Studies on Hop Analysis

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ABSTRACT

Modifications to the standard procedures of the American Society of Brewing Chemists for the analysis of hops and hop pellets are presented. Improvements over existing methods are clearly outlined.

Key words: Alternative solvents, Modifications, Sample preparation, Sampling, Standard procedures

Methods recommended by the American Society of Brewing Chemists contain complete descriptions of well-tested procedures for the analysis of brewing raw materials and brewery products. Collaborative tests of these methods are done prior to acceptance. Laboratories may modify the individual methods because new equipment becomes available or because they need to analyze large numbers of samples. This paper presents specific modifications in sampling of hops and hop pellets, preparation of hops and hop pellet samples, sample dilution by a commercially available auto-diluter, use of a microprocessor-controlled, double-beam recording spectrophotometer, methods of spectrophotometric calibration, and information on alternative solvents for use in spectroscopy.

EXPERIMENTAL

Sampling of Hops and Hop Pellets

The method presented in ASBC "Methods of Analysis" Hops-1 (1) suggests using a 25-mm × 25-cm resin sampler and taking two cores from each bale sampled. Lots are sampled at the rate of 10% when the total number of bales is less than 100, and at the rate of the square root of the bales if the number of bales exceeds 100. A sample taken in this fashion from a lot of 100 bales could weigh up to 0.5 kg. Only 50–75 g of the total sample is actually ground. Proper sampling is a very important part of evaluating hops and hop pellets. Our technique takes 10 20-g core samples (using a coring tool 3 × 18 cm) from each lot of hops up to 100 bales. Lots are subdivided so that no 10-core sample represents more than 100 bales. The hop samples are placed in a polyethylene bag (8 × 4 × 18 in., volume about 7.5 L). The size of the bag is very important to proper sample preparation.

Sampling of hop pellets is most effectively done on a continuous basis during production. A collection device allows removal of a random sample during filling of cartons from an overhead hop pellet storage bin. About 20 lb of pellets is collected from each pallet of hop pellets produced (880 lb). A Jones ore splitter, available from scientific supply houses, is used to reduce the sample to about 1 lb for laboratory use. This method is comparable to the ASBC method, which suggests removing 200 g from each 10th box. This technique is adequate for sampling hop pellets received in a shipment to a brewery, but is not considered adequate for production quality control.

Sample Preparation

As previously mentioned, the ASBC method (1) suggests that a small amount of the total hop sample be ground as a representative sample. Because hop lupulin tends to separate in a sample container, it is very important that the entire hop sample be

ground. We use a no. 3 Universal food chopper, adapted to an electric motor run at a reduced speed of about 300 rpm. The cores are ground twice using a six-tooth grinder head. A six-tooth grinder is more versatile than the three- or 12-tooth heads suggested for use by ASBC; the grind from the three-tooth cutter is too large for proper mixing, and the 12-tooth grinder head is often plugged because of the small openings, particularly when grinding older hops. The rapid speed of the grinder allows a 200-g sample to be ground twice in less than 2 min.

After grinding, the ground hops are replaced in the 8 × 4 × 18 in. bag, inflated, and mixed by tumbling for 1 min. Samples of 5.00 g are weighed directly into 250-ml Erlenmyer flasks on a digital electric scale accurate to 0.01 g. Solvent is added by 100-ml dispensing pipette, and flasks are sealed with no. 5 polyethylene stoppers and shaken for 30 min using a Burrell Wrist-Action shaker.

The composite of hop pellets is placed in an open flat container. About 100 g are selected randomly and placed in a 1-pint Osterizer container. An Osterizer Liquifier-Blender is used at highest speed to grind the pellets for two periods of 30 sec each, mixing between each grind. The ground hop pellet material is weighed into a 250-ml widemouthed polyethylene bottle (4). Seven Burundum grinding cylinders are placed in the jar; these aid the final comminution of the hop pellets. Solvent is added as for hop samples. A model RX-24 Tyler portable sieve shaker, modified to hold eight samples in two layers of four, is used to agitate the samples.

Dilution of Samples

The spectrophotometric method of analysis of hops and hop products requires a dilute solution of hop constituents. The double-dilution method (2) requires 200 ml of methanol per sample. The most widely used method of dilution is done by the use of a microliter pipette. The proposed method uses only 10 ml of methanol. Hand-held pipettors or dispensers that deliver a preset, fixed volume of sample are also used.

For reasons of speed, precision and economy, use of a microliter syringe diluter is suggested. The Hamilton digital diluter/dispenser is one example of a precise liquid delivery system. It can be adjusted to a wide range of volumes selected by digital thumb switches. This machine consists of two syringes, each independently driven by step-motors. When used as a diluter, a gas-tight syringe, adjustable from 50 μl to 25 ml, is mounted on the left or diluent side. On the right or sample side, a gas-tight syringe adjustable from 50 μl to 5 ml is mounted. Volumes are set by turning the thumb switches, which indicate a percentage of the total volume of the corresponding syringe. The movement of the syringe plungers is not limited by mechanical switches but is controlled digitally by C-MOS logic circuits. The extreme simplicity of operation enables fast, error-free use with a minimum of training. Accuracy of the digital diluter is demonstrated in Figure 1. A near linear relationship exists between the dilution and resulting absorbance.

