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Varietal Discrimination of Hop Pellets by Essential Oil Analysis I. Comparison of Fresh Samples

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ABSTRACT

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The aim of this study was to differentiate hop pellets by essential oil analysis. Volatile compounds of five aromatic cultivars (Styrie, Saaz, Lublin, Mount Hood, and Hallertau) and seven bitter cultivars (Northern Brewer, Nugget, Pride of Ringwood, Northdown, Galena, Target, and Challenger) were extracted with a Likens-Nickerson simultaneous solvent extractor. The extracts had a strong hop aroma that varied according to the type of hop. Approximately 100 compounds were separated by gas chromatography (GC) and identified by GC-mass spectrometry. An identification flowchart including seven terpenic compounds, four esters, and one methyl ketone was established to discriminate between fresh samples of the 12 investigated cultivars. High amounts of bergamotene and farnesene were found only in Saaz, Lublin, and Styrie samples. Quantification of 4-decenoic acid methyl ester and 3-methyl butyl isobutyrate proved a quick means of distinguishing non-European and European bitter hops from aromatic cultivars.

Keywords: 3-Methyl butyl isobutyrate, Bergamotene, Flavor, Hop aroma, Hop cultivars

RESUMEN

El propósito de este estudio fue diferenciar lúpulo en pellets por análisis de aceites esenciales. Compuestos volátiles de 5 variedades aromáticas (Styrie, Saaz, Lublin, Mount Hood, y Hallertau) y siete variedades amargas (Northern Brewer, Nugget, Pride of Ringwood, Northdown, Galena, Target, y Challenger) fueron extraídas con el extractor simultáneo de solventes Likens-Nickerson. Los extractos tuvieron un fuerte aroma a lúpulo, variando de acuerdo al tipo de lúpulo. Casi cien compuestos fueron separados por cromatografía de gases e identificados por espectrometría de masas. Un diagrama de flujo de identificación incluyendo 7 compuestos terpénicos, 4 ésteres, y una metilcetona fueron establecidas para discriminar entre muestras frescas de las 12 variedades investigadas. Altas cantidades de bergamoteno y farneseno fueron encontradas solamente en muestras de Saaz, Lublin, y Styrie. La cuantificación del éster metilico del ácido 4-decenoico y del isobutirato de 3- metilbutilo proveen un medio rápido para distinguir lúpulos amargos europeos y no-europeos de variedades aromáticas.

It is now well established that the composition of hop oil, and the hop flavor derived therefrom, in beer depend on the hop cultivar. Many works authenticated hop oils by their flavor constituents (2, 4–6), but rarely by using cones (11) or pellet samples (14), the methods used in the present work. Several hop cultivars were compared: three European aroma hops, usually selected to impart the "noble" hop aroma; two other low-bitterness cultivars; plus four European and three non-European bitter cultivars. Three consecutive crops (1994–1996) were investigated for most of the cultivars (Table I), even though many authors (17) indicate good varietal uniformity of composition over a range of environmental conditions. The relationship between hop aroma and the aromatic compounds organoleptically active in beer is not known. Most hop oil constituents are not recovered in beer due to oxidation, hydrolysis, transesterification, and reduction during boiling and fermentation. The "kettle hop" aroma is therefore quite different from the aroma of hop itself. Hydrolysis of humulene and caryophyllene epoxides (7,13) produces organoleptically active, though not too pleasant, products in beer (9). Other flavoring compounds are probably required to impart the pleasant "kettle hop" aroma. The lack of both qualitative and quantitative knowledge makes it hard to choose which hop to use. For this reason, our identification flowchart was established both to highlight the distinctive features of each hop cultivar and to point out any common features between the aromatic hops or the various bitter hops.

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EXPERIMENTAL

Hop Samples

A total of 32 commercial samples were collected, including 12 cultivars. Samples of the cultivars Saaz, Lublin, and Hallertau were in T45 pellet form, while the other cultivars were in T90 pellet form. For a better comparison, all our data were calculated for T90 pellet conditioning. All samples (5 kg) were stored under nitrogen at 4°C in hermetic dark packing.

