Aging of Hops and Their Contribution to Beer Flavor

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Two American hop varieties, Washington-grown Cascade and Idaho-grown Hallertauer mittelfrüh, were chosen for this aging study and its effect on beer flavor quality. Prior to brewing, hops were refrigerated at 27 °F (fresh), while one portion of the hops from the same bale was aged at 90 °F for 2 weeks (aged I) and another portion for 9 weeks (aged II). Samples at each brewing stage were taken for chemical profile analysis. The finished beers were also submitted for flavor profile evaluations. Geraniol, linalool, and citronellol are mainly responsible for the floral/citrus note, while the oxidation products of α-humulene, especially the humulenol II and humulene diepoxides, contribute to the herbal/spicy note in beer. A grapefruit-like fruity flavor is also detected in beers brewed with extensively aged Cascade and Hallertauer hops. The survival of various hop-derived aroma compounds is also discussed.

INTRODUCTION

Historically, one of the main purposes of using hops in brewing is to add bitterness to the finished beer. In addition, a unique and distinctive hoppy aroma and taste can also be obtained when raw cone hops, hop pellets, or certain hop extracts are used. Many European hop varieties such as Hallertauer mittelfrüh, Tettnanger, etc., are noted as aroma hops because of their ability to impart the "kettle hop" or "noble hop" flavor to the final beer.

The chemical composition of the essential oil collected from different hop varieties and the effects of aging on its composition are well documented (Likens and Nickerson, 1967; Buttery and Ling, 1967; Naya and Kotake, 1972; Tressl et al., 1978a, 1983). It has also been suggested that noble hop flavor is not caused by compounds in the major hydrocarbon fraction but, instead, by compounds in the minor oxygenated fraction of hop oil (Howard and Stevens, 1959; Tressl et al., 1978b; Peacock et al. 1980). Although numerous hop-derived oxygenated compounds have been identified in various beers, the argument about which ones contribute to the hoppy aroma of beer has not been settled (Shimazi et al., 1974; Verzele and Sandra, 1981; Sharpe and Laws, 1981; Tressl et al., 1978b, 1983; Fukuoka and Kowaka, 1983). The effects of using different hopping procedures on beer flavor quality have also been reported (Seaton et al., 1982; Haley and Peppard, 1983; Kowaka et al., 1983; Moir et al., 1983).

The purpose of this study was to observe the effects of aging on the chemical composition of the essential oil of both Cascade Hallertauer mittelfrüh hops and to correlate these changes with the qualtiy and type of hoppy flavor obtained in the final beer. The contributions of various compounds to hoppy beer aroma are also discussed. In addition, the survival of certain well-known hop oil compounds throughout the brewing process is also documented.

EXPERIMENTAL SECTION

Raw Hop Treatments. Freshly harvested Washington Cascade and Idaho Hallertauer mittelfrüh (henceforth called Hallertauer) hops were stored refrigerated at 27 °F (fresh) until they were submitted for accelerated aging study, where portions of the hops from the same bale were aged at 90 °F, one for 19 days (aged I) and another for 60 days (aged II). A total of eight samples of hops was obtained for pilot brewing. Each variety had four brewing trials: two brews with warmed stored hops (aged I and aged II) and two brews with refrigerated hops (fresh).

Hops Analysis. Essential oils were isolated from hops by using the method of Likens and Nickerson (1967). An aliquot of a stock solution containing 500.0 μg of naphthalene (internal standard) in hexane was added to 100.0 mg of hop oil, to which hexane was added to make a 1.0-mL solution. Samples were analyzed by capillary gas chromatography–mass spectrometry (Cap-GC–MS). The Hop Storage Index (HSI) (Nickerson and Likens, 1979) was used to monitor the degree of aging.

Pilot Beers. Two hop varieties, Cascades and Hallertauer, at three different levels of oxidation were added to a 30-barrel (3534-L) pilot-scale brew kettle at the Adolph Coors Co. Eight pilot beers were obtained when brewed with each of these hop samples, two fresh and one each of aged I and aged II, under identical conditions. Normal commercial malt and adjunct were used under standard pilot brewery operations. Wort from each brew was boiled, hopped, and adjusted to a required degree Plato. Ten barrels of the cooled, aerated wort were pitched with production yeast and allowed to ferment (fermentor drop) and lager (storage drop) normally. Each storage drop was blended with water and adjusted to an alcohol content of 3.7% by weight (finished beer).

Beers were evaluated by the taste panel of Oregon State University and by the Flavor Profile panel of Adolph Coors Co. Samples of wort, fermentor drop, storage drop, and finished beer were also taken and analyzed by Cap-GC–MS at Oregon State University.

A blank pilot brew was made in which no hops were added during the kettle boil process. Unhopped wort and unhopped storage drop samples were taken so that compounds derived from malts and yeast metabolism could be differentiated.

