Development of Near-Infrared Calibrations for Hop Analysis

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ABSTRACT

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Two approaches were examined for the preparation of near-infrared (NIR) calibrations for the prediction of α-acids content, β-acids content, and hop storage index (HSI) in baled hop samples. In the first approach, NIR calibrations were developed using a hop sample calibration set that was representative of the full analytical range of values for each constituent (α-acids: 1.86–19.57%; β-acids: 1.45–11.58%; and HSI values: 0.220–0.480). In the second approach, hop samples were segregated by α-acids content before calibration development. NIR calibrations were produced from calibration sets with high (9–20%), medium (6–10%), and low (1–7%) α-acids contents. For α-acids predictions, the overall performance of the full-analytical-range α-acids calibration (first approach) was comparable to calibrations prepared using either the high- or medium-α-acids-range calibration sets. The standard error of cross validation (SECV) and coefficient of determination (r^2) values for the full-analytical-range α-acids calibration were 0.31% and 0.99, respectively. The α-acids calibration prepared from the low-α-acids-range set had the best overall performance statistics of all the α-acids calibrations developed. The SECV and r^2 values for that calibration were 0.22% and 0.97, respectively. NIR calibrations for β-acids and HSI were not substantially improved when calibrations were produced using hop samples segregated into high, medium, and low α-acids ranges. The SECV and r^2 values for the full-analytical-range ß-acids and HSI calibrations were 0.20% and 0.99, and 0.010 and 0.89, respectively.

Keywords: α-Acids, β-Acids, Hop storage index, Near-infrared spectroscopy

RESUMEN

Se han evaluado dos alternativas en la construcción de curvas de calibrado para la predicción del contenido en α-ácidos, en β-ácidos y del índice de almacenamiento del lúpulo (HSI) en muestras de lúpulo en fardos mediante espectroscopía en el infrarrojo cercano (NIR). En el primer procedimiento, las curvas patrón para el NIR se construyeron empleando un conjunto de muestras de lúpulo para el calibrado que abarcaba todo el rango de valores analíticos para cada parámetro (α-ácidos: 1,86-19,57 %; β-ácidos: 1,45-11,58 %, y valores del HSI entre 0,220-0,480). En el segundo método, las muestras de lúpulo, antes de ser empleadas en la calibración, se agruparon de acuerdo con su contenido en α-ácidos. Se construyeron curvas patrón para cada uno de los grupos de calibración, en función de que su contenido en α-ácidos fuera alto (9-20 %), medio (6-10 %) o bajo (1-7 %). En la predicción del contenido en α-ácidos, los resultados globales que se obtuvieron empleando en el NIR la calibración que abarcaba todo el rango de valores analíticos para los α-ácidos (primera alternativa), fueron similares en comparación con los resultados obtenidos utilizando cualquiera de las curvas patrón construidas a partir de los grupos de calibración que abarcaban tanto el rango de α-ácidos alto como el medio. Los valores correspondientes a la desviación estándar de la comprobación (SECV) y al coeficiente de correlación (r^2) para la calibración que abarcaba todo el rango de valores analíticos para los α-ácidos, fueron de 0,31 % y 0,99 , respectivamente. Las curvas patrón construidas a partir del grupo de calibración que abarcaba el rango de contenidos bajos en α-ácidos, presentaron el mejor conjunto de resultados, en cuanto a los parámetros estadísticos se refiere, de entre todas las calibraciones para los α-ácidos. Los valores del SECV y del r^2 para dicha calibración fueron de 0,22 % y 0,97 , respectivamente. Las curvas patrón para el NIR correspondientes a los β-ácidos y al HSI, no mostraron una mejora significativa cuando las muestras de lúpulo utilizadas para la calibración se agruparon en intervalos según sus contenidos, alto, medio y bajo, en α-ácidos. Los valores de la SECV y del r^2 para las calibraciones que abarcaban todo el rango de valores analíticos de los β-ácidos y del HSI, fueron de 0,20 % y 0,99 , y de 0,010 % y 0,89, respectivamente.