Hop Aroma Extraction

An optimized version (1) of the Likens and Nickerson (12) extraction procedure was used, allowing high recovery of most essential oils (>90%, except for humulene diepoxides, for which recovery was only 50%) and high reproducibility (variation coefficients <10%). The results presented here for each cultivar are the means of two different extractions.

Steam-distillation solvent-extraction was conducted in a microextractor to remove hop oil components. Ground pellets (0.5 g) were mixed with 1.5 ml of internal standard solution (20 ppm of carvone) in 50 ml of ultrapure deoxygenated water (Milli-Q water purifier, Millipore, Bedford, MA) and transferred to flask A (Fig. 1). Dichloromethane (1.5 ml) was transferred to flask B. Dichloromethane and ultrapure deoxygenated water (1.5 ml each) were introduced into area C using arm H. A few clean carborundum grains were successively introduced into flasks A and B. Prior to the procedure, the entire system was purged with nitrogen (2–3 ml/min) for 5 min. Flask A was then heated in a 140°C oil bath. After 3 min, flask B was heated in a 70°C water bath. The vapors were then condensed in area C by means of a cold finger maintained at –15°C by a cryostat. The entire steam-distillation solvent-extraction procedure was conducted under a 2 ml/min nitrogen flow. The steam-distillation was stopped after 45 min and 2 ml of dichloromethane extract was removed from flask B. The dichloromethane layer in area C was then collected in flask B; flask B was finally washed with 1 ml of 4× dichloromethane. The extract was then concentrated to 1 ml in a Danish-Kuderna column, and 1 ml was analyzed by gas chromatography (GC) and GC-mass spectroscopy (MS).

GC Analytical Conditions

For GC, we used an HP5890 gas chromatograph equipped with an HP7673 automatic sampler, a cold on-column injector, a flame-ionization detector, and a Shimadzu CR4-A integrator. Hop oil components were analyzed on a wall-coated, open tubular CP-SIL5 CB capillary column (50 m × 0.32 mm; 1.2-µm film thickness) allowing separation in one run of ≈250 peaks. The oven temperature was set to rise from 36 to 85°C at 50°C/min, to 145°C at 1°C/min, to 250°C at 3°C/min, and then to remain constant at 250°C for 30 min. The injector temperature was set at 280°C. The detector temperature was 280°C. The carrier gas was helium at a flow rate of 1.1 ml/min.

GC-MS Analytical Conditions

The column was directly connected to an HP5988 quadrupole mass spectrometer. Electron impact mass spectra were recorded at 70 eV. Spectral recording throughout elution was automatically performed with the HP59970C software. Peaks were identified by their enhancement after co-injection of standard compounds and with the help of the NBS/EPA/NIH mass spectra library.

RESULTS AND DISCUSSION

Gas chromatography of 32 hop samples (in duplicate) yielded typical fingerprints for each cultivar, although unexplained varia-