Brewing Products Analysis. The following extraction and cleanup procedure was used for all samples of wort, fermentor drop, storage drop, and finished beer. An aliquot of a stock solution in ethanol containing 1.0 mg of naphthalene (internal standard) was added to 2 L of liquid sample that was then mixed thoroughly with 2 kg of Celite 545 (J. T. Baker Chemical Co., Phillipsburg, NJ). The mixture was packed into three large liquid chromatography (LC) columns, and each column was eluted with 2 L of methylene chloride. The combined eluent was dried over anhydrous sodium sulfate and reduced in volume to about 0.5 mL on a rotary evaporator. An LC column (2.5-cm i.d.) was filled first with 25 cm of 80–200-mesh adsorption alumina (Fisher Scientific Co., Fair Lawn, NJ) followed

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Table I. Chemical Information of Selected Hops

variety	aging level	HSI[a]	oil content, mL/100 g	% α-acids[b]	% β-acids[b]	hops used in brewing, g
Cascade	fresh[c]	0.33	0.60	5.3	5.1	7194
	aged I	0.53	0.38	4.5	3.6	7432
	aged II	1.04	0.13	1.9	1.2	9806
Hallertauer	fresh[c]	0.33	1.08	6.1	5.4	6831
	aged I	0.68	0.74	3.6	3.1	7432
	aged II	1.21	0.44	1.3	1.2	11000

[a] Hop Storage Index. [b] Hop bitterness components: reported by the UV spectrophotometric method of the American Society of Brewing Chemists (1976). [c] Reported as the average of two analyses.

Table II. Maximum Available Amount of Selected Aroma Components in Hops

	Cascade			Hallertauer		
	fresh[a]	aged I	aged II	fresh	aged I	aged II
myrcene	1754.0[b]	471.0	3.0	1807.0	507.0	37.0
linalool	40.9	46.7	1.4	61.9	279.3	85.1
geranial	7.3	39.5	2.5	12.8		28.1
neral	0.7	2.1				1.0
methyl geranate	30.4	53.6	4.7	18.3	101.8	34.1
geranyl acetate	110.1	173.3	9.2			
geranyl isobutyrate	67.5	132.9	5.4	1.6	18.7	8.1
geraniol	8.2	20.8	0.2	6.4	23.3	8.9
α-terpineol	2.7	1.8	0.1		10.8	4.2
α-caryophyllene	296.9	342.8	1.9	384.0	915.3	175.7
α-humulene	623.1	665.7	10.2	1514.6	2536.9	539.5
caryophyllene epoxide	44.8	119.6	6.4	209.0	611.9	112.3
humulene monoepoxide I	39.6	198.9	9.4	171.9	951.2	488.1
humulene monoepoxide II	187.1	569.4	34.5	1699.3	4061.9	668.0
humulene monoepoxide III	26.6	120.3	6.6	126.9	354.1	346.5
humulenol II	21.3	603.0	44.6	166.4	2984.6	1435.2
humulene diepoxide A		2.3	2.7		80.6	5.6
humulene diepoxide B			0.2		0.5	10.8
humulene diepoxide C					5.2	
humulene diepoxide D			0.4		23.7	
humulene diepoxide E			0.1		9.1	
∑OP[c]	322.0	1615.3	105.0	2373.5	9093.6	3070.7
∑FC[d]	265.4	468.9	23.4	101.0	423.1	165.3
∑OP:∑FC	1.2	3.4	4.5	23.5	21.5	18.6

[a] Reported as the average of two analyses. [b] Concentration (μg/L) added to the brew kettle; total amount (mg) × 1000 μg/mg × 1/3534 L. [c] Total amount of herbal/spicy compounds including caryophyllene oxide, humulene monoepoxides I, II, and III, humulenol II, humulene diepoxides A–E, and α-terpineol. [d] Total amount of floral/citrus compounds including linalool, geranial, neral, methyl geranate, geranyl acetate, geranyl isobutyrate, and geraniol.

by 2.5 cm of sodium carbonate and 2.5 cm of anhydrous sodium sulfate. The column was wetted with hexane, and the sample was then applied to the column. The column was eluted with 100 mL of hexane followed by 200 mL of ether. The combined eluents were reduced to a volume of 100 μL on a rotary evaporator. All extracts were analyzed by Cap-GC–MS.

Cap-GC–MS. All compounds were identified and quantified on a Finnigan Model 4023 quadrupole mass spectrometer. A 0.32 mm × 60 m Durawax-4 fused silica WCOT column (J & W Scientific, Inc., Rancho Cordova, CA) was used to chromatograph all samples. Helium was used as the carrier gas, with a linear velocity of 20 cm/s. The split ratio was 1:100. Oven temperature was programmed from 80 to 190 °C at 5 °C/min, with a 5-min hold at the initial temperature. The end of the capillary column was inserted directly into the ionization chamber via a heated stainless-steel transfer line. Other temperatures were set as follows: transfer line, 210 °C; ionizer, 190 °C; injection port, 150 °C. The mass spectrometer was operated in electron-impact mode with the electron energy set at 70 eV. Mass spectra were taken once every second, with a scan range of m/e from 35 to 450. Data were acquired and stored on disks for later retrieval. Quantitation was carried out by comparing the ion intensities between the selected ion chromatogram of the aroma compound and the ion chromatogram at m/e 128 of the internal standard naphthalene. As an aid to identification of compounds, mass spectra were compared with reference spectra from the National Bureau of Standards and with a collection of reference spectra compiled in the author's laboratory at Oregon State University.

