cascade and Nugget (data not published).

Another important parameter is cohumulone content, which has been related to harsh bitterness [29], although currently not supported. Cohumulone has a higher isomerization rate and utilization than humulone [30]. Patagonian Cascade dried cones contained 35.2 ± 0.6 % w/w cohumulone, which fell within the reported range for this variety (31–40 % w/w) and the same occurred for the Nugget variety (26.8 ± 0.7 % w/w) with a reported range of 22–30 % w/w [21–24].

3.2 Essential oils

Essential oil yields were 1.0 ml/100 g for Cascade cones, whereas Nugget cones resulted in higher yields (1.9 ml/100 g); similar to what was reported by others in different regions [21,22,23,24]. The analysis of volatile compounds from both varieties was performed (Table 3, see page 98, Supplementary Table 3, see page 102), including main terpenes, and sulfur compounds encountered in trace amounts.

Patagonian samples showed high myrcene content, above 50 % for Nugget and 60 % for Cascade. Compounds α-humulene and β-caryophyllene were both higher in Nugget samples; with an H/C ratio of 2.1–2.3; whereas Patagonian Cascade showed an H/C ratio closer to 3. Also linalool content was higher for the Nugget cultivar (~ 0.5 % vs ~ 1 %). The most striking difference was (E)-β-farnesene content, which accounted for near 5 % for Cascade and only 0.2 % for Nugget. These values and ratios all fall within the

Table 3 Main volatile compounds found in Cascade and Nugget varieties (cones) determined by GC-FID-MS

LRI‡		Compound	Area %†	
Non polar	Polar		Cascade	Nugget
895	1100	isobutyl isobutanoate	0.4	0.4
935	1043	α-pinene	0.2	0.2
943	1200	isopentenyl propionate	0.5	0.4
979	1133	β-pinene	0.3	0.4
980	1170	myrcene	69.6/62.5	60.1/52.5
996	1194	isopentenyl isobutanoate	0.3	0.7
1001	1198	2-methyl butyl isobutanoate	1.1	1.4
1006	1286	methyl heptanoate	0.2	0.6
1011	1175	δ-3-carene	0.2	0.5
1022	1227	β-phellandrene	0.5	0.2
1024	1221	limonene	0.3	0.2
1081	1407	nonanal	0.2	–
1081	1549	linalool	0.5/0.4	1.1/0.8
1103	1397	methyl octanoate	0.2	0.6
1206	1488	methyl nonanoate	–	0.3
1231	1852	geraniol	-/0.2	0.2/tr
1271	1607	undencan-2-one	–	0.4
1291	1620	methyl *Z*-dec-4-enoate	0.6	1.8
1291	1718	undecan-2-ol	–	0.4
1295	1690	methyl deca-4,8-dienoate	0.5	0.4
1306	1506	methyl decanoate	–	0.3
1359	1760	geranyl acetate	0.7	–
1376	1511	α-copaene	–	0.1
1417	1614	β-caryophyllene	3.8/3.7	7.5/7.6
1431	1596	*E*-α-bergamotene	0.2	–
1448	1668	(E)-β-farnesene	4.5/4.8	0.2/0.2
1449	1683	α-humulene	11.7/9.8	16.9/16
1468	1699	γ-muurolene	0.3	0.3
1475	1811	tridecan-2-one	–	0.2
1480	1732	β-selinene	0.4/0.5	0.6/0.8
1490	1794	geranyl isobutanoate	0.8	–
1492	1736	α-selinene	0.5/0.6	0.6/0.8
1504	1766	γ-cadinene	0.3	0.4
1512	1763	δ-cadinene	0.5	0.7
1582		humulene epoxide I§	0.1	0.2
1611		humulene epoxide III§	0.1	–
		monoterpenes	71.5	63.1
		sesquiterpenes	22.2	27.7
		esters	5.7	7.2
		α-humulene/β-caryophyllene (H/C) ratio	3.1/2.6	2.3/2.1
		Total	99.3	97.9

† Values represent percentages of total chromatogram area obtained in 2015 and 2018 from cone samples (2015/2018). ‡ LRI: Linear Retention Index, non-polar column: DB-1; polar column: HPWAX. § Tentatively identified by only one LRI and mass spectrum. tr. trace compounds found less than 0.05 %

ranges reported for these two varieties in the main producing regions [3,21,23,24,31,32].