Palabras clave: α-ácidos, β-ácidos, Índice de almacenamiento del lúpulo, Espectroscopía en el infrarrojo cercano

Hops have long been a key ingredient in the production of beer (15). Among their many contributions to finished beer quality, a characteristic bitterness is one of the unique traits of a hopped beer. The principal precursors of the beer bittering compounds are the hop α-acids (15). The ability to determine α-acids concentrations in hop products by accurate, reliable, and cost-effective means is of fundamental importance to the hop and brewing industries.

A number of analytical techniques have been developed for determining α-acids concentrations in hops. The methods most often employed by the hop and brewing industries for α-acids quantification are based on lead conductance, ultraviolet (UV) spectrophotometry, and high-performance liquid chromatography (HPLC) (1,9,12). In North America, the UV spectrophotometric method, as outlined by the American Society of Brewing Chemists (ASBC), has commonly been the method of choice (1).

In addition to α-acids quantification, the UV spectrophotometric method also provides a measure of β-acids concentrations and the degree of deterioration of α-acids and β-acids in hops. Deterioration of hop bitter acids can be quantified through the calculation of the hop storage index (HSI) value. The HSI value is simply a ratio of absorbance values ($A_{275\ nm}/A_{325\ nm}$) determined for a hop toluene extract diluted with alkaline methanol (1,13). The deterioration of hop acids increases with improper storage or handling practices. Even when hops are properly handled, HSI values gradually increase as hops age.

Although the UV spectrophotometric method of hop analysis does provide valuable information on hop quality, a number of disadvantages are associated with its use. The UV spectrophotometric method is time-consuming, requires a considerable degree of technical training to perform analyses, and requires the use of organic solvents.

Near-infrared (NIR) spectroscopy has been used for rapid analytical determinations for a wide variety of food and agricultural products (8,16). NIR analysis employs energy in the near-infrared region of the electromagnetic spectrum (750–2,500 nm) for predicting the quantity or quality of organic constituents in a product of interest. NIR analysis has distinct advantages over other analytical techniques. It is fast, easy to perform, requires little or no sample preparation or operator training, and is chemical-free. In addition, a single NIR absorbance spectrum obtained from a sample can produce analytical data pertaining to more than one constituent of interest for that sample (simultaneous multiconstituent analysis). The application of NIR technology for predicting UV spectrophotometric hop analysis provides a means of obtaining desired information on hop quality without the analytical disadvantages associated with the UV method.

A number of published accounts have described NIR calibrations developed for a variety of hop analyses (4,5,7,11,14,17). In the majority of these accounts, the efforts directed toward calibration development had met with success. Most of the calibrations

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described were developed using lead conductance and/or HPLC as the reference laboratory method. The only report of UV spectrophotometric methodology being used as the reference method was by Axcell et al (5). In this article, calibrations were described for β-acids and HSI determinations but not for α-acids determinations.

The objective of the present study was to determine whether NIR calibrations of suitable analytical performance could be prepared for the determination of α-acids concentrations, β-acids concentrations, and HSI values in North American hops. The ASBC UV spectrophotometric method of hop analysis was selected as the reference laboratory method for this study. Particular emphasis was placed on developing an optimal calibration for α-acids predictions, as this is the constituent of greatest analytical importance for the hop and brewing industries.

EXPERIMENTAL

Hop Sample Database

Probe samples from hop bales were the source of hop material for this study. The bales were composed of dried hop cones produced by hop farms in the United States in the states of Washington, Oregon, and Idaho. A total of 3,014 probe samples from 30 hop varieties were collected from the 1994–1998 crop years. The hop varieties sampled included representation from the major American hop varieties: Cascade, Columbus/Tomahawk, Chinook, Cluster, Chelan, Nugget, Galena, and Willamette. Reference laboratory analyses and NIR spectral data were collected for all hop samples.