Flavor Panel Evaluations. A trained panel at Oregon State University with an average of 15 panelists participated in each of the triangular tests. A 90-mL (3-oz) sample was carefully poured into a coded 360-mL (12-oz) amber glass. Each panelist was served with two triangular sets at one time. Each triangular set consisted of two identical and one different sample, using all six possible combinations. The total number of samples from both test beers was the same in each serving. A flavor profile analysis was also conducted for each pilot beer with a specialized Coors Flavor Profile panel. Five subgroups of hoppy flavor were used to describe the changes in hoppy character between both varieties and the changes in hoppy flavors due to aging within each variety.

RESULTS AND DISCUSSION

Chemical properties of the two fresh hop samples of each variety were so similar that their results were averaged and reported as fresh in Table II. Likewise, results of the two fresh samples at each brewing stage were also averaged (Tables V and VI).

Hop Oil Composition. In Table I the HSI, α- and β-acids content, oil content, and total amount of hops used in pilot brews are summarized. As expected, the HSI increased with the degree of aging, while the amount of essential oil and the content of both α- and β-acids de-

creased. Adjustments in the hopping rate were based on the extent of decrease in α-acids content.

Results of the hop oil analysis from three oxidation levels of both Cascade and Hallertauer hops are shown in Table II. For ease of comparison, hop aroma compounds were reported as their maximum available amount for brewing (μg/L). They were calculated by multiplying their concentrations in hop (μg/g) with the total amount of hops used (g) and then dividing by 3534 L.

Among these hops the two aged I hops showed the highest amount of aroma compounds. Compared with the aged I hops, there were substantial losses of aroma compounds for the aged II hops, and the situation was more drastic for Cascade aged II than for Hallertauer aged II hops. The loss may be due to evaporation, polymerization, and other degradation processes. This can also be caused by the intrinsic differences between the two hop varieties upon storage (Foster and Nickerson, 1985).

Both the floral/citrus compounds (mainly geraniol-type compounds and linalool) and the herbal/spicy compounds (mainly oxidation products of α-humulene and β-caryophyllene) were found in hops at all three oxidation levels for each variety. The content of floral/citrus compounds was comparatively higher in Cascade hops, while the content of herbal/spicy compounds was higher among Hallertauer hops. The ratio between the total amounts of herbal/spicy compounds (∑OP) and floral/citrus compounds (∑FC) showed a significant difference between the two hop varieties. These ratios, ∑OP:∑FC, around 20 for Hallertauer hops and less than 5 for Cascade hops, remained fairly constant within samples of the same variety.

Comparing the fresh and aged I hop samples, the rate of increase of humulene monoepoxide II upon aging was much slower than that of its other two isomers. At the same time, the rate of increase of humuladienone, humulol, and humulenol II was much higher than that of humulene monoepoxide II. From examination of the chemical structures of α-humulene and its oxidation products (Tressl et al., 1978a; Peacock and Deinzer, 1981), it is evident that humulene monoepoxide II is the precursor of humuladienone, humulol, and humulenol II. Data from Table II suggested that a portion of the humulene monoepoxide II was rearranged to its secondary products during aging.

Several diepoxides of α-humulene were observed in all aged hops but not in fresh hops, suggesting that they were formed during hop aging. These diepoxides were tentatively identified in the laboratory at Oregon State University by using Cap-GC–MS in both electron-impact and chemical ionization modes. They are likely to be formed by further oxidation of the three monoepoxides.

The hop oil data generally agree with the findings of Tressl et al. (1978a), Peacock and Deinzer (1981), and Foster and Nickerson (1985). These authors reported an increase in both the floral/citrus compounds and the oxidation products of α-humulene in hops upon storage.

Compounds Contributed by Malt and Yeast. Analysis on the unhopped wort reveals compounds derived from malt. The major compounds are listed in Table III. They consist mainly of various aliphatic and unsaturated hydrocarbons and alkylated benzenes. Table IV shows the major fermentation products in the unhopped storage drop. Most of the malt-derived compounds are still detectable, but at a much lower level. As can be seen, more hydroxy-containing compounds have been produced or transformed from their precursors by the yeast. The components that are most abundant in the unhopped storage drop are 4-hydroxy-4-methyl-2-pentanone, 2-methyl-1-propanol, 1-butanol, isoamyl alcohol, 3-hexen-2-one, and 2-phenethanol.