A number of compounds were only detected in Nugget samples, including methyl nonanoate, methyl decanoate and undecan-2-ol; this latter considered a marker for this variety [33]. Also, two other typical markers of Nugget variety were found, although in minimal amounts (nonan-2-ol and methyl dodeca-3,6-dienoate) (Supplementary Table 3, see page 102). Patagonian Nugget cones showed a higher proportion of sesquiterpenes when compared to Cascade, which may provide more intense earthy and spicy notes [31, 34, 35].

As for Cascade, this aromatic variety widely distributed has been repeatedly analysed regarding its brewing aromatic potential [31, 34, 36, 37]. Also, new evidence is arising on the effect of the different terroirs on its aromatic and biochemical profile [25,38]. Both monoterpene alcohols linalool and geraniol are important floral odorants [39]; and synergistically with β-citronellol and other monoterpene alcohols are considered responsible for the typical citrusy flavor and aroma in beers hopped with Cascade, as well as other aroma hops [40,41,42]. Geranyl esters such as geranyl acetate and geranyl isobutanoate, may also contribute. Geraniol is considered a Cascade varietal characteristic compound; and its conversion to other monoterpene alcohols during fermentation is key to its final aroma and flavor contribution to beer [40,43]. Patagonian Cascade cones showed marginal amounts of geraniol, though geranyl acetate and geranyl isobutanoate were found in higher amounts; together with linalool. Terroir effects on the amount of these compounds in Cascade were observed, even within USA samples, and a similar low geraniol profile was found for Italian Cascade hop samples [38]. The above-mentioned geranil esters, together with nonanal and *E*-α-bergamotene were all found solely in Cascade and not in Nugget samples.

Also, “sweet floral like muscatel grapes” is a characteristic aroma of Cascade, related to the presence of geraniol, 4-mercapto-4-methylpentan-2-one and *Z*-hex-3-enol [44]. *Z*-hex-3-enol was detected in our samples, but only in traces. The Patagonian Cascade presented di and trimethyl sulfide, the last compound also detected by *Steinhaus* et al. (2007) [45]. Other important volatile compounds in Cascade variety

are myrcene, α-humulene, nonanal and limonene and octanal [39,45], the majority present in the Argentinian samples here analysed. Nevertheless, typical Cascade aroma compounds like 2-isopropyl-3-methoxy pyrazine, 4-mercapto-4-methyl pentan-2-one, *E-Z*-undeca-1,3,5-triene, anethol and 4-methyl-4-sulphanyl penta-2-none were not detected. Detection of these minor compounds is very likely influenced by the analytical platform, including extraction methods, and thus more samples and specific analysis are needed to confirm these results. In addition to the cited terroir effects, differences in polyphenol content were reported for Cascade hop material from Germany and the USA [46]; and it is of interest to analyse in the future the Argentinian samples for these components.

Because of the chemical complexity of hops essential oils, there are numerous misidentified compounds encountered in the bibliography (mostly esters with side chains) or omitted compounds with overlapping signals. We have unequivocally identified, through LRI in a polar and a non-polar column and MS: methyl sulfide, perillene and geranyl α-terpinene, all compounds which have never been cited for the Cascade variety. Other constituents identified but present in less than 0.05 %, are shown separately in Supplementary table 3, see page 102.

4 Conclusions

This study represents the first comprehensive analysis of the cv Cascade and cv Nugget hop products from Patagonia, Argentina. The overall composition of these hop products were similar to those reported for the same varieties in other producing regions including USA, Australia, New Zealand, Germany, Italy and Spain, and comply the accepted commercial range of the main constituents. These include α- and β-acids, cohumulone and the major essential oil components. Hop Storage Index for all samples (cones and pellets) was within the accepted range for "freshness"; which indicates current handling and pelletizing are not impacting negatively in α- and β-acids composition. Of notice are the high α-acid content and the high α/β ratio observed in Patagonian Cascade and its low geraniol content; yet other Cascade characteristic essential oil components were found; including geranyl esters. Further research is needed to evaluate the flavor impact this may cause on beer; when compared with other Cascade hops. Patagonian Nugget acids and volatile composition were similar to that reported for this variety in the high producing regions.

Land characteristics, possible genetic variations (ecotypes), purity of the planting material and the analytical platform, among other factors, may influence the obtained results. Further studies are being conducted to continue gaining new valuable information about these highly added value agronomic products from Argentina.

