Varietal Differences in the Proportions of Cohumulone, Adhumulone, and Humulone in Hops[1]

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ABSTRACT

Hop α-acids are composed of three major analogues: cohumulone, adhumulone, and humulone. The proportion of cohumulone is a varietal characteristic and ranges from 16 to 50% of the α-acids. The standard deviation of the mean cohumulone ratio of 25 hop varieties analyzed for six years is about 2%. Adhumulone ranges from 6 to 15% of the α-acids, but has greater year-to-year varietal variability than cohumulone or humulone ratio. Losses of α-acids during storage are compatible for all analogues; therefore, the cohumulone content is unaffected. Hop varieties used as kettle hops have higher cohumulone ratios than varieties used as aroma hops. The cohumulone ratio is helpful in varietal identification but cannot be used as the only criterion.

Key words: Adhumulone, Cohumulone, HPLC, Humulone

Hops contain many unusual constituents, but the α-acids are most important to the brewer. α-Acids are converted to bitter tasting, water-soluble iso-α-acids during wort boil. Although α-acid or humulone was first isolated in 1904 (8), Rigby and Bethune discovered in 1952 (12) that the α-acid fraction was a mixture of several similar compounds. In a subsequent paper, the three major α-acids were identified as humulone, cohumulone and adhumulone. They account for 95% of the α-acids (17). The analogues differ only in the acidic side chain, as shown in Figure 1. Two additional analogues, prehumulone and posthumulone, with three and six carbons in the acidic side chain, respectively, were identified later (13). Commercial hop varieties contain from less than 4 to over 14% α-acids, depending upon variety and environmental conditions.

The relative proportion of the cohumulone in α-acids is usually reported as the "cohumulone ratio" or "cohumulone content," representing the percentage of cohumulone in the α-acids. The cohumulone ratio is important for several reasons. Cohumulone is more efficiently utilized in brewing; i.e., the proportion of isocohumulone in wort and beer is higher than the proportion of cohumulone in the hops. This may be caused by increased isomerization of cohumulone or greater losses of the other analogues in brewing and fermentation (9,14). Beer made from hops with low cohumulone ratios has somewhat greater foam stability than beer from hops with high cohumulone ratios (2,14). Rigby (14) suggested that high levels of isocohumulone are responsible for harsh, unpleasant bitterness in beer. High cohumulone ratios were also associated with poor aroma by Zattler (20). All the hop varieties associated with noble aroma, such as Hallertau and Tettnang, have cohumulone ratios of 25 or less (9), whereas varieties such as Cluster and Bullion that are used as general purpose kettle hops have cohumulone ratios of 35–50.

The cohumulone ratio has long been recognized as a varietal characteristic, although there is disagreement on whether environmental conditions can influence the proportion (3,4,9). Usually humulone and adhumulone are not reported individually because of the difficulty of separating them. Adhumulone may be slightly more efficiently isomerized than humulone (9). The adhumulone content may vary from 6 to 23% of the α-acids (7). Although adhumulone is a small proportion of the total, the reported values differ by a large factor.

A number of techniques are used to determine the proportions of cohumulone, adhumulone, and humulone. Counter-current distribution analysis is a lengthy procedure requiring special equipment (13). Various forms of chromatography have been used to separate the analogues, including gas-liquid chromatography of pyrolysis products and derivatives (4,5,11,13) and low-pressure reversed-phase liquid chromatography of hop extracts (16). Nuclear magnetic resonance spectroscopy and proton magnetic resonance spectroscopy have been used but require expensive equipment, and preliminary separation by precipitation is necessary (6,7,10,15). Initial purification is not necessary with high-pressure liquid chromatography (HPLC). Separation of all three analogues can be achieved in 60 min (19), whereas separation of cohumulone from humulone plus adhumulone can be achieved in 20 min (18).

The Agricultural Research Service and Oregon Agricultural Experiment Station maintain a collection of world hop varieties and of new varieties that have been developed through breeding and selection. We have determined the α-acid analogues by several analytical techniques and have up to 20 years of data on some varieties. Brewing value certification of commercial samples is also performed by our laboratory, and the cohumulone ratio has been determined on many commercial samples.

The purpose of the work reported here was to determine if the adhumulone ratio was a varietal characteristic and to measure the variability in the analogue proportions of different hop varieties.