Table III. Major Compounds Found in Unhopped Wort

Aliphatic Hydrocarbons		
decane	decalin	branched alkenes
undecane	1,1′-bicyclohexane	cycloalkanes
dodecane	branched alkanes	cycloalkenes
tridecane		

Alcohols and Ketones	
1-chloro-2-propanol	6-methyl-3-heptanol
1-(2-propenyloxy)-2-propanol	2-ethyl-1-hexanol
4-hydroxy-4-methyl-2-pentanone	4-methyl-4-penten-2-one
	3,5,5-trimethylcyclohexen-1-one
5-hexen-2-ol	2-(2-hydroxypropoxy)-1-propanol
2-ethoxyethyl ether	furfuryl alcohol
4-hydroxy-4-methyl-2-butanone	4-(hexyloxy)-1-butanol

Aromatic Compounds		
toluene	xylenes	$C_6H_5C_4H_9$
ethylbenzene	$C_6H_5C_3H_7$	$C_6H_5C_5H_{11}$

Table IV. Major Fermentation Products Found in Unhopped Storage Drop

Alcohols	
2-methyl-1-propanol	2-butoxyethanol
1-butanol	4-methyl-2-heptanol
1-isopropoxy-2-propanol	2-methyl-4-heptanol
isoamyl alcohol	3-(methylthio)-1-propanol
2-ethoxyethanol	2-phenethanol
2-isopropoxy-1-propanol	2-phenyl-2-propanol
1-isobutoxy-2-propanol	

Ketones	
4-methyl-3-penten-2-one	3,5-dimethyl-2-cyclohexen-1-one
5-methyl-3-hexen-2-one	3-methyl-2-cyclohexen-1-one
4-(acetyloxy)-2-butanone	

Phenolics	
diisopropylphenols	(hexyloxy)benzene
tert-butylphenol	BHA

Brewing Process vs. Compound Survival. Results from monitoring the pilot beers brewed with Cascade and Hallertauer hops at four different brewing stages, wort, fermentor drop, storage drop, and finished beer, are summarized in Tables V and VI, respectively. The level of aroma compounds in all brewing products of Cascade aged II hops could not be correlated with their maximum available amount in the hop oil. This discrepancy might be due to the way the aroma compounds were being collected. In the raw hop samples, hop oil was collected by steam distillation. Both volatility and initial amount of each compound determine its final content in the hop oil. On the other hand, during kettle boil of the worts, compounds are extracted directly into the wort. Besides their initial amounts the volatility and hydrophilicity of these compounds along with duration of boil determine the efficiency of extraction. Thus, kettle boil might be a more efficient step than steam distillation for extracting certain hop aroma compounds.

(*A*) *Worts.* Considerable quantities of the floral/citrus compounds listed in Tables V and VI survived the kettle boil with the exception of geranyl acetate. Among Cascade worts both linalool and geraniol were detected in all worts, while methyl geranate and geranial were only found in fresh wort and geranyl isobutyrate was detected in both fresh and aged I wort. For Hallertauer worts, linalool and methyl geranate were found among all worts, geranial was detected in fresh and aged I worts, geraniol was observed for aged I and aged II worts, and geranyl isobutyrate was not found in any worts. The amount of geraniol was higher

Table V. Aroma Compounds at Various Brewing Stages of Cascade-Hopped Pilot Beers

	wort			fermentor drop			storage drop			beer		
	FR[a]	A I	A II	FR	A I	A II	FR	A I	A II	FR	A I	A II
linalool	12.6[b]	17.0	12.1	28.1	38.1	20.4	14.4	23.0	27.2	17.7 (9.6)[c]	21.9 (15.4)	16.4 (18.2)
geranial	1.5											
methyl geranate	6.7											
geranyl acetate												
geranyl isobutyrate	8.5	54.7										
geraniol	4.3	30.5	40.8	4.6	8.2		3.4	4.3	5.7	2.3 (2.3)	5.4 (2.9)	6.1 (3.8)
α-terpineol	36.8		7.4	15.2	30.5	26.9	14.7	37.3	9.9	7.8 (9.8)	43.5 (25.0)	11.0 (6.6)
β-caryophyllene		7.7										
α-humulene		27.5										
caryophyllene epoxide	93.4	178.4										
humuladienone												
humulene monoepoxide I	16.4	57.8			5.7			0.8			0.7 (0.5)	
humulene monoepoxide II	275.1	624.5										
humulene monoepoxide III	8.3	22.6	7.2									
humulenol II	116.0	63.3	148.1		55.2	11.61		25.9	147.9		25.8 (17.4)	63.7 (99.1)
humulol		tr	1.1									
caryolan-1-ol								0.3			tr (0.2)	
humulene diepoxide A	183.9	295.3		27.1	84.2	24.6	tr	11.4			23.0 (7.6)	
humulene diepoxide B	29.4	76.2	13.0	1.6	5.3	15.0					7.5	
humulene diepoxide C								16.6	6.7			
humulene diepoxide D	16.6	27.7				10.4						
humulene diepoxide E												
citronellol				4.8	10.1	2.6	2.9	5.9	14.8	8.1 (1.9)	7.1 (4.0)	4.5 (9.9)
ethyl geranate				7.0	15.7	3.5	6.0	9.8	4.8	3.8 (4.0)	4.9 (6.6)	3.0 (3.2)
ethyl octanoate				140.4	231.5		9.2	20.0	19.5	8.2 (6.2)	45.6 (13.4)	(13.1)
ethyl decanoate				61.9	95.8		8.8	3.3	47.7	3.6 (5.9)	12.6 (2.2)	(32.0)
2-phenylethyl acetate				304.5	537.6	3.8	9.8	2.2	28.7	3.1 (6.6)	65.0 (1.5)	(19.2)
2-phenethanol	27.3	62.7		7664.0	10660.0	10629.0	13045.0	6132.0	12763.0	7800.0 (8740.0)	5400.0 (4108.0)	7051.0 (8551.0)

[a] Key: FR = fresh; A I = aged I; A II = aged II. Note: The fresh sample represents the average of two analyses. [b] In μg/L. [c] Calculation: storage drop value (μg/L) × 0.67 (dilution factor).