Table I shows results of diluting a hop sample 10 times each by double dilution and by digital diluter. Dilution by the digital diluter is at least as precise as the ASBC double-dilution method.

In our method of using the digital diluter, only 10 ml of methanol per sample is used. This is one-tenth the amount of methanol used in the microliter dilution methods and one-twentieth the amount used in double dilution. For a laboratory analyzing 4,000 or more hop samples per year, the savings of HPLC methanol will almost pay for the digital diluter.

Spectrophotometry

Proper sampling, preparation, and dilution lead to the final step in analysis of hops and hop products—measurement of the bittering constituents by spectrophotometry. The spectrophotometer used in hop laboratories today has undergone many improvements since the days of the Beckman D.U. A typical instrument, such as the IBM 9420 used in this study, is a UV-vis double-beam spectrophotometer, designed for very accurate measurement of transmittance, absorbance, and concentration of substances. Major features include automatic self-calibration on startup, self-diagnosis of component failures, and computer setting of selected wavelengths. A quick-flow sampler is used for rapid measurement of multiple samples. A built-in thermal printer/plotter is used to obtain a permanent record of spectral data and operating conditions, and a high-resolution CRT display provides prompting during parameter entry, and data display.

A holmium oxide filter is recommended in the ASBC methods (3) to check accuracy of wavelength setting. The filter has discreet absorbance maxima in the ultraviolet and visible region. These filters allow detection of a peak at a specified wavelength but do not allow measurement of maximum absorbance. This filter can be of some value, but recent developments in wavelength calibration are far superior.

The National Bureau of Standards (NBS) has developed a standard reference material (SRM) 2031 metal-on-quartz filter (5). These filters, available from NBS, are recommended for use as a standard to verify accuracy of spectrophotometers from 200 to 800 nm. Each set consists of three filters with nominal transmittances of 10, 30, and 90%. Each individual filter is measured with a spectrophotometer, which is the primary transmittance standard of the NBS. A certificate accompanying the filter set gives transmittance values at 10 wavelengths from 250 to 635 nm.

We use a simple and inexpensive method for checking spectrophotometer accuracy on a daily basis. A solution of 60 mg/L of NBS potassium dichromate in 0.01 *M* perchloric acid medium kept in an amber bottle in dark cupboard provides a liquid reference standard. Table II shows the relative stabiiity of the standard in this laboratory over a nine-month period.

Toluene in alkaline methanol can also serve as a source of information concerning the accuracy of a spectrophotometer. The instrument is started up with alkaline methanol in the sample cell. After completion of initialization and base-line setting, an appropriate blank solution of toluene in methanol is placed in the sample cell and measured at the three appropriate wavelengths.

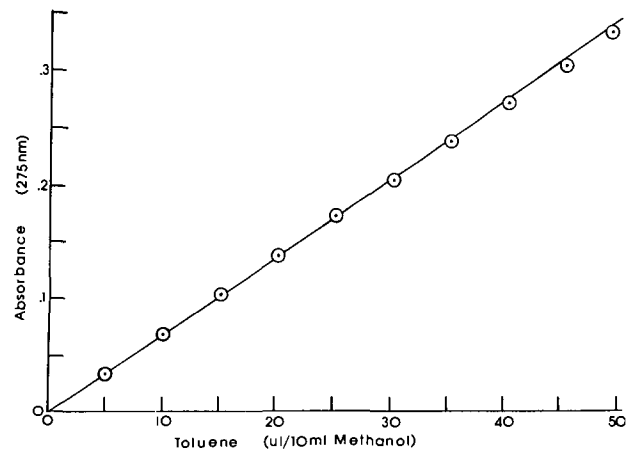

Fig. 1. Demonstration of linearity of the digital microliter diluter.

TABLE I
Double Dilution Versus Digital Dilution

	α-Acid Values of 10 Repetitions	
Parameter	**Double Dilution**	**Digital Dilution**
Mean	11.3232	11.4005
Range	11.2403–11.3894	11.3603–11.4681
SD	0.04215	0.0322
c.v., %	0.37224	0.28244

The absorbance at 275 nm is substracted from each hop sample absorbance at 275 nm before calculation of α- and β-acids. The absorbance at 275 nm by toluene in methanol is very consistent if dilutions are made with great care. This can serve as an additional standard to check spectrophotometers. Results of 389 checks over nine months are given in Table III.

The ASBC Hop Analysis Check Service is available to check analytical performance of a laboratory compared with that of all subscribers. For a fee each member receives samples of hop and hop products on a monthly basis. The samples are analyzed using ASBC procedures, and results are sent to the manager of the service who makes a monthly report to the service subscribers. This report lists the results of each coded collaborator including mean, standard deviation, and coefficient of variance for each parameter. Table IV shows a comparison between this laboratory and other participating laboratories over a seven-month period of the 1985–1986 check service year.