EXPERIMENTAL

Hop samples were machine picked at optimum maturity and dried at 50° C. After equilibrating to approximately 8% moisture content, 600 g samples were pressed into miniature bales (15 × 10 × 20 cm). The bales were sliced into two parts and placed in plastic bags. One sample, "harvest," was stored at −6° C until analysis. The second sample, "storage," was kept at ambient temperature (~20° C) for six months before analysis. Commercial samples were composited from bale cores. Approximately 100 g was ground in a food chopper and mixed well. Ground samples were stored in glass jars at −6° C until analysis.

A 5.0-g sample was extracted 30 min on a shaker with 100 ml toluene containing 1% antioxidant (Antiox 330). Appropriate dilutions were made for spectrophotometric determination of α- and β-acids (1). For rapid HPLC analysis, 2.0 ml of the toluene extract was diluted to 25 ml with methanol. For the complete separation of all three analogues, 1.0 ml of the toluene extract was diluted to 10 ml with glacial acetic acid in methanol (1:1, v/v).

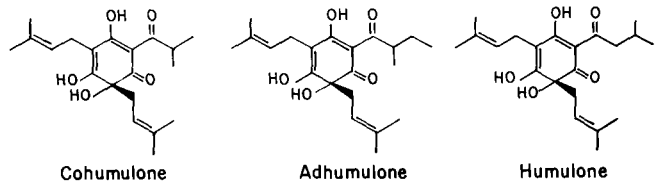

Fig. 1. Structures of cohumulone, adhumulone, and humulone.

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All chromatographic analyses were performed on a Beckman model 420 HPLC equipped with two model 110A pumps, an Altex model 500 autosampler, a Hitachi 110-10 variable wavelength spectrophotometer with flow-through cell, and a Hewlett-Packard 3390A integrator. A 10-μm C18 reversed-phase column (0.45 × 25 cm, Alltech #600RP) was used. A 0.45 × 5 cm precolumn containing 10-μm C18 RP packing material was placed between the injection loop and column. A solvent system composed of methanol, water, and phosphoric acid (85:17:0.25) was used for rapid analysis with 1.0 ml/min flow rate and a column temperature of 40° C (18). The solvent system used for complete separation was sodium acetate buffer (0.04*N*, pH 7.0) mixed with methanol (41:59) with a flow rate of 1.0 ml/min at 40° C. This is a modification of the solvent system used by Verzele et al (19), with less concentrated buffer (0.04*N* instead of 0.2*N* sodium acetate) and acidified sample (G. B. Nickerson and P. A. Williams, *unpublished*). Minor adjustments, less than 1%, in the proportions of methanol and buffer were necessary when new buffer preparations or new columns affected the resolution. The spectrophotometer was set at 334 nm, where the analogues have the same extinction coefficients (19). The proportions were determined directly by integrator counts. Retention times were verified using purified α- and β-acids. Typical chromatograms are shown in Figure 2.

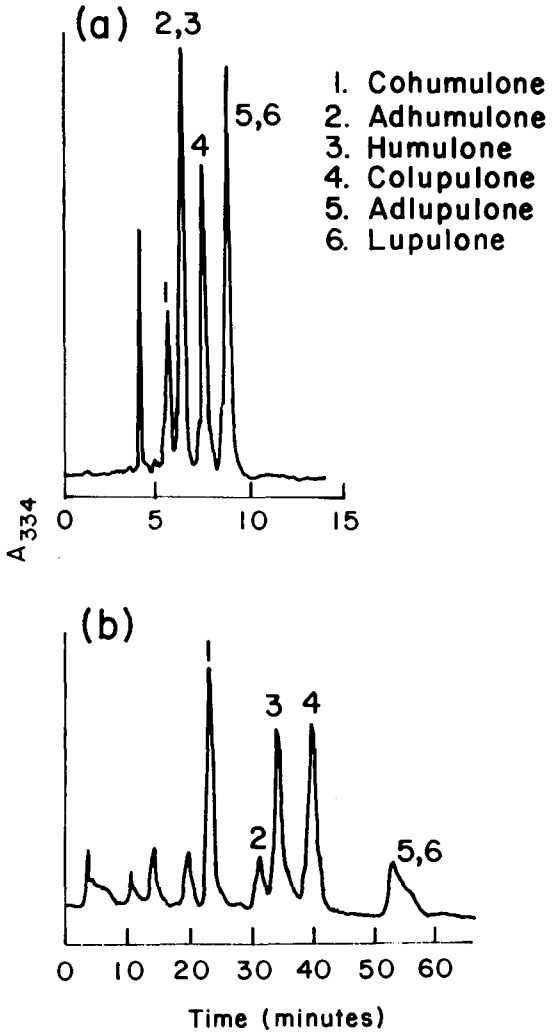

Fig. 2. High-pressure liquid chromatography of hop extracts on a 10 μm C18 reversed-phase column: (**a**) rapid separation with methanol, water, and phosphoric acid (85:17:0.25) at a 1.0 ml/min flow rate; and (**b**) separation of adhumulone from humulone with sodium acetate buffer (0.04*N*, pH 7.0) mixed with methanol (41:59).