Table VI. Aroma Compounds at Various Brewing Stages of Hallertauer-Hopped Pilot Beers

	wort			fermentor drop			storage drop			beer		
	FR[a]	A I	A II	FR	A I	A II	FR	A I	A II	FR	A I	A II
linalool	17.5[b]	61.1	43.0	27.1	62.2	49.6	25.9	39.9	64.7	18.2 (17.4)[c]	17.9 (26.7)	15.0 (43.3)
geranial	2.3	10.6										
methyl geranate	3.8	13.3	11.6									
geranyl acetate												
geranyl isobutyrate												
geraniol		9.2	16.1	1.4	6.4	3.3	0.8	0.5	2.2	0.9 (0.5)	3.8 (0.3)	
α-terpineol	20.4	35.8	17.5	11.5	24.0	9.0	15.3	11.9	11.2	8.4 (10.3)	4.6 (8.0)	6.5 (7.5)
β-caryophyllene	7.6	24.3	37.4									
α-humulene	56.7	94.7	184.3									
caryophyllene epoxide	482.6	500.9	74.6		16.3							
humuladienone			2.9									
humulene monoepoxide I	256.4	286.2	199.8	3.3	20.6	34.4	0.8	4.8	25.7	tr (0.5)	5.6 (3.2)	28.8 (17.2)
humulene monoepoxide II	2274.3	2286.3	456.4									
humulene monoepoxide III	103.8	117.6	83.2			5.2						
humulenol II	809.8	197.2	1785.7	1.2	123.3	781.5		43.5	737.7		19.9 (29.1)	223.8 (494.3)
humulol					2.2							tr
caryolan-1-ol					3.4							
humulene diepoxide A	947.4	980.3	188.8	75.1	229.0	183.8	41.3	48.7	156.4	1.6 (27.7)	27.5 (32.6)	3.1 (104.6)
humulene diepoxide B	328.0	360.8	58.6	12.4	68.6	53.1	20.1	29.8	30.6	1.0 (13.5)	5.3 (20.0)	28.1 (20.5)
humulene diepoxide C	37.7	55.90	30.9		11.5	33.1						
humulene diepoxide D	94.1	141.0	99.6	0.4	66.1	46.0		23.4	105.3		0.6 (15.7)	(70.6)
humulene diepoxide E	46.2	tr	22.7			13.6		2.9	9.6			
citronellol			1.2	1.4	8.3	5.7	6.6	4.2	48.8	52.2 (4.4)	22.2 (1.9)	(32.7)
ethyl geranate				0.6	9.6	2.0	11.3	12.1		16.2 (7.6)	8.9 (8.1)	(94.3)
ethyl octanoate				99.3	188.8	62.8	33.6	11.8	19.7	2.2 (22.5)	8.4 (7.9)	(13.2)
ethyl decanoate				41.9	76.2	22.8	20.6	1.2	16.0	1.1 (13.8)	2.0 (0.8)	(10.7)
2-phenethyl acetate				246.3	514.1	75.3	33.6	1.1	93.7	31.0 (22.5)	1.5 (0.7)	(62.8)
2-phenethanol	11.1	81.4	31.5	7342.0	9758.0	18514.0	13165.0	9331.0	12617.0	11316.0 (8820.0)	11504.0 (6252.0)	8218.0 (5506.0)

[a] Key: FR = fresh; A I = aged I; A II = aged II. Note: The fresh sample represents the average of two analyses. [b] In μg/L. [c] Calculation: storage drop value (μg/L) × 0.67 (dilution factor).

in the Cascade worts, while that of linalool was higher in the Hallertauer worts.

None of the myrcene in the hop oil samples survived the kettle boil. Only small amounts of α-humulene and β-caryophyllene were found in the Cascade aged I wort. As Hallertauer hops aged, an increase in the level of α-humulene was observed in the corresponding worts. The oxidation products of α-humulene and β-caryophyllene appeared to be well extracted into worts. The level of the three humulene monoepoxides, caryophyllene epoxide, and humulenol II was higher in the Hallertauer than Cascade worts. Caryolan-1-ol was not detected in any worts. Only small amounts of humuladienone were found in Hallertauer aged II wort. Trace amounts of humulol were found in Cascade aged I and aged II worts.

Five humulene diepoxides were found in all Hallertauer worts, but not more than three diepoxides were detected in Cascade worts. Since they were not observed in either Cascade or Hallertauer fresh hops, their presence in both Cascade and Hallertauer fresh worts suggested that oxidation of hop-derived aroma compound occurred during kettle boil. This was further supported by the observation that the level of α-terpineol among all worts was much higher than that in their corresponding hops. Dieckmann and Palamand (1974) had reported that oxidation of limonene produced α-terpineol.