TABLE I
Peak Identification and Concentrations (ppm) of Relevant Compounds

	Compounds[a]														
Cultivar, Year	**1[b]**	**2*[c]**	**3*[d]**	**4[e]**	**5*[f]**	**6*[g]**	**7**[h]**	**8**[i]**	**9**[j]**	**10[k]**	**11**[l]**	**12**[m]**	**13**[n]**	**14**[o]**	**15**[p]**
Non-European bitter hops															
Pride of Ringwood (T90) 94	9.5	…[q]	129	144	1	155	59	…	8	134	186	65	787	823	16
Pride of Ringwood (T90) 95	9.2	3	16	173	…	205	14	1	35	85	105	38	669	669	2
Pride of Ringwood (T90) 96	7.6	9	49	150	…	204	24	…	18	81	178	51	723	721	13
Nugget (T90) 94	12.5	83	176	71	77	244	27	…	21	3,268	86	73	173	170	143
Nugget (T90) 95	12.6	135	297	78	171	510	23	…	75	3,858	128	97	273	294	88
Nugget (T90) 96	11.5	55	108	72	72	211	13	…	20	2,378	156	60	244	263	32
Galena (T90) 94	11.7	97	407	72	4	447	17	…	…	1,849	80	392	148	100	133
European bitter hops															
Northern Brewer (T90) 94	10.3	24	62	69	2	58	21	…	10	4,996	27	39	71	…	368
Northern Brewer (T90) 94	10.3	15	75	76	2	45	19	…	10	3,357	41	38	44	…	203
Northern Brewer (T90) 95	5.9	30	188	83	…	43	59	…	8	3,296	96	23	70	70	187
Northern Brewer (T90) 96	5.2	11	58	65	…	11	29	…	9	2,248	90	9	56	63	265
Northdown (T90) 95	6.2	50	180	79	…	20	76	1	119	3,508	188	7	551	584	257
Northdown (T90) 96	6.4	42	150	65	2	19	65	…	132	2,094	184	7	519	563	208
Target (T90) 94	9.9	14	58	153	4	113	16	…	8	1,470	49	45	98	…	341
Target (T90) 95	10.3	40	233	147	12	28	53	…	19	1,394	110	54	114	114	113
Target (T90) 96	8.3	19	91	162	7	43	25	…	26	1,439	148	27	129	125	101
Challenger (T90) 95	6.2	30	120	127	…	45	13	4	85	1,605	84	25	379	399	184
Challenger (T90) 96	5.4	22	92	98	…	26	10	…	52	1,332	96	16	356	380	240
Low-bitter hops															
Saaz (T45) 94	3.2	…	…	41	2	47	7	38	203	1,066	28	21	16	…	128
Saaz (T45) 94	3.4	…	…	38	5	11	6	40	150	711	13	13	13	…	241
Saaz (T45) 95	2.8	…	…	53	2	61	4	49	861	990	26	24	15	20	81
Saaz (T45) 96	2.6	…	…	45	3	36	4	54	727	833	28	20	12	18	87
Lublin (T45) 94	3.2	…	…	30	2	47	5	42	590	1,015	29	19	23	…	42
Lublin (T45) 95	3.2	2	18	43	3	31	14	50	1,055	1,176	39	23	39	42	53
Lublin (T45) 96	3.2	…	3	41	…	23	9	74	688	976	40	16	37	48	129
Styrie (T90) 94	4.7	…	15	53	2	23	40	25	183	1,390	21	18	37	…	123
Styrie (T90) 95	4.9	9	79	133	1	40	95	45	618	1,626	54	30	114	110	171
Styrie (T90) 96	4.0	7	87	97	4	23	98	53	980	1,414	48	22	25	14	167
Mount Hood (T90) 95	4.2	10	28	55	2	139	32	…	76	3,589	239	22	83	96	332
Mount Hood (T90) 96	4.3	10	30	58	…	97	30	…	28	2,677	202	17	63	95	703
Hallertau (T45) 95	3.1	4	45	65	6	44	7	…	10	2,101	81	12	58	72	366
Hallertau (T45) 96	2.8	4	29	39	6	24	6	…	18	1,218	72	9	48	55	206

[a] All concentrations are calculated for T90 conditioning; * = carvone equivalent for quantification; ** = caryophyllene equivalent for quantification.
[b] α-Acids.
[c] 3-Methyl butyl isobutyrate; numbering, 12; identification, gas chromatography (GC)-mass spectroscopy (MS); ret. index (CPSil5-CB) 973.
[d] 2-Methyl butyl isobutyrate; 13; GC-MS; index 977.
[e] 2-Undecanone; 45; GC-MS; index 1301.
[f] Unknown 46; 46; m/z 57, 75, 83, 69; index 1317.
[g] 4-Decenoic acid, methyl ester; 47; MS; index 1322.
[h] Methyl geranate; 79; GC-MS; index 1337.
[i] Bergamotene; 64; MS; index 1458.
[j] β-Farnesene; 65; MS; index 1463.
[k] α-Humulene; 67; GC-MS; index 1480.
[l] α-Amorphene; 69; MS; index 1497.
[m] 3,6-Dodecanoic acid, methyl ester; 70; MS; index 1505.
[n] β-Selinene; 71; MS; index 1515.
[o] α-Selinene; 74; MS; index 1525.
[p] Humulene epoxyde II; 85; GC-MS; index 1639.
[q] Not detected or detected but not quantified.