RESULTS AND DISCUSSION

There is a direct relationship between cohumulone and humulone, but adhumulone varies in proportion to either cohumulone or humulone (Fig. 3). We found greater year-to-year variation in the adhumulone proportion than for cohumulone or humulone, which is shown by the data for 1982 and 1984 analyses (Table I). There is not the same degree of correlation between the two year's data for adhumulone ($r = 0.544$) as for cohumulone and humulone ($r = 0.876$ and 0.803, respectively). This variability may account for the range of values reported in the literature. Analyses of commercially grown Bullion and Cascade show that the proportions of cohumulone, adhumulone, and humulone are very consistent for a particular environment (Table II). The coefficient of variation (c.v.) is greater for Bullion, an English variety introduced before 1940, than for Cascade, a variety released in 1976. The c.v. for adhumulone is higher for both varieties than the cohumulone or humulone variation. Because the adhumulone proportion does not show the same year-to-year consistency as the cohumulone ratio, we felt justified in using the rapid HPLC procedure that does not separate adhumulone from humulone.

Figure 4 shows the relationship between harvest and storage results for cohumulone ratio and α-acid content for 1982 and 1983 samples. The cohumulone ratio is unaffected by storage, unlike the α-acid content. The α-acid analogues must be oxidized at the same rate.

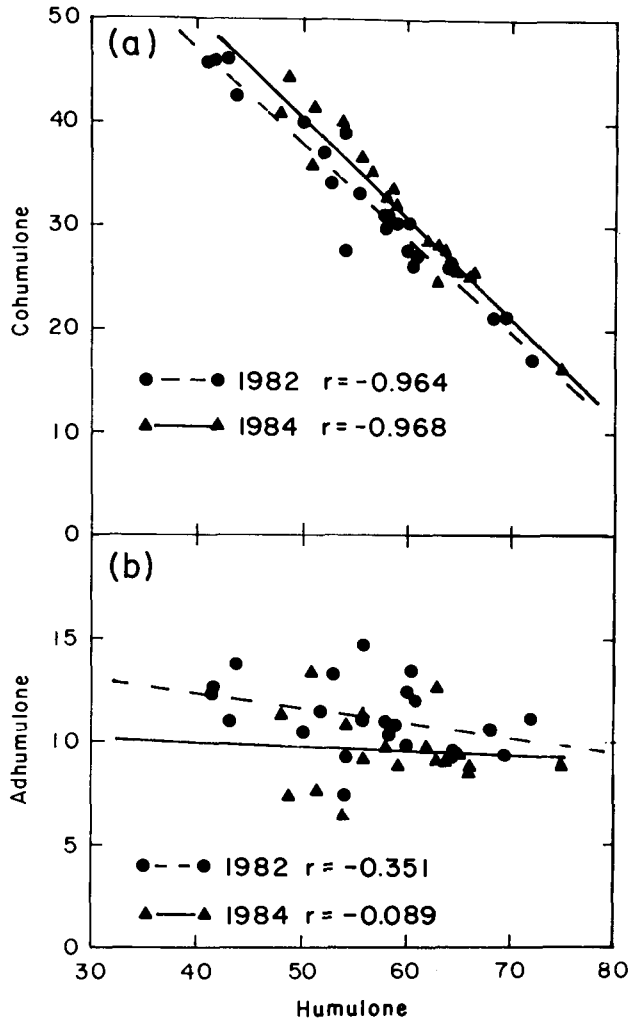

Fig. 3. Relationship between the proportions of (**a**) cohumulone and humulone and (**b**) adhumulone and humulone.

