

# Analysis of Hop Bittering Constituents

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## CONCLUSIONS

Stepwise Sephadex® ion-exchange chromatographic (IEC) determination of iso- $\alpha$ -acids in beer showed significant between-laboratory and within-laboratory errors.

## RECOMMENDATIONS

1. Further work should be done on a reference method for the determination of iso- $\alpha$ -acids in beer.

2. The Sephadex IEC methods (gradient and stepwise elution) for the determination of  $\alpha$ -acids in hops and iso- $\alpha$ -acids in isomerized extracts should be considered International Methods because they have been adopted by the European Brewery Convention (EBC).
3. The quantitative determination of seed content in hops (HOPS-2) should be considered an International Method in principle. The EBC has adopted the ASBC "Methods of Analysis" procedure with sample size of 500 g instead of 20 g.

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Last year the subcommittee determined iso- $\alpha$ -acids in isomerized hop extracts by two modifications of the Sephadex IEC method. Enough results were obtained to permit adoption of the stepwise modification (1). However, only four collaborators reported gradient results. After the Annual Meeting, the EBC Analysis

TABLE I  
Estimated Bitterness Units and Iso- $\alpha$ -Acids<sup>a</sup> in Beer

Collaborator	Estimated Bitterness Units				Iso- $\alpha$ -Acids			
	Pair I		Pair II		Pair I		Pair II	
	X	Y	X	Y	X	Y	X	Y
1	14.6	13.5	11.8	11.7	13.0	12.3	13.9	13.4
2	14.6	14.2	12.9	12.3	11.8	11.6	11.9	12.4
3	13.2	12.8	13.6	13.4	10.0	11.9	12.6	8.4
4	10.6	11.4	11.2	10.3	12.5	12.1	15.1	12.9
5	14.7	8.8	10.8	12.1	14.5	8.8	15.5	11.6
6	14.6	14.8	11.6	11.6	10.5	10.5	13.0	12.2
7	14.0	14.7	14.2	13.4	10.4	7.8	10.5	8.9
8	...	...	...	...	11.1	11.5	13.1	11.6
9	11.2	7.9	8.4	9.5	10.7	3.7 <sup>b</sup>	10.6	4.7
10	16.3	15.2	13.2	12.6	12.9	12.6	14.0	13.6
11	13.6	14.8	13.5	14.0	11.5	12.5	10.9	12.1
Mean	13.74	12.81	12.12	12.09	11.82	11.16	12.83	11.07
8 <sup>c</sup>	...	...	...	...	8.8	9.9	14.8	12.7

<sup>a</sup>mg/L.

<sup>b</sup>Outlier by Dixon's test (2).

<sup>c</sup>Results from high-performance liquid chromatography.

TABLE II  
Statistical Summary of Bitterness Units (BU) and Iso- $\alpha$ -Acids in Beer

Analysis	Pair	No. of Labs.	Grand Mean <sup>a</sup>	Error			c.v. <sup>d</sup> (%)	Calculated F <sup>b</sup>	Critical F <sup>c</sup>
				Within-Lab <sup>b</sup>	Between-Lab. <sup>b</sup>	Combined <sup>c</sup>			
BU	I	10	13.23	1.53	1.59	2.21	16.7	3.1590	3.4381
	II	10	12.11	0.55	1.48	1.58	13.0	15.6704	3.4381
Iso- $\alpha$ -acids, mg/L	I	10	11.49	1.50	0.30	1.53	13.3	1.0819	3.4381
	II	11	11.95	1.52	1.67	2.26	18.9	3.4256	3.1789

<sup>a</sup>Grand mean =  $GM = (\bar{X} + \bar{Y})/2$ .

<sup>b</sup>Calculated per Youden and Steiner (4).

<sup>c</sup>Combined error ( $S_c$ ) calculated from within-lab. error ( $S_r$ ) and between-lab. error ( $S_b$ );  $S_c = \sqrt{S_r^2 + S_b^2}$ .

<sup>d</sup>Coefficient of variation of  $S_c = c.v. = 100(S_c/GM)$ .

<sup>e</sup>Critical F from tables of F distribution (3) at  $P = 0.05$ .

Committee sent 12 results determined by stepwise and gradient modifications. They found coefficients of variation of 6.1 and 9.9% for the combined-laboratory error of the stepwise and gradient methods, respectively. The EBC has adopted both of these methods. Therefore an IEC method for the determination of  $\alpha$ -acids in hops and hop products and of iso- $\alpha$ -acids in isomerized extracts has been adopted by both the ASBC and EBC.

This year the subcommittee investigated the determination of iso- $\alpha$ -acids in beer by Sephadex IEC. Collaborators tested the extraction procedure by estimating bitterness units (BUs) on the extract and then determined iso- $\alpha$ -acids by IEC. The subcommittee felt that work on an extraction procedure would be useful not only for IEC analysis but for future high performance liquid chromatography (HPLC) analysis.

## EXPERIMENTAL

Collaborators were sent two pairs of commercial beer samples and asked to extract 200 ml of acidified beer three times with pentane or trimethylpentane. The combined extracts were dried over anhydrous sodium sulfate and evaporated. The residue was dissolved in 1.0 ml of 80% methanol and 0.25 ml was used to estimate BUs. A 0.5-ml aliquot was used for the determination of iso- $\alpha$ -acids by stepwise Sephadex IEC (1). Collaborators were also asked to analyze samples by HPLC if possible.

## RESULTS AND DISCUSSION

Results of the analyses are given in Table I. The absorbance of the extract was used to calculate estimated BUs (except for collaborator 2 who used the ASBC method, BEER-23,A). BUs were expected to be higher than the iso- $\alpha$ -acids determined by IEC, but the experimental results do not show this.

Collaborator 8 also reported HPLC results. Collaborators reported difficulty in getting rid of the emulsion with anhydrous sodium sulfate. One collaborator used Silicone Antifoam A to break up the emulsion. Collaborators criticized the small volume used to dissolve the residue after the extract had been evaporated. One collaborator suggested that the residue be dissolved in 10.0 ml rather than in 1.0 ml. Statistical treatment of the data is summarized in Table II. Combined laboratory errors are large, but this is not unexpected with a new procedure.

## LITERATURE CITED

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