tions are measured in a few cases between successive crops (e.g., farnesene, 3-methyl butyl isobutyrate, 2-methyl butyl isobutyrate). As previously shown by Krajl et al (6), the variability of the aroma composition among samples, as well as years, can be large; therefore, an analysis based on only one year's production seems insufficient. In order to facilitate the authentication of aged samples, additional information about the stability of all our markers will be soon published. Figure 2 shows the chromatograms obtained for cvs. Saaz 95 and Nugget 95 hops. Peak identification and concentration values of the relevant compounds are summarized in Table I.

Only a few peaks required quantification to authenticate our 12 hop cultivars (Fig. 3). However, all discriminant compounds could be helpful in detecting when hop cultivars have been mixed prior to pelleting. Three major groups can be defined depending on whether the 4-decenoic acid methyl ester concentration is above or below 150 ppm (carvone equivalent) and the 3-methyl butyl isobutyrate concentration above or below 11 ppm (carvone equivalent). Surprisingly, factor analysis of sensory data also led Peppard et al (15) to group hops into three broad categories: the first including cvs. Hallertau and Saaz, the second cv. Northern Brewer, and the third cv. Galena.

The three non-European bitter cultivars (Pride of Ringwood, Nugget, and Galena) are characterized by higher concentrations of 4-decenoic acid methyl ester (>150 ppm, carvone equivalent).

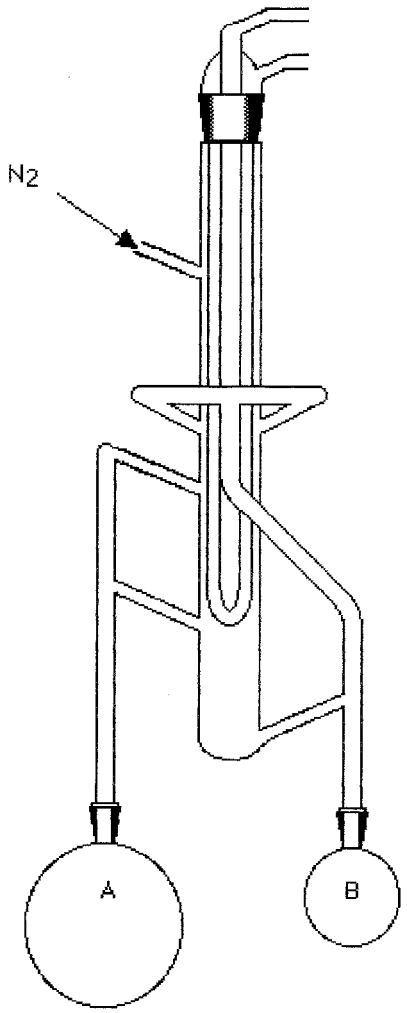

Fig. 1. Microextractor used for simultaneous steam-distillation solvent-extraction.

Relatively high concentrations (97–139 ppm) are also measured in cv. Mount Hood, the sole non-European aromatic cultivar investigated here. Nickerson and Likens (12) suggest that this compound is transesterified to the corresponding ethyl ester during fermentation. The three Pride of Ringwood samples are easily distinguishable from all nine other cultivars by their very low concentrations (<150 ppm) of α-humulene and its main epoxide, humulene epoxide II (<100 ppm, caryophyllene equivalent). High concentrations of α and β selinenes (>650 ppm, caryophyllene equivalent) further characterize Pride of Ringwood hop. Identification of these two selinadienes was based on the mass spectra published by Davies and Menary (3). These authors also detected higher levels of these compounds in Pride of Ringwood samples than in cvs. Northern Brewer, Brewers' Gold, Bullion, and Cluster. More than 25 ppm (carvone equivalent) of an unidentified compound (retention index = 1317) distinguished our three Nugget samples. Although only one Galena hop was analyzed, our data suggest that high levels of 3,6-dodecadienoic acid methyl ester (392 ppm as opposed to <100 ppm, carvone equivalent) could help to recognize this cultivar.