(B) Fermentor Drops and Storage Drops. During fermentation, ethanol and carbon dioxide were produced and the acidity of the fermentor drop decreased to pH 4.3–4.5 from 5.5–5.8 in the wort. Although the fermentation products of yeast metabolism were predominant in the samples analyzed, there were significant changes in the hop aroma profiles of the hopped fermentor and storage drops.

An initial increase in the level of linalool was observed between all worts and their corresponding fermentor drops, followed by a decrease from fermentor drops to storage drops. The amount of linalool was higher among Hallertauer than Cascade samples.

Among the geraniol-type aroma compounds, only geraniol survived the fermentation. The level of geraniol showed a consistent decrease in all samples from worts to fermentor drops to storage drops, and the degree of change was higher among all Hallertauer samples. Both geranyl acetate and geranyl isobutyrate were possibly hydrolyzed to geraniol, geranial reduced to geraniol, and methyl geranate transesterified to its ethyl ester. Ethyl geranate was observed in moderate amounts in almost all samples after fermentation. Haley and Peppard (1983) concluded that hydrolysis was one of the major metabolic processes of yeast, while transesterification was a minor one.

Citronellol was only observed in samples after fermentation, with the exception of minute quantities detected in the Hallertauer aged II wort. Since it was not found in any hop oils or in the unhopped storage drop, it may be a transformation product from yeast acting on some hop-derived precursor. Seaton et al. (1982) postulated that both neral and geranial were reduced to nerol, geraniol, and citronellol. With the exception of the Hallertauer fresh and aged I fermentor drop in our study, the amount of citronellol in the fermentor drop far exceeded the amount of geranial in the corresponding wort. In addition, an increase in the level of citronellol from the fermentor drop to the storage drop was observed in Hallertauer fresh and aged II and Cascade aged II samples, accompanying a decrease of geraniol. At the same time, geranial was no longer detected past the wort stage. It seemed that the conversion of geraniol to citronellol, also mediated by yeast, should be considered. A proposed transformation scheme of citronellol and its possible precursors is shown in Figure 1.

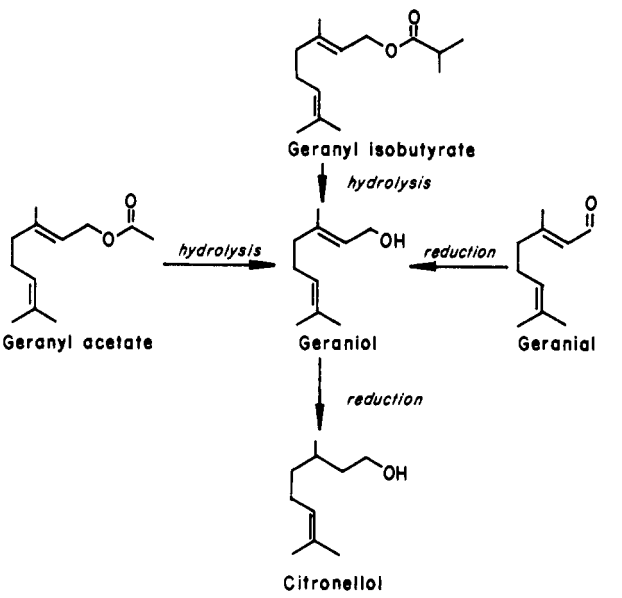

Figure 1. Proposed transformation scheme of some floral compounds.

Most oxidation products of α-humulene and β-caryophyllene poorly survived the fermentation process. As noted by Moir et al. (1983), this might be due to adsorption onto yeast, owing to their relatively high lipophilicity. Among these oxidation products, moderate amounts of humulenol II, humulene monoepoxide I, and humulene diepoxides were detected in all fermentor drops and in all storage drops. The levels of these compounds were much higher in Hallertauer than Cascade samples and were higher in the aged than the fresh samples. Humuladienone was not observed in any samples past kettle boil. Caryophyllene epoxide and humulol were only detected in Hallertauer aged I fermentor drop and were not found in any storage drops. Small amounts of caryolan-1-ol were found in Cascade aged I storage drop and in Hallertauer aged I fermentor drop.

Both ethyl octanoate and ethyl decanoate were detected in all samples after fermentation, with the exception of Cascade aged II fermentor drop. These two esters were transesterified from their hop-derived methyl ester (Haley and Peppard, 1983). α-Terpineol was also found in moderate amounts in all fermentor drops and in all storage drops.

(C) Finished Beers. Both the observed and the calculated concentrations of aroma compounds were reported for all beer samples. The calculated concentration, as shown in parentheses in Tables V and VI, was obtained by multiplying the observed storage drop value by 0.67. (Since finished beers were obtained by blending their corresponding storage drops with water and adjusted to a required alcohol content, the level of aroma compounds in beers should be lowered by a value equal to the dilution factor, 0.67, when compared with their storage drops.) However, results showed that there was no general recognizable pattern for the transfer of aroma compounds from storage drop to its corresponding beer. As Moir et al. (1983) has pointed out, the equilibrium between sorption–desorption of aroma compounds on the filtration materials might have significant influence on the overall flavor profile in beer.