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5 References

1. Neve, R. A.: Hops, Chapman and Hall, London, New York, 1991.
2. Hough, J. S.; Briggs, D. E.; Stevens, R. and Young, T. W.: Malting and Brewing Science, Vol. 2 (2nd ed), Chapman and Hall, London, 1982.
3. Briggs, D. E.; Boulton, C.; Brookes, P. A.; Stevens, R.: Brewing Science and practice, Woodhead Publishing Limited, Cambridge, 2004.
4. IHGC Hop List 2019: http://www.hmelj-giz.si/ihgc/doc/2019%20NOV%20IHGC%20Hop%20variety%20list.pdf
5. AKC.: Cerveza de Argentina [Online], (cited 2020 August 10), Available: http://www.cervezadeargentina.com.ar/articulos/historiadellupuloenargentina.html.
6. Testa H.; Trochine A. and Bergamini H: Overview on Mapuche, Traful and Nahuel, local hop varieties cultivated in Patagonia, Argentina, Proceedings of the Scientific-Technical Commission IHGC (2019), pp. 96-100.
7. Barth haas group: H O P S 2 0 1 8 / 2 0 1 9 [Online], (cited 2020 August 09), Available: https://www.barthhaas.com/fileadmin/user_upload/news/2019-07-23/barthreport20182019en.pdf
8. Technical Committe A.: α- and β-Acids in Hops and Hop Pellets by Spectrophotometry and by Conductometric Titration, ASBC Methods of Analysis, (2011), pp. 3-5.
9. Technical Committe A.: Hop Storage Index (HSI), ASBC Methods of Analysis, (2011), pp. 1094.
10. Alderton, G.; Bailey, G. F.; Lewis, J. C. and Stitt, F.: Spectrophotometric Determination of Humulone Complex and Lupulone in Hops, Analytical Chemistry, **26** (1954), no. 6, pp. 983-992.
11. Technical Committe A.: α- and β-Acids in Hops and Hop Extracts by HPLC (International Method), ASBC Methods of Analysis, (2011), pp. 10-11.
12. Farmacopea Argentina ANMAT: Farmacopea Argentina, 7th ed, Vol. 1, 2003. Available from: http://www.anmat.gov.ar/webanmat/fna/pfds/Libro_Primero.pdf
13. van Baren, C. M.; Di Leo Lira, P.; Elechosa, M. A.; Molina, A.M.; Juárez, M. A.; Martínez, A.; Perelman, S. and Bandoni, A. L.: New insights into the chemical biodiversity of Minthostachys mollis in Argentina, Biochemical Systematics and Ecology, **57** (2014), pp. 374-383.
14. Joulain, D. and König, W. A.: The Atlas of Spectral Data of Sesquiterpene Hydrocarbons, E.B. Verlag, Hamburg, 1998.
15. Wiley, J. and Sons Inc.: The Wiley/NBS registry of mass spectral data, 8th edn, John Wiley & Sons, New York, 2008. ISBN 978-0-470-04785-9.
16. Babushok, V. I.; Linstrom, P. J. and Zenkevich, I. G.: Retention Indices for Frequently Reported Compounds of Plant Essential Oils, Journal of Physical and Chemical Reference Data, **40** (2011), no. 4, p. 043101.
17. Adams, R. P.: Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy, Carol Stream, IL: Allured Publishing Corporation, 2007.
18. Tkachev, A. V.: The Investigation of Plant Volatiles, Novosibirsk Institute of Organic Chemistry, Novosibirsk, Russia, 2008. ISBN 978-5-85957-056-0 [In Russian.]
19. Gross, R. W. and Schwiesow, M. H.: High Performance Liquid Chromatographic Analysis of Hop Alpha-and Beta-Acids, Journal of the American Society of Brewing Chemists, **40** (1982), no. 3, pp. 116-117.
20. Salanta, L. S.; Tofana, M.; Socaci, S. A. and Truta, D.: Hop Pellets

Bitter Acids – Comparison of Different Determination Methods, Bulletin UASVM Agriculture, **68** (2011), no. 2, pp. 404-408.

