Analysis of Hop Bittering Constituents

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CONCLUSIONS

1. Three methods were tested for solvent extraction of hop bittering substances from beer. Based on Youden Unit Block statistical design (5), the standard ASBC bitterness unit (BU) extraction method (1) gave more consistent results than either the Morton baffled flask stirring method (4) or the separatory funnel extraction method (2).
2. A sufficient number of membership laboratories have high performance liquid chromatographs to justify collaborative trials using this equipment.

RECOMMENDATIONS

1. The ASBC method (BEER-23,A) should be used for extraction of hop bittering substances from beer.
2. The subcommittee should evaluate a high performance liquid chromatographic procedure for determining total BUs and the portion that is iso-α-acids.

This subcommittee planned for several years to develop a single analytical technique that could be applied to raw hops, processed hops, hop extracts (including isomerized and reduced), and to hop bittering principles in wort and beer. Sephadex and Dowex anion exchange chromatographic procedures were selected and subsequently applied to hops and hop products including isomerized extracts. These methods required 1 to 3 mg of hop acid component for satisfactory detection. Consequently, when the techniques were applied to the analysis of iso-α-acids in beer in 1979–1980, it was necessary to extract 100–150 ml of beer. A separatory funnel extraction using pentane as the extracting solvent was unsuccessfully attempted last year (2). This year, a modification of that separatory funnel extraction and the Hansen and Ramos method (4) were tried, as well as the ASBC BEER-23,A method. The first two extractions represent high-volume methods while the later requires a significantly smaller sample.

During the past two years, however, many laboratories have obtained high performance liquid chromatographic equipment. Methodology using this equipment is appearing for analyses of hop bittering constituents in various types of products. Consequently, superior methods may become available that will accept microgram samples, in which case high-volume extraction procedures would be unnecessary.

EXPERIMENTAL

Collaborators were sent two pairs of commercial beer samples and were asked to extract specific aliquots of acidified beer with trimethylpentane (TMP) using three different extraction techniques (the standard ASBC BU method utilized 10-ml aliquots, while the Hansen-Ramos stirring method and the modified separatory funnel method each required 150-ml aliquots). Modifications in the separatory funnel extraction method included: use of TMP instead of pentane, elimination of anhydrous sodium sulfate for drying purposes, and implementation of centrifugation to break emulsions. All resultant mixtures were centrifuged and the TMP extracts were spectrophotometrically analyzed according to BEER-23,A. Collaborators were also requested to analyze samples by HPLC, if possible.

RESULTS AND DISCUSSION

Results of the analyses are given in Table I. The UV spectrophotometric absorbance values of the extracts were used to calculate the estimated BUs. Some collaborators reported minor

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TABLE I
Estimated Bitterness Units Found by Three Extraction Procedures

Collaborator	Beer-23,A				Stirring Method				Separatory Funnel Method			
	Pair I		Pair II		Pair I		Pair II		Pair I		Pair II	
	A	B	C	D	A	B	C	D	A	B	C	D
1	13.5	13.5	14.5	14.0	12.5	13.5	14.2	14.0	13.4	13.0	14.9	14.4
2	12.4	13.3	15.5	15.5	11.2	11.2	13.0	11.2	12.3	10.8	13.0	13.0
3	11.8	12.1	14.5	14.7	13.8	13.0	14.3	14.2	13.6	13.2	14.9	14.8
4	12.5	12.3	15.0	14.7	12.1	12.3	14.1	13.8	13.4	13.5	15.0	15.2
5	12.7	11.8	13.4	13.8	12.5	11.4	13.3	14.2	...	...	...	...
6	14.8	13.4	17.0	14.7	...	...	...	...	11.1	10.2	13.1	12.6
7	13.0	12.4	14.6	15.4	12.7	11.8	14.5	...	11.6	12.4	14.1	14.7
8	12.7	12.2	13.8	14.1	10.2	11.2	11.5	10.7	11.2	9.6	11.1	12.0
9	14.2	15.2	15.8	15.6	12.8	14.2	15.2	14.4	11.4	14.0	17.2	17.6
10	11.8	11.2	13.9	11.6	10.8	9.9	11.7	8.9	12.3	10.8	12.4	11.3
11	13.9	12.1	14.1	14.0	11.1	10.7	10.9	12.2	...	...	...	...
12	12.7	12.6	13.9	13.8	11.5	10.1	12.8	12.5	9.9	10.0	9.2	8.3
13	13.5	13.5	15.1	14.1	12.2	10.9	13.4	10.5	13.9	13.9	16.6	17.3
Mean	13.04	12.74	14.70	14.31	11.95	11.68	13.13	12.42	12.19	11.95	13.77	13.75

TABLE II
Statistical Summary of Results

Extraction Method	Pair	No. of Labs.	Grand Mean[d]	Error: Within-Laboratory[b]	Error: Between-Laboratory[b]	Error: Combined[c]	c.v.[d] (%)	Calculated F[b]	Critical F[e]
BU[f]	I	13	12.89	0.57	0.78	0.97	7.5	4.77	3.28
	II	13	14.50	0.67	0.75	1.01	7.0	3.49	3.28
Stirring[g]	I	12	11.82	0.69	0.96	1.18	10.0	4.92	4.46
	II	11	12.78	0.95	1.32	1.63	12.7	4.89	4.85
Separatory funnel[h]	I	11	12.07	0.86	1.21	1.49	12.4	4.96	4.85
	II	11	13.76	0.47	2.48	2.52	18.3	57.19	4.85

[a] Grand mean = GM = $(\bar{A} + \bar{B})/2$ or $(\bar{C} + \bar{D})/2$.
[b] Calculated per Youden and Steiner (5).
[c] Combined error (S_c) calculated from within-laboratory error (S_r) and between-laboratory error (S_b); $S_c = \sqrt{S_r^2 + S_b^2}$.
[d] Coefficient of variation of S_c = c.v. = 100 (S_c/GM).
[e] Critical F from tables of F distribution (3) at $P = 0.05$.
[f] Bitterness unit determination (1).
[g] Morton baffled flask stirring method (4).
[h] Slight modification of last year's extraction method (2).

difficulties with emulsions in the Morton flask stirring method while many collaborators encountered more severe emulsion problems with the separatory funnel procedure. No handling problems were noted with the ASBC BEER-23,A method. Statistical treatment of the data is summarized in Table II. Within-laboratory errors were consistently less than the between-laboratory errors. The BEER-23,A method performed better than the other two methods studied: it gave lower within-laboratory errors, indicating superior precision; lower between-laboratory errors, indicating less bias; and substantially lower c.v. values (Tables I and II). No HPLC data were received.

LITERATURE CITED

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