Linalool, geraniol, and α-terpineol survived the whole brewing process and were found in all beers. The level of linalool was similar in Cascade and Hallertauer beers. The

Table VII. Survival Rates (%) of Selected Aroma Compounds in Pilot Beers

	Cascade			Hallertauer		
	fresh	aged I	aged II	fresh	aged I	aged II
linalool[a]	116.7	128.8	135.5	104.0	29.3	34.9
geraniol[a]	53.5	17.7	15.0	0.0	41.3	0.0
citronellol[b]	168.9	70.3	173.1	371.4	267.5	0.0
α-terpineol[b]	51.3	142.6	40.9	73.0	19.2	72.2
humulene monoepoxide I[a]	0.0	4.3	0.0	0.0	2.0	14.4
humulenol II[a]	0.0	40.8	43.0	0.0	10.1	12.5
humulene diepoxide A[a]	0.0	7.8	0.0	0.2	2.8	1.6
humulene diepoxide B[a]	0.0	9.8	0.0	0.3	1.5	48.0
humulene diepoxide D[a]	0.0	0.0	0.0	0.0	0.4	0.0

[a] Percent survival from wort to finished beer. [b] Percent survival from fermenter drop to finished beer.

level of geraniol and α-terpineol was higher in the Cascade beers.

Caryophyllene epoxide, humuladienone, humulene monoepoxide II and III, humulol, and caryolan-1-ol were no longer detectable in any beers. The amounts of humulene monoepoxide I and humulene diepoxides found in all Hallertauer beers were much higher than those found in Cascade aged I beer. These compounds were absent in both Cascade fresh and aged II beer. The level of humulenol II was similar between the two aged I beers, but it was almost 4 times higher in Hallertauer aged II than in Cascade aged II beer. No humulenol II was found in the two fresh beers.

Ethyl geranate was detected in all beers except Hallertauer aged II beer, and its level was higher among Hallertauer beers. Both ethyl octanoate and ethyl decanoate were detected in higher concentration in the Cascade fresh and aged I beer than in the Hallertauer fresh and aged I beer.

Although 2-phenethanol is not a hop-derived compound, its large concentration in beer makes it important due to its distinct floral flavor. Its level in Cascade and Hallertauer fresh beers was much higher than those in the corresponding aged beers. 2-Phenethyl acetate, a possible secondary product of 2-phenethanol, was also found in the two fresh and in the two aged I beers.

Survival Rates of Selected Hop Aroma Compounds. Table VII lists the overall survival rates of some selected hop aroma compounds. These rates were higher for the floral compounds in both Cascade and Hallertauer beers, but much lower for the oxidation products of α-humulene. Cascade beers generally showed a higher survival of linalool, geraniol, and α-terpineol, while Hallertauer beers, with the exception of Hallertauer aged II, had higher survival rates of citronellol. Although the oxidation products of α-humulene survived poorly in beers of both hop varieties, the actual amounts of humulenol II and humulene monoepoxide I found in the Hallertauer aged II beer are 4 and 3 times, respectively, higher than those found in Cascade aged II beer.

Beer Flavor Evaluations. Results from Triangular Tests showed that significant differences in flavor were detected between any two beers analyzed. Beers brewed with hops at different aging levels of the same variety, as well as those brewed with different hop varieties, could be differentiated. Results showed that the confidence level was 95% or better.

Results from the flavor profile analysis along with the total amount of herbal/spicy and floral/citrus compounds in beer are summarized in Table VIII. The overall hoppy flavor of the pilot beers was subdivided into herbal/spicy, floral, citrus, grapefruit, and grassy flavors. The first three flavors were commonly detected in all beers analyzed.

The intensity of the herbal/spicy flavor note decreased from the fresh to aged I to aged II Cascade beer but increased in intensity in the corresponding Hallertauer beers. Humulenol II, humulene diepoxides, and humulene monoepoxide I may be major contributors to the herbal/spicy flavor, since they are the oxidation products of α-humulene that survived the whole brewing process and were found in substantial amounts in beers. Moreover, α-terpineol produced a mild herbal/spicy flavor. Its level in various beers suggested that it might be a minor contributor to the herbal/spicy flavor.

Table VIII. Flavor Profile Analysis and Level of Aroma Compounds in Finished Beers

	Cascade			Hallertauer		
	fresh[a]	aged I	aged II	fresh[a]	aged I	aged II
(A) Flavor Profile Analysis						
herbal/spicy	3.2[d]	2.9	2.3	2.6	2.7	3.0
floral	2.8	3.2	3.0	2.9	3.2	2.4
citrus	3.0	2.3	5.3	3.5	2.9	4.3
grapefruit	0.3	0.7	2.4	0	0	2.3
grassy	0.2	0	0	0.5	0.4	1.1
(B) Level of Aroma Compounds						
ΣOP[b]	7.8[e]	100.5	74.7	11.0	63.5	247.3
ΣFC[c]	28.1	34.4	27.0	24.3	43.9	15.0

[a] Reported as the average of two analyses. [b] Total amount of herbal/spicy compounds. See footnote in Table II. [c] Total amount of floral/citrus compounds including linalool, geraniol, and citronellol. [d] Intensity of sensory response. [e] In μg/L.