21. Hieronymus, S.: For the Love of Hops – The Practical Guide to Aroma, Bitterness and the Culture of Hops, Brewers Publications, Colorado, 2012.
22. Yakima Chief Hops: American Hops from the Pacific Northwest [Online], (cited 2020 August 09), Available: https://www.yakimachief.com/.
23. Hop Growers of America: Variety Manual 2016 [Online], Available: https://www.usahops.org/cabinet/data/2016_VarietyManual%20%281%29.pdf.
24. Kenny, S. T.: Identification of U.S.-Grown Hop Cultivars by Hop Acid and Essential Oil Analyses, Journal of the American Society of Brewing Chemists, **48** (1990), no. 1, pp. 3-8.
25. Van Holle, A.; Muylle, H.; Ruttink, T.; Van Landschoot, A.; Haesaert, G.; Naudts, D.; De Keukeleire, D. and Roldán-Ruiz, I: Single Nucleotide Polymorphisms and Biochemical Markers As Complementary Tools To Characterize Hops (*Humulus lupulus* L.) in Brewing Practice, Journal of Agricultural and Food Chemistry, **67** (2019), no. 13, pp. 3761-3771.
26. De Keukeleire, D.: Fundamentals of beer and hop chemistry, Quimica Nova, **23** (2000), no. 1, pp. 108-112.
27. Cocuzza, S.; Lutz, A. and Müller-Auffermann, K.: Influence of Picking Date on the Initial Hop Storage Index of Freshly Harvested Hops, Technical quarterly published by the Master Brewers Association of the Americas, **2** (2013), pp. 66-71.
28. Garret, O.: The Oxford Companion to Beer, Oxford University Press Inc., Madison, 2011.
29. Rigby, F. L.: A Theory on the Hop Flavor of Beer, Proceedings. Annual meeting-American Society of Brewing Chemists, Taylor & Francis, **30** (1972), no. 1, pp. 46-50.
30. Haseleu, G.; Lagemann, A.; Stephan, A.; Intelmann, D.; Dunkel, A. and Hofmann, T.: Quantitative sensomics profiling of hop-derived bitter compounds throughout a full-scale beer manufacturing process, Journal of Agricultural and Food Chemistry, **58** (2010), no. 13, pp. 7930-7939.
31. Nance, M. and Setzer, W.: Volatile components of aroma hops (*Humulus lupulus* L.) commonly used in beer brewing, Journal of Brewing and Distilling, 2 (2011), no. 2, pp. 16-22.
32. Vázquez-Araújo, L.; Rodríguez-Solana, R.; Cortés-Diéguez, S. M. and Domínguez, J. M.: Use of hydrodistillation and headspace solid-phase microextraction to characterize the volatile composition of different hop cultivars, Journal of the Science of Food and Agriculture, **93** (2013), no. 10, pp. 2568-2574.
33. Eri, S.; Khoo, B. K.; Lech, J. and Hartman, T. G.: Direct thermal desorption-gas chromatography and gas chromatography-mass spectrometry profiling of hop (Humulus lupulus L.) essential oils in support of varietal characterization, Journal of Agricultural and Food Chemistry, **48** (2000), no. 4, pp. 1140-1149.
34. Eyres, G. T.; Marriott, P. J. and Dufour, J. P.: Comparison of odor-active compounds in the spicy fraction of hop (*Humulus lupulus* L.) essential oil from four different varieties, Journal of Agricultural and Food Chemistry, **55** (2007), no. 15, pp. 6252-6261.
35. Goiris, K.; De Ridder, M.; De Rouck, G.; Boeykens, A.; Van Opstaele, F.; Aerts, G.; De Cooman L. and De Keukeleire D.: The Oxygenated Sesquiterpenoid Fraction of Hops in Relation to the Spicy Hop Character of Beer, Journal of the Institute of Brewing, **108** (2002), no. 1, pp. 86-93.
36. Inui, T.; Tsuchiya, F.; Ishimaru, M.; Oka, K. and Komura, H.: Different beers with different hops. Relevant compounds for their aroma characteristics, Journal of Agricultural and Food Chemistry, **61** (2013), no. 20, pp. 4758-4764.
37. Sharp, D. C.: Harvest Maturity of Cascade and Willamette Hops, Oregon State University, Master Thesis, 2013.
38. Rodolfi, M.; Chiancone, B.; Liberatore, C. M.; Fabbri, A.; Cirlini, M. and Ganino, T.: Changes in chemical profile of Cascade hop cones according to the growing area, Journal of the Science of Food and Agriculture, **99** (2019), no. 13, pp. 6011-6019.
39. Peacock, V. E.; Deinzer, M. L.; Likens, S.T.; Nickerson, G. B. and McGill, L. A.: Floral Hop Aroma in Beer, Journal of Agricultural and Food Chemistry, **29** (1981), no. 6, pp. 1265-1269.
40. Lam, K. C.; Foster, R. T. and Deinzer, M. L.: Aging of hops and their contribution to beer flavor, Journal of Agricultural and Food Chemistry, **34** (2005), no. 4, pp. 763-770.
41. Takoi, K.; Itoga, Y.; Koie, K.; Kosugi, T.; Shimase, M.; Katayama, K.; Nakayama, Y. and Watari, J.: Contribution of geraniol metabolism to citrus flavour of beer: synergy of geraniol and b-citronellol under coexistence with excess linalool, Journal of the Institute of Brewing. **116** (2010), pp. 251-260.
42. Takoi, K.: Screening of geraniol-rich flavor hop and interesting behavior of beta-citronellol during fermentation under various hop-addition timings, Journal of the American Society of Brewing Chemists, **72** (2014), p. 22-29.
43. Inui, T.; Tsuchiya, F.; Ishimaru, M; Oka, K. and Komura, H.: Different Beers with Different Hops. Relevant Compounds for Their Aroma Characteristics, Journal of Agricultural and Food Chemistry, **61** (2013), no. 20, pp 4758-4764.
44. Kishimoto, T.: Hop-Derived Odorants Contributing to the Aroma Characteristics of Beer, University of Kyoto, Master Thesis, 2008.
45. Steinhaus, M.; Wilhelm, W. and Schieberle, P.: Comparison of the most odour-active volatiles in different hop varieties by application of a comparative aroma extract dilution analysis, European Food Research and Technology, **226** (2007), no. 1-2, pp. 45–55.
46. Forster, A. and Gahr, A.A.: Comparison of the analytical and brewing characteristics of Cascade and comet hop varieties as grown in Yakima (USA) and Hallertau (Germany), BrewingScience – Monatsschrift für Brauwissenschaft, **67** (2014), no. 11/12, pp. 137-148.