TABLE I
Proportion of Cohumulone, Adhumulone, and Humulone in Hop α-Acids[a]

	% of α-Acids					
	Cohumulone		Adhumulone		Humulone	
Variety	1982	1984	1982	1984	1982	1984
Ahil	26.2	28.1	13.4	9.1	60.4	62.8
Apolon	26.4	32.5	9.3	9.8	64.3	57.7
Blisk	34.2	35.2	13.1	10.8	52.7	54.0
Brewers Gold	45.9	40.8	12.8	11.3	41.4	47.9
Bullion	46.3	···	10.8	···	42.9	···
Cascade	38.8	41.4	7.3	7.5	54.0	51.2
Cluster	39.7	39.9	10.4	6.3	50.0	53.7
Columbia	36.8	···	11.4	···	51.8	···
Dunav	31.4	···	10.3	···	58.3	···
Eroica	45.8	44.2	12.4	7.2	41.4	48.6
Fuggle H	27.2	25.2	12.0	8.8	60.8	66.0
Fuggle N	27.6	25.8	12.4	9.4	60.0	64.9
Galena	42.5	···	13.9	···	43.6	···
Golding	27.6	25.4	9.3	8.6	54.0	66.1
Hallertau MF	16.9	16.3	11.1	8.8	72.0	74.9
Huller Bitter	26.0	27.2	9.6	9.0	64.4	63.8
Neoplanta	30.2	36.4	9.8	9.0	60.0	55.6
Nordgard	21.3	28.5	10.5	9.7	68.2	61.9
Nugget	31.1	24.5	11.0	12.7	58.0	62.9
Perle	33.3	32.1	11.0	8.8	55.7	59.1
Styrian	30.3	32.9	10.8	11.1	58.9	56.0
Tettnang	21.3	···	9.3	···	69.5	···
Willamette	29.6	35.8	14.7	13.4	55.7	50.7

[a] Results of high-pressure liquid chromatography analyses of 1982 and 1984 crop grown at Corvallis, OR.

TABLE II
Proportions of Cohumulone, Adhumulone, and Humulone in Commercially Grown Bullion and Cascade Hops[a]

Variety	Cohumulone	Adhumulone	Humulone
Bullion	43.1	12.4	44.4
	44.3	11.6	44.1
	45.2	12.3	42.6
	45.6	12.9	41.5
	46.1	12.3	41.6
	46.1	13.7	40.1
	44.3	13.5	42.2
	44.0	13.5	42.4
	44.3	11.5	44.3
	41.8	11.6	46.7
Mean	44.48	12.53	42.99
SD	1.351	0.837	1.895
% c.v.	3.0	6.7	4.4
Lots	10		
Cascade	39.2	9.7	51.1
	39.6	9.8	50.6
	40.4	9.3	50.3
	40.5	9.7	49.8
	40.4	9.5	50.0
	39.6	9.7	50.7
	39.9	10.0	50.1
	40.5	9.6	49.9
	39.7	9.6	50.6
	40.4	9.6	49.9
	40.2	9.7	50.2
Mean	40.04	9.66	50.29
SD	0.454	0.175	0.411
% c.v.	1.1	1.8	0.8
Lots	11		

[a] High-pressure liquid chromatography of 1983 crop.