TABLE II
Daily Potassium Dichromate Test

	Absorbance Averages of 91 Repetitions		
Parameter	**355 nm**	**325 nm**	**275 nm**
Mean	0.624	0.395	0.691
Range	0.622–0.628	0.393–0.398	0.687–0.695
SD	0.00142	0.000973	0.00169
c.v., %	0.228	0.246	0.245

TABLE III
Toluene Dilution Tests, 389 Repetitions

Parameter	Absorbance at 275 nm
Mean	0.172
Range	0.166–0.178
SD	0.0032
c.v., %	0.1860

Alternative Extraction Systems

Toluene has been the standard solvent for extraction of hops and hop products for many years. Use of both toluene and benzene was accepted at one time, but benzene was eliminated when it was found to be hazardous to health.

Toluene, with its ultraviolet cutoff point of 290 nm, can cause problems when used as an extractant for samples analyzed by high-pressure liquid chromatography. A very large toluene peak, seen in Figure 2, interferes with early eluting compounds at 280 nm. Other solvents, such as pentane, elute earlier and have much less absorption than toluene.

Hops and hop products extracted with a solvent system of 100 ml of pentane and 5 ml of 0.1*M* acetate buffer, pH 4.0, yield amounts of α- and β-acid comparable to that extracted with

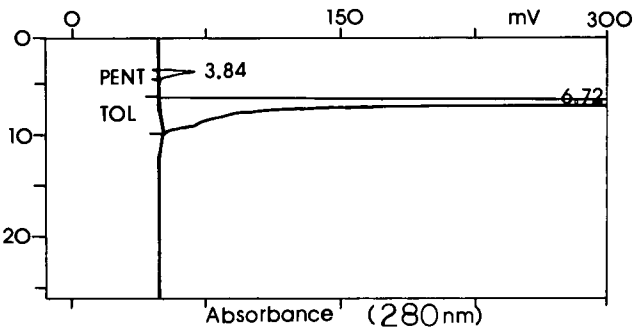

Fig. 2. High-pressure liquid chromatogram of 10% pentane and 10% toluene in methanol. Column: 25 cm × 4.6 mm, Nucleosil 5 μm C18; mobile phase: MeOH/H_2O/H_3PO_4 (85%), 85:15:0.25; flow rate: 1 ml/min; injection volume: 10 μl; attenuation: 2.

TABLE IV
1985–86 Hop Analysis Check Service

	Sample Number						
Parameter	**1**	**2**	**3**	**4**	**5**	**6**	**7**
% α-Acids							
mean	7.21	6.55	41.47	11.15	19.90	41.57	13.47
s	0.56	0.33	0.96	0.28	0.29	1.39	0.44
mean + *s*	7.77	6.88	42.43	11.43	20.19	42.96	13.91
mean − *s*	6.65	6.22	40.51	10.87	19.61	40.18	13.03
reporting lab[a]	7.2	6.6	41.6	11.0	20.3	41.3	13.5
% β-Acids							
mean	2.27	4.77	31.27	7.67	13.64	26.44	6.86
s	0.31	0.12	0.79	0.22	0.39	1.05	0.21
mean + *s*	2.58	4.89	32.06	7.89	14.03	27.49	7.07
mean −*s*	1.96	4.65	30.48	7.45	13.25	25.39	6.65
reporting lab	2.2	4.6	32.3	7.4	13.4	26.0	6.8
HSI[b]							
mean	0.595	0.321	0.306	0.265	0.253	0.365	0.260
s	0.035	0.013	0.018	0.014	0.011	0.018	0.014
mean + *s*	0.630	0.334	0.324	0.279	0.264	0.383	0.274
mean − *s*	0.560	0.308	0.288	0.251	0.242	0.347	0.246
reporting lab	0.62	0.31	0.30	0.25	0.24	0.36	0.26
C.V.[c]							
mean	9.13	6.62	40.96	11.27	20.49	41.77	13.86
s	0.40	0.33	1.78	0.08	0.23	0.68	0.60
mean + *s*	9.53	6.95	42.74	11.35	20.72	42.45	14.46
mean − *s*	8.73	6.29	39.18	11.19	20.26	41.09	13.26
reporting lab	9.2	6.8	42.8	11.3	20.8	42.8	14.3

[a] Reporting lab = S. S. Steiner, Inc. Laboratory.
[b] HSI = Hop storage index.
[c] C.V. = Conductometric value.

toluene. Care must be taken to avoid evaporation of the pentane during sample handling.

The use of toluene to extract samples for analysis by spectrophotometry also requires care in the preparation of blank solutions. Just 25 μl of toluene in 10 ml MeOH has an absorbance of about 0.170 at 275 nm; the same concentration of pentane solution has an absorbance of 0.000. Use of pentane as an extractant causes a slight lowering of the hop storage index values of hops and hop products.

CONCLUSIONS

This study showed that laboratories that do not follow ASBC procedures exactly can still produce acceptable results. Modifications of methods are often necessary to enable laboratories to analyze more samples in less time and at lower cost.

Reducing the use of methanol by a factor of 10 is of great importance. The amount of money saved on purchase of solvent and the reduction of waste material is very substantial.

This study showed that no sacrifice is made of accuracy or precision, in fact, improvement of such may be realized by use of these modifications.

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