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Supplementary Tables

Supplementary Table 1 Meteorological data of season 2019/2020

	Avg T (°C)	Avg Max T (°C)	Avg Min T (°C)	Max T (°C)	Min T (°C)	Total pp (mm)	Avg Atm p (hPa)
sep-19	6.6	13.8	−0.1	24.7	−6.6	37.4	1020.3
oct-19	9.2	16.3	1.5	26.4	−4.9	11.6	1018.3
nov-19	12.4	19.5	4.7	26.9	−0.7	56.0	1014.8
dec-19	14.1	22.5	4.2	29.4	0.0	19.8	1015.9
jan-20	15.6	24.8	5.6	32.3	−0.6	40.2	1014.7
feb-20	14.6	23.7	5.5	31.4	0.3	10.6	1015.9
mar-20	13.3	22.9	4.4	32.3	−2.2	29.2	1016.1
apr-20	9.0	16.4	2.5	21.8	−3.7	44.2	1018.9

Data were obtained from a Meteorological station (Davis vantage pro2) located at the hop farm (Lat: -41.9007 Long: -71.5194; Elevation (ground): 451 m). Avg Temp: monthly average temperature, Avg Max T: monthly average of daily Max temperatures, Avg Min T: monthly average of daily Min temperatures, Max T: maximum detected temperature, Min T: minimum detected temperature, Total pp: Monthly total precipitation, Avg Atm p: monthly average atmospheric pressure at sea level

Supplementary Table 2 Harvesting and pelletizing periods

	Harvest	Pelletization
Cascade	02/27−03/28	03/27−04/13
Nugget	03/09−03/27	03/30−04/18

Data represent date (mm/dd) ranges corresponding to the studied samples (2015, 2017, 2018, 2019 and 2020). Harvest days also represent hop kilning dates. After drying, hops were stored in bales at −20 °C before pelletizing

Supplementary Table 3 **Trace volatile compounds (less than 0.05 %) found in Cascade or Nugget varieties (dried cones) determined by GC-FID-MS**