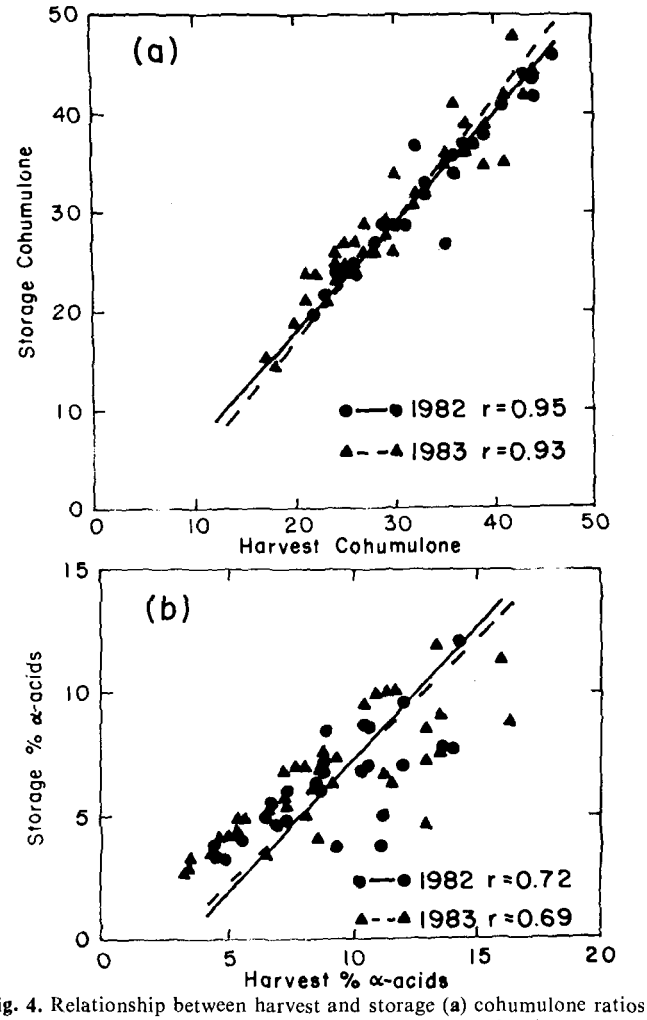

Fig. 4. Relationship between harvest and storage (a) cohumulone ratios and (b) α-acids. Cohumulone ratio determined by rapid high-pressure liquid chromatography (18) and percent α-acids by spectrophotometry (1).

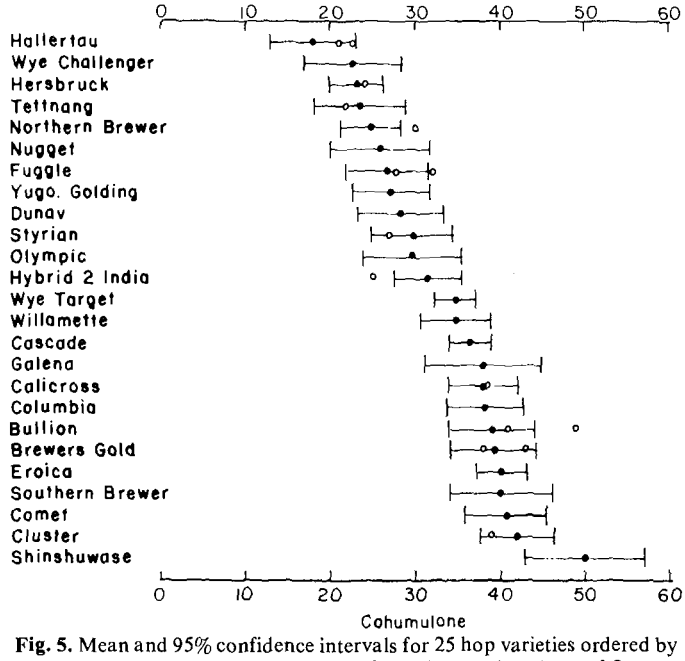

Fig. 5. Mean and 95% confidence intervals for 25 hop varieties ordered by mean cohumulone. Mean cohumulone from six years' analyses of Oregon State University samples, closed circles. Open circles are average values reported by Meilgaard (9).

TABLE III
Year-to-Year Variation in Cohumulone Ratio of 39 Hop Varieties Grown at Corvallis, OR[a]

Variety	Cohumulone Ratio						Mean	SD
	1979	1980	1981	1982	1983	1984		
Backa	26	23	26	···	23	···	24.5	1.73
Brewers Gold	41	37	37	42	40	39	39.3	2.07
Bullion	41	39	37	42	39	37	39.2	2.04
Calicross	40	37	36	38	40	37	38.0	1.67
Cascade	35	36	37	38	36	36	36.3	1.03
Chinook	···	29	36	32	34	33	32.8	2.59
Cluster	41	39	43	43	44	42	42.0	1.79
Columbia	38	37	41	40	38	36	38.2	1.86
Comet	41	39	41	41	44	39	40.8	1.83
Dunav	27	25	30	30	30	27	28.2	2.14
Elsasser	26	22	28	24	···	···	25.0	2.58
Eroica	40	38	40	41	41	41	40.2	1.17
Fuggle H	30	25	25	28	26	26	26.7	1.97
Galena	35	35	37	41	41	39	38.0	2.76
Yugo. Golding	27	29	29	27	27	24	27.2	1.83
Hallertau	···	19	21	22	22	···	21.0	1.41
Hallertau MF	20	18	16	21	17	16	18.0	2.10
Hersbruck	23	21	25	23	23	23	23.0	1.27
Huller Bitter	31	···	29	26	27	26	27.8	2.17
Hybrid 2 India	34	31	32	31	29	32	31.5	1.64
Kirin II	···	···	···	46	42	45	44.3	2.08
Northern Brewer	24	24	26	27	25	23	24.8	1.47
Nugget	25	27	25	30	26	23	26.0	2.37
Olympic	30	28	26	33	31	30	29.7	2.42
Perle	···	···	27	30	27	25	27.3	2.06
Pride of Ringwood	32	30	33	33	27	···	31.0	2.55
Record	21	24	30	26	30	···	26.2	3.90
Shinshuwase	53	48	54	47	48	50	50.0	2.90
Southern Brewer	36	39	41	43	42	40	40.2	2.48
Spalt	···	23	25	25	21	22	23.2	1.79
Styrian	31	27	32	31	29	28	29.7	1.97
Talisman	54	52	50	52	52	···	52.0	1.41
Tettnang	22	24	25	26	24	20	23.5	2.17
Willamette	35	32	37	34	35	36	34.8	1.72
Wye Challenger	25	23	25	22	21	19	22.5	2.35
Wye Northdown	24	23	24	···	24	···	23.8	0.50
Wye Saxon	···	···	···	22	17	···	19.5	3.54
Wye Target	33	35	35	36	34	35	34.7	1.03
Wye Viking	···	···	···	25	23	···	24.0	1.41