Reference Laboratory Hop Analysis

All hop samples used for developing NIR calibrations were analyzed using the UV spectrophotometric method as described by the ASBC (1). α-Acids and β-acids determinations were performed according to the procedure outlined under method Hops-6A. HSI determinations were performed in accordance with method Hops-12. The following paragraph provides an overview of how the analyses were performed.

A composite sample (200–500 g), taken from hop bales, was prepared for analysis by grinding the sample using a universal no. 3 grinder with a six-tooth cutter. Within 1 hr of grinding, a moisture detector (model G-34, Delmhorst Instrument Co., Towaco, NJ) was used to perform a rapid moisture determination on the ground hop sample to ensure that moisture content was within the range of 8–10%. Immediately following grinding, a 5-g aliquot of ground hop material was extracted with 100 ml of toluene for 30 min on a mechanical shaker. Each sample was then centrifuged at 2,000 rpm for 5 min. A 5-ml aliquot of the clarified toluene extract was then diluted with 100 ml of methanol (dilution A). An alkaline methanol solution was used to further dilute dilution A such that absorbance measurements could be made within the range of 0.2–0.8 absorbance units at wavelengths corresponding to 355, 325, and 275 nm. A set of equations was then used to calculate the α-acids concentration (%), the β-acids concentration (%), and the HSI value for the sample. The α-acids concentrations, β-acids concentrations, and HSI values were not corrected for moisture and were recorded for the hops on an "as-is" basis. All analyses were performed in duplicate.

Near-Infrared Spectrophotometric Hop Analysis

A spectrophotometer (model 5000, Foss-NIRSystems, Silver Spring, MD) in the reflectance mode, was used for the collection of NIR spectral data for hop samples. Diffusely reflected energy from hop samples, in 2-nm increments, was collected over a wavelength range of 1,100–2,498 nm. The spectral data collected were recorded in the form of the logarithm of reciprocal reflectance [log (1/*R*)].

As part of the sample preparation procedure for laboratory analysis, hop samples were ground using a universal no. 3 grinder. For NIR analysis, ~4 g of these coarsely ground hop samples were subjected to an additional grinding for 10 sec using a household (Braun, Woburn, MA) coffee grinder. A portion of the finely ground hop material from the coffee grinder (1–2 g) was then loaded into each of two "small ring cup" sample cells (Foss-NIRSystems). NIR reflectance data, obtained from both sample cells, were used to calculate an average spectrum for each hop sample, which was recorded in the form of log (1/*R*).

Chemometrics

Software (NIRS 2 version 3.0, Infrasoft International [ISI], Port Matilda, PA) was used for near-infrared spectral data collection, spectral processing, and calibration development. The software was used to compile NIR spectra data and reference laboratory data for all samples in the hop sample population. The ISI algorithms CENTER and SELECT (18,20,21) were used to define and reduce the hop sample population before calibration development. However, before the application of CENTER and SELECT, spectral data for each of the hop samples, recorded in the form of log (1/R), underwent a series of mathematical treatments. First, standard normal variate transformation (SNV) and detrend algorithms were applied to the spectral data (6,20). These algorithms enhance the differences in spectra related to the chemical composition of samples by reducing differences in spectra related to physical characteristics of the sample (primarily particle size). Next, the first derivative of the spectral data was taken using a four-data-point gap and a four-data-point smooth (running average). Finally, the number of spectral data points associated with each sample was reduced from 700 (1,100–2,498 nm, absorbance readings of every second nanometer) to 173 (1,108–2,492 nm, every eighth nanometer) before further spectral processing.

Spectral data for each hop sample in the population, refined and reduced to 173 data points, was then processed using CENTER and SELECT algorithms. Both CENTER and SELECT use principal component analysis to condense the spectral information to a set of scores (eigenvalues). The scores are then used to calculate a standardized *H* distance (Mahalanobis distance) for each sample. Relative *H* distances, calculated for all hop samples in the population, were used to define and reduce the population.