LRI				
Non polar	Polar	Compound	Cascade	Nugget
590	738	dimethyl, sulfide	–	+
600	1061	2-methylbut-3-en-2-ol	–	+
634	917	isovalerianal	+	+
666		methyl isobutanoate†	+	+
690		2-ethyl furane†	+	–
702		2,4-dimethylpenta-1,3-diene†	–	+
712	1014	methyl isobutyl ketone	+	+
756		3-methylbut-2-en-2-ol†	+	+
769	1100	hexanal	+	+
800	800	octane	+	+
822	1670	isovaleric acid	+	+
826	1240	*E*-hex-2-enal	+	+
831	1392	*Z*-hex-3-enol	+	–
844	1077	isobutyl propionate	+	+
852		2-methyl butyl acetate†	+	+
868	1043	2-methyl propyl 2-methyl butanoate	+	+
873	1136	ethyl valerianate	–	+
876		heptanal†	+	+
905	1186	methyl caproate	+	+
926	1036	α-thujene	+	+
930		butyl isobutanoate†	+	+
945	1202	amyl propionate	+	+
951	1100	camphene	+	+
955		methyl trisulfide†	+	+
957	1448	oct-3-en-1-ol	+	+
957	1349	methyl heptenone	+	+
961		methyl, 5-methyl hexanoate†	–	+
1012	1206	α-terpinene	+	+
1018	1286	*p*-cymene	+	+
1020	1235	*Z*-β-ocimene	+	+
1026	1307	amyl isobutanoate	–	+
1032	1260	*E*-β-ocimene	+	+
1047	1264	γ-terpinene	+	+
1069	1403	nonan-2-one	+	+
1073		epoxy myrcene†	+	+
1081	1280	2-methyl butyl 2-methyl butanoate	+	+
1081		nonan-2-ol†	–	+
1082	1305	terpinolene	+	+
1083	1307	2-methyl butyl, isovalerianate	+	+
1087	1425	perillene	+	–
1098		karahana ether†	+	+
1103	1591	fenchol	+	+
1121	1396	hex-Z-3-enyl isobutanoate	+	+
1125	1346	hexyl isobutanoate	+	+
1141	1771	methyl phenylacetate	+	–
1150	1711	borneol	+	+
1158		octanoic acid†	+	–
1164	1614	terpinen-4-ol	–	+
1170	1504	decan-2-one	+	+
1172	1705	α-terpineol	+	+
1175	1456	methyl 6-methyl octanoate	+	+
1183		heptyl propionate†	+	+
1187		decan-2-ol†	+	+
1192		methyl (E)-non-4-enoate†	+	+
1223	1448	hexyl isovalerate	+	+
1238		methyl citronelate†	+	+
1239	1740	geranial	+	–
1269		methyl 8-methyl nonanoate†	+	–
1281	2007	perilla alcohol	+	–
1302	1699	methyl geraniate	+	+
1331		octyl 2-methyl propionate†	+	+
1340	1729	neryl acetate	+	+
1350	1470	α-cubebene	+	+
1376	1704	dodecan-2-one	+	+
1387	1603	β-elemene	+	+
1405	1591	α-caryophyllene	+	+
1409		methyl undecanoate†	+	+
1449	1822	geranyl propanoate	–	+
1458		*Z*-cadina-1(6),4-diene†	+	+
1473	1700	α-amorphene	+	+
1484	1727	*Z*-*E*-α-farnesene	+	+
1485	1660	*Z*-cadina-1,4-diene	+	+
1488		γ-amorphene†	+	+
1488		methyl dodeca-3,6-dienoate†	–	+
1489	1693	epizonarene	+	+
1492	1730	α-muurolene	+	+
1496	1754	*E*-*E*-α-farnesene	+	+
1502	1740	δ-amorphene	+	+
1508	1841	*Z*-calamenene	+	+
1510	1770	7-*epi*-α-selinene	+	+
1524	1787	*E*-cadina-1,4-diene	+	+
1525	1922	α-calacorene	+	+
1528	1798	α-cadinene	+	+
1546	1940	β-calacorene	+	+
1559	2056	caryophyllenol	+	+
1561	1954	dendrolasine	+	–
1563	2088	furopelargone B	+	–
1563	1977	iso caryophyllene oxide	+	–
1565	1989	caryophyllene oxide	+	+
1570		clovenol†	–	+
1581	1911	tetradecan-2-one	+	+
1587		humulol†	+	+
1617		humulenol II†	+	–
1619		caryophylladienol II†	+	+
1620	2171	Γ-cadinol	+	+
1630	2058	cubenol	+	+
1680	2011	pentadecan-2-one	+	+
1698	2355	*E*-2-*E*-6-farnesol	+	+
1937	2205	geranyl α-terpinene	+	+
1972	2252	*p*-camphorene	–	+

Non polar column: DB-1; polar column; HPWAX. † Tentatively identified only by one LRI and the mass spectrum. (+): detected; (–): not detected