[a] Analyses by rapid high-pressure liquid chromatographic method (18).

We have been using the rapid HPLC method for six years, and cohumulone values for that period are summarized in Table III. This table includes commercial varieties and older varieties of mainly historic interest. The samples include seeded and seedless hops grown at Corvallis, OR. When the varieties are ranked by mean cohumulone ratio, the aroma types tend to cluster at the low end of the scale and the kettle hops are at the other extreme, as shown in Figure 5. Figure 5 shows the mean and 95% confidence interval for six years' results on 25 varieties in Table III compared with average values reported by Meilgaard (9) in 1960. The cohumulone ratio is helpful in varietal identification, but the overlapping ranges do not allow absolute verification. Varieties with similar cohumulone ratios differ in the amount and proportion of α- and β-acids. Figure 6 shows the mean and 95% confidence intervals for α-acids, β-acids, and the ratio of α-acid/(α- + β-acid) for the varieties in Figure 5. The cohumulone ratios of Hallertau and Hersbruck are very similar, but Hersbruck generally has a higher proportion of α-acids than Hallertau. In many instances, the combination of cohumulone ratio with the amount and proportion of α- and β-acids is sufficient for varietal identification. With additional information, such as oil composition, it is possible to identify varieties having similar α- and β-acid content and proportions.

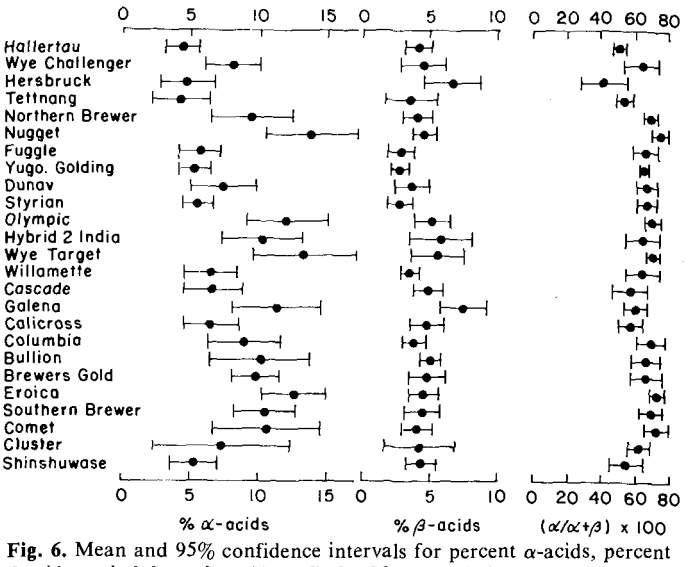

Fig. 6. Mean and 95% confidence intervals for percent α-acids, percent β-acids, and alpha ratio, $\alpha/(\alpha + \beta)$, for 25 hop varieties.

CONCLUSIONS

The cohumulone ratio, the proportion of cohumulone to the total α-acids, is a varietal characteristic. For a specific variety, a range of values may be obtained even under identical environmental conditions. The range of values found in one environment does include all of the values found in the literature. The loss of α-acids during storage does not affect the cohumulone ratio. When cohumulone ratio is combined with other chemical characteristics, hop varieties may be identified with greater certainty.

ACKNOWLEDGMENTS

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