Data obtained in this study generally agree with various published results. Peacock et al. (1980) reported 100 μg/L of humulene monoepoxide I and 250 μg/L of humulenol II in a beer brewed with a mixture of Hallertauer, Tettnanger, and Styrian hops. They concluded that the oxidation products of α-humulene collectively were important to the herbal/spicy flavor in beer. Moir et al. (1983) showed that 5–10 μg/L of humulene monoepoxide I and 20–50 μg/L of humulenol II were detected in an ale sample.

Of the various floral/citrus compounds in hops, only linalool and geraniol survived into beer in moderate amounts. Citronellol, a transformation product of geraniol, is also included here, and it produced both citrus and floral flavor. The highest floral rating was given to the two aged I beers, while a very intense citrus flavor was found in the two aged II beers. The total amounts of floral/citrus compounds seemed to correlate better with the floral than with the citrus flavor. Various compounds exhibit both floral and citrus flavor, but little is known specifically about the extent that these compounds contribute to each of the two flavor notes in beer. Perhaps this is one of the inconsistencies between chemical and sensory analysis for floral and citrus beer flavor.

A distinctive grapefruit flavor note was found in all Cascade and in the Hallertauer aged II beers. For Cascade beers, the intensity of the grapefruit flavor increased from fresh to aged I to aged II samples. This increase correlated

well with the level of aging of Cascade hops, although compounds responsible for this flavor note have not been identified. Both Seaton et al. (1982) and Moir et al. (1983) reported a similar fruity/citrus flavor in beer. A weak grassy flavor note was detected in all Hallertauer beers, as well as in Cascade fresh beer.

SUMMARY

It was found that moderate aging of fresh hops prior to brewing is needed to maximize the level of various aroma compounds. However, excessive aging leads to a significant loss of these compounds. The loss was more severe in Cascade than in Hallertauer hops. By monitoring the levels of various selected hop-derived aroma compounds at each brewing stage, a better understanding of the fate of these compounds can be obtained. Our data suggest that humulenol II and humulene diepoxides and to a lesser extent humulene monoepoxides I and α-terpineol may be responsible for the herbal/spicy flavor in beers, whereas linalool, geraniol, and citronellol are mainly responsible for the floral/citrus flavor. An intense grapefruit flavor was also detected among the two aged II beers brewed with extensively aged hops, although the compound responsible for this flavor has not been identified.

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Registry No. Geraniol, 106-24-1; linalool, 78-70-6; citronellol, 106-22-9; humulenol II, 19888-00-7; α-terpineol, 98-55-5; humulene monoepoxide I, 19888-33-6; humulene diepoxide, 11066-50-5; myrcene, 123-35-3; geranial, 141-27-5; neral, 106-26-3; methyl geranate, 1189-09-9; geranyl acetate, 105-87-3; geranyl isobutyrate, 2345-26-8; α-caryophyllene, 6753-98-6; α-humulene, 6753-98-6; caryophyllene oxide, 1139-30-6; humulene monoepoxide II, 19888-34-7; humulene monoepoxide III, 21624-36-2.

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Effect of Tissue Disruption on Volatile Constituents of Bell Peppers

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(*Z*)-3-Hexen-1-ol, (*E*)-2-hexenal, hexanol, (*E*)-2-hexen-1-ol, and hexanal were formed after tissue disruption of bell peppers (*Capsicum annuun* Var. grossum, Sendt). The ratio of a total amount of unsaturated C_6 aldehydes and alcohols to a total one of saturated C_6 compounds is 3 in disrupted bell peppers. Stannous chloride showed its enzyme-inhibition activity in bell pepper disruption.

Six-carbon aldehydes and alcohols are important flavor components of fruits, vegetables, and green leaf products, especially when plant tissues are processed (Galliard et al., 1977; Schreier and Lorenz, 1981; Tressl et al., 1981; Hatanaka et al., 1983; Josephson et al., 1984). Lipoxygenase and hydroperoxide lyase are responsible for six-carbon aldehyde formations from C_{18} fatty acids (Hatanaka et al., 1983). Josephson et al. (1984) used stannous chloride to inhibit these enzymic reactions.

In the investigation of volatile constituents of green bell peppers (*Capsicum annuun* Var. grossum, Sendt) by Buttery et al. (1969), they reported 2-isobutyl-3-methoxypyrazine has an extremely potent bell pepper aroma and useful flavoring properties. Several authors (Seifert et al., 1970, 1972; Pittet and Hruza, 1974; Parliment and Epstein, 1973; Buttery et al., 1976; Pelosi et al., 1983) have investigated the relationship of bell pepper odor and low olfactory threshold to chemical structure and have recog-

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