Higher amounts of 3-methyl butyl isobutyrate are found in almost all European bitter hops, while concentrations of <11 ppm are found in all five low-bitterness cultivars. Except in two samples of cv. Styrie, a 2-methyl butyl isobutyrate level of <50 ppm could provide additional proof. Particularly high amounts of both isobutyrates are evidenced in the two α-rich cultivars, Nugget and Galena, suggesting that such compounds could be from α-acids. Seaton et al (16) have shown that the hop fraction containing myrcene and 2-methyl butyl isobutyrate possess the major part of the "resinous" and "fruity/estery," citrus-like character of the "hop loft" aroma. However, such compounds could be unstable during boiling and fermentation. According to Seaton et al (16), 2-methyl butyl isobutyrate was identified in a pilot-scale pale ale when late hopping was carried out with Aurora liquid CO_2 extract. Murakami et al (10) also detected higher levels of isoamyl isobutyrate in Galena-brewed than in Hallertau-brewed beers. Experiments in our laboratory have confirmed that residual 2-methyl and 3-methyl butyl isobutyrate are present in beers when late hopping is carried out with Northern Brewer or Nugget hops, but absent when the Saaz cultivar or no hop is used.

In the five low-bitterness cultivars, bergamotene (>10 ppm, caryophyllene equivalent) and farnesene (>150 ppm, caryophyllene equivalent) appear as key markers for identifying the most pleasant aromatic cultivars: Saaz, Lublin, and Styrie. Peacock and McCarty (14) also categorized hops in two groups according to the farnesene content: high-farnesene cultivars included Saaz, Willamette, and Cascade; low-farnesene cultivars included Hersbrucker, Cluster, Hallertau, and most of the high α-acid cultivars. Due to its higher stability, bergamotene quantification should be preferred when hop freshness is unknown. As previously shown for humulenes (13), sesquiterpenes are oxidized and hydrolyzed during boiling. This suggests that a sensory investigation of bergamotene and farnesene degradation products should be conducted soon.

Methyl geranate emerges here as a reliable indicator for distinguishing Styrie (>20 ppm, caryophyllene equivalent) from Saaz and Lublin. Of all the relevant esters, methyl geranate is probably the most stable due to the presence of an α,β-unsaturation. No significant difference has been found between Lublin and Saaz, which are in fact the same cultivar grown at different geographical sites (14).

In the "European bitter hops" group, Northdown and Challenger are characterized by large amounts of α and β selinenes (>200 ppm, caryophyllene equivalent), as compared to Target and Northern Brewer. Northdown is distinguishable from Challenger by the concentration of a sesquiterpene, which could be α-amor-