The CENTER algorithm is used to rank the spectrum of samples according to their *H* distance from the average scores calculated for the population. Standardized *H* distance values calculated by CENTER are called the "global *H*" values. The smaller the global *H* value for a sample, the closer the sample's spectrum resembles the "average" spectrum for the population. Samples with global *H* values greater than 3 (a limit suggested by the software manufacturer) were considered to be outliers. These samples were eliminated from the sample population and, consequently, from further spectral processing.

Following CENTER and the removal of outliers, the remaining hop spectral data were processed using the SELECT algorithm. The SELECT algorithm removes what are deemed to be "redundant" spectra/samples from the population before calibration development. Unlike CENTER, the SELECT algorithm uses principal component scores to calculate a Mahalanobis distance between two sample, not between a sample and the average scores for a population. Standardized Mahalanobis distances calculated by SELECT are called "neighborhood *H*" values. The degree of spectral redundancy in the population is determined by the choice of a specific neighborhood *H* cut-off, or desired minimum *H* distance between samples. The lower the neighborhood *H* cut-off, the more samples retained in the population and thus the more samples carried forward for calibration development.

Following SELECT, a modified form of the partial least square regression (MPLSR) technique developed by Wold et al (23) and modified by Shenk and Westerhaus (19) was used for calibration development for the hop constituents α-acids, β-acids, and HSI. The same mathematical treatments that were applied to the spectral data before CENTER and SELECT (SNV and detrend, derivatization, and selection of 173 data points) were applied to spectral data before MPLSR of the calibration data. The number of terms to be included in each of the calibration equations was determined by the lowest standard error of cross validation (SECV) that was obtained (22).

Preparation of Calibration and Validation Sets

Full-analytical-range (FAR) calibration and validation sets. The hop sample population contained 3,014 samples before spectral processing using the CENTER algorithm. The CENTER algorithm sorts the samples from lowest to highest global *H* value, and samples with global *H* values >3 (outliers) were removed from the sample population. Following the removal of outliers, a subset of 289 samples, representative of the entire range of global *H* values, was selected for a FAR validation set (i.e., every 10th sample was selected from the sample population for the FAR validation set). Samples in the FAR validation set were transferred to a separate computer file and were not used for calibration development. This "independent" sample set was later used to evaluate the performance of the NIR calibrations developed. The composition of the FAR validation set can be found in Table I.

Following CENTER and the removal of the validation set, the 2,646 samples remaining in the population were processed using the SELECT algorithm. For this study, a neighborhood *H* value of 0.3 was chosen as the cut-off value for SELECT. The SELECT algorithm reduced the size of the population to 1,320 samples, which formed the FAR calibration set. This set was representative of the entire analytical range for each hop constituent (α-acids, β-acids, and HSI values) examined during this study. The composition of the FAR calibration set can be found in Table I.

High-alpha-range (HAR), medium-alpha-range (MAR), and low-alpha-range (LAR) calibration and validation sets. Calibration and validation sets containing samples with high, medium, and low α-acids concentrations were prepared from both the 2,646-hop-sample population (pre-SELECT population) and the 289-sample FAR validation set. The first step in preparing the high, medium, and low alpha calibration sets was to redistribute samples from the 2,646-hop-sample population into high, medium, and low subpopulations. A total of 1,994 samples with α-acids concentrations within the range of 9–20% were chosen for the HAR subpopulation. The MAR subpopulation contained 445 samples (alpha range of 6–10%), and the LAR subpopulation contained 347 samples (alpha range of 1–7%). Some of the same samples can be found in more than one subpopulation due to overlapping alpha ranges. Each of the HAR, MAR, and LAR subpopulations underwent CENTER and SELECT to further refine and reduce the populations. The final result of this process was a HAR calibration set containing 1,130 samples, a MAR calibration set containing 285 samples, and a LAR calibration set containing 237 samples. The composition of HAR, MAR, and LAR calibration sets can be found in Tables II, III, and IV, respectively.