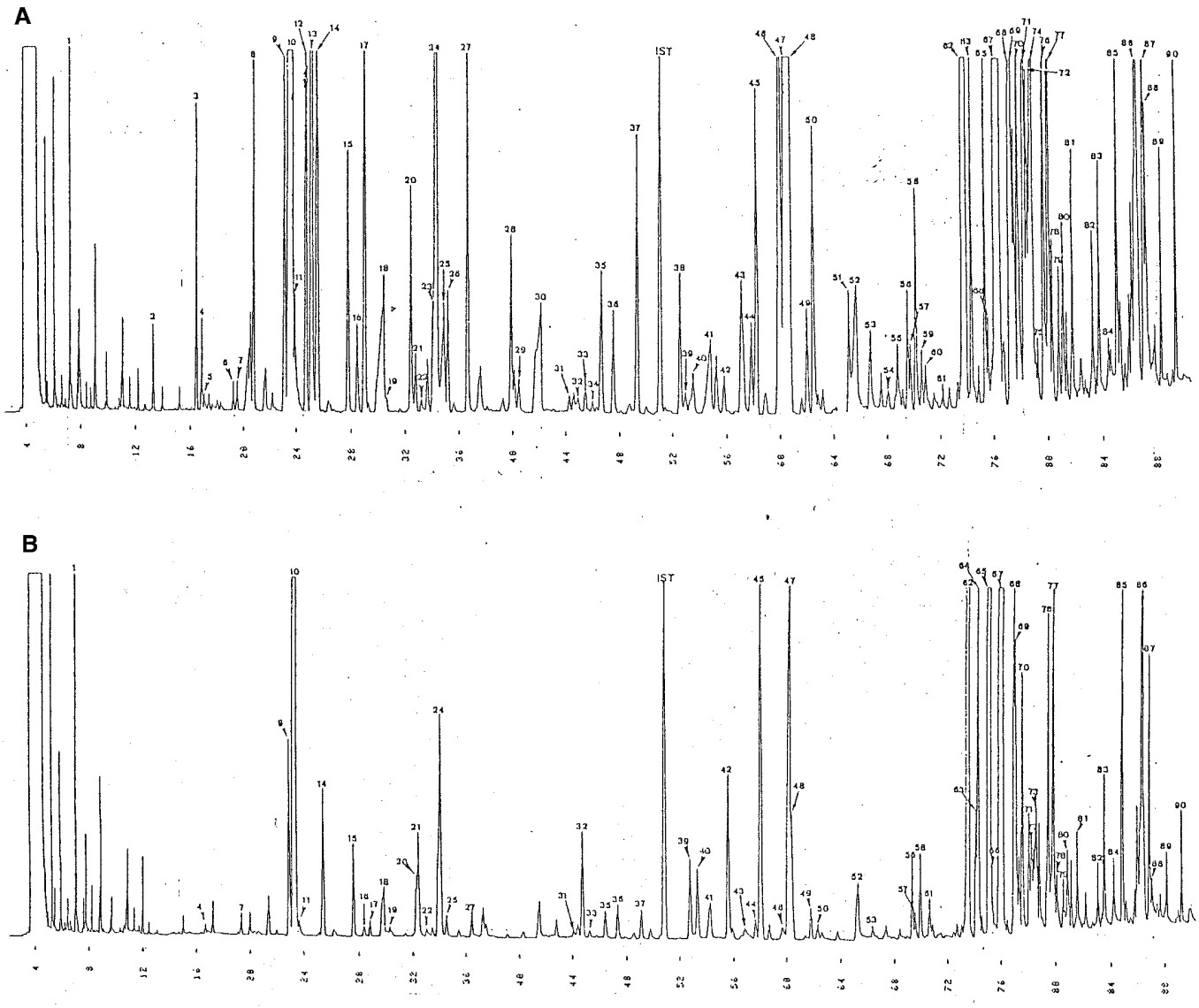

Fig. 2. Chromatograms obtained for Nugget 95 **(A)** and Saaz 95 **(B)** hops.

phene (>125 ppm, caryophyllene equivalent). The same terpene also occurs as a significant constituent distinguishing Mount Hood (>125 ppm, caryophyllene equivalent) from Hallertau in the "low bitterness" group. Perhaps the most interesting marker of Northern Brewer hops emerging from our study is the humulene-to-farnesene ratio (>249); all other cultivars show a ratio <220. The comparison between Northern Brewer and the low-humulene Target samples is even more relevant. An additional marker for distinguishing Target (>100 ppm) from Northern Brewer hops is 2-undecanone. High 2-undecanone concentrations can also occur in Pride of Ringwood samples. This methylketone could give rise to an organoleptically active reduced compound in beer, the 2-undecanol flavor threshold being estimated at 70 ppb in beer (8).

Obviously, a great deal of research is still required to obtain the full picture of hop-derived compounds. As cultivar markers emerge, this should encourage researchers to focus on the fate of such compounds through boiling and fermentation.

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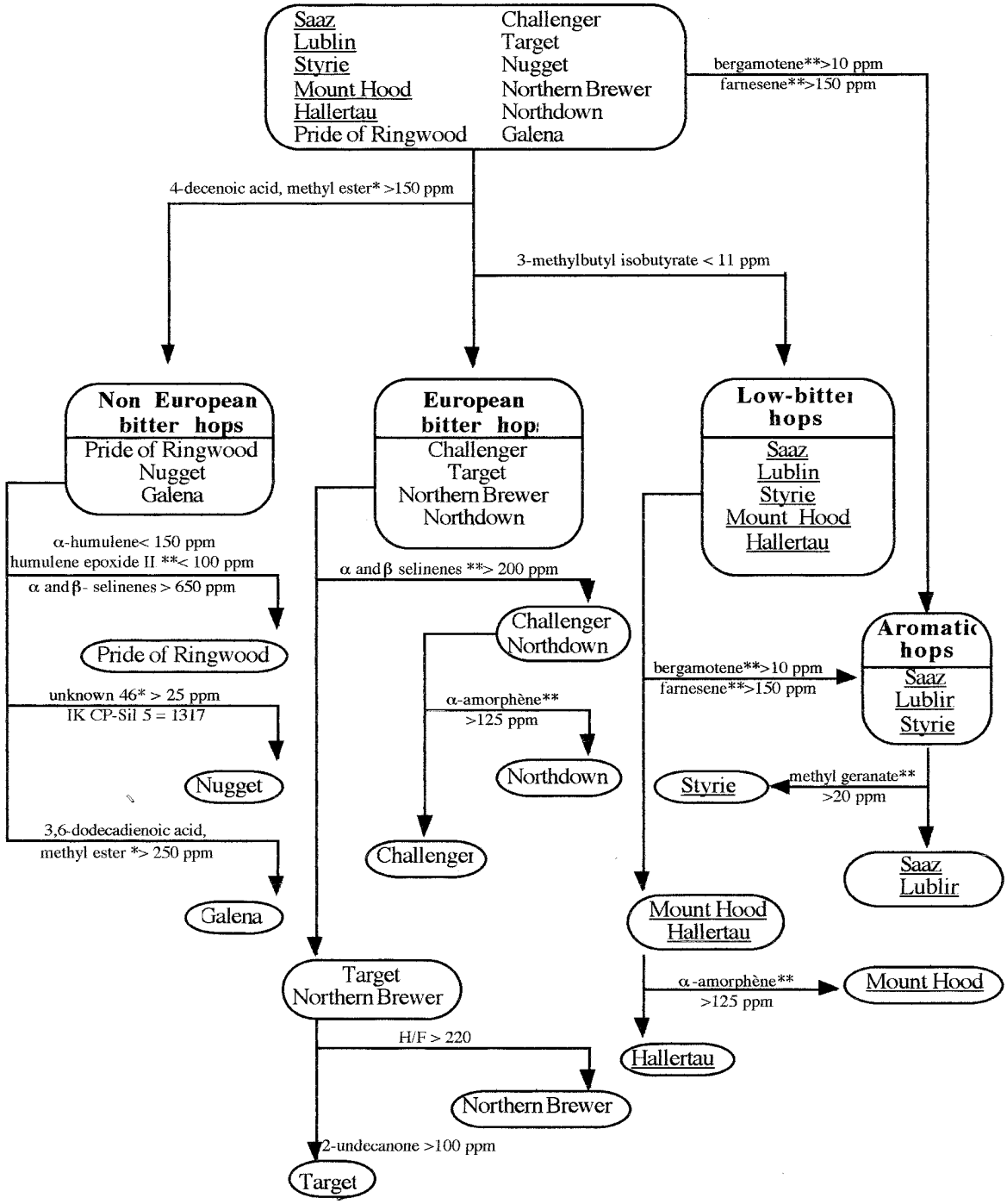

Fig. 3. Flowchart for distinguishing 12 hop cultivars.

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