HAR, MAR, and LAR validation sets were prepared from the FAR validation set (289 sample population). The same α-acids ranges used for the preparation of the HAR, MAR, and LAR subpopulations and calibration sets were used for selection of samples for the respective validation sets. The composition of the HAR, MAR, and LAR validation sets can be found in Tables II, III, and IV, respectively. As with the calibration sets, some of the same samples can be found in more than one validation set due to overlapping alpha ranges.

Using α-acids concentration as the selection criteria for calibration and validation sets produces data sets defined primarily according to α-acids concentration. No attempt was made to refine or define data sets according to their β-acids concentration and/or HSI values.

Statistical Evaluation of NIR Calibration Performance

The SECV and the percent of variance reduction achieved by the cross validation prediction (1–VR) were used to evaluate the performance of all calibrations developed. The SECV represents the standard deviation of the prediction residual values (i.e., the differences between NIR predicted values and laboratory values) for samples in the calibration set. The SECV was calculated for each calibration by dividing the calibration set into groups for prediction. Each group was predicted once based on a calibration prepared from the remaining groups. When all groups had been predicted, the predicted results were summarized, and the result was reported as the SECV for the calibration (21).

The interpretation of the 1–VR (one minus the variance ratio) statistic is analogous to that of the coefficient of determination (r^2). The formula for the calculation of 1–VR is also very similar to that of r^2, differing only in the standard error term:

$$r^2 = 1 - (\mathrm{SEC}/\mathrm{SD})^2 \quad \text{and}$$

$$1 - \mathrm{VR} = 1 - (\mathrm{SECV}/\mathrm{SD})^2$$

where SEC = standard error of calibration and SD = standard deviation of the reference values for the calibration set.

The predictive capabilities of calibrations were also evaluated using samples found in the validation sets. Calibration performance was evaluated by examining the standard error of prediction (SEP), the bias (average difference between NIR and laboratory values), and the fraction of explained variation (r^2) observed when NIR predicted values were compared to reference laboratory values for validation set samples. When determining r^2 for the validation sets, the value of the SEP corrected for bias (SEP(C)) (i.e., the resulting standard error value following the mathematical removal of systematic error) is used in place of the SEC value,

TABLE I
Composition of the Full-Analytical-Range (FAR) Calibration and Validation Sets as Determined by UV Spectrophotometric Analysis[a]

	FAR Calibration Set (1,320 samples)			FAR Validation Set (289 samples)		
Constituent	Range	Mean	SD[b]	Range	Mean	SD
α-Acids/FAR (%)	1.86–19.57	11.36	3.77	3.01–18.79	11.74	3.50
β-Acids/FAR (%)	1.45–11.58	5.50	1.97	2.11–11.24	5.51	2.00
HSI[c]/FAR	0.220–0.480	0.281	0.0344	0.230–0.470	0.280	0.0349

[a] The calibration and validation sets contained hop samples that were representative of the entire analytical range of α-acids, β-acids, and HSI values determined.
[b] Standard deviation.
[c] Hop storage index.

such that $r^2 = 1 - (SEP(C)/SD)^2$. A SEP with magnitude similar to that of the calibration SECV, a low bias, and high correlation values (1–VR and r^2), were considered to be necessary elements of calibrations having accurate and reliable analytical performance.

RESULTS AND DISCUSSION

Hop Sample Database Composition

Database production and preparation is an important part of NIR calibration development. In order for an NIR calibration to be a robust analytical tool, it should be prepared from a database that contains samples that are both spectrally and analytically representative of samples that will be encountered during future analyses (16). During the course of this study, an extensive hop sample database containing 3,014 samples, which included representation from 30 hop varieties and covered five crop years, was collected. Through the use of computer algorithms (i.e., CENTER and SELECT), samples were selected from the 3,014-hop-sample database for the FAR calibration and validation sets. Samples in these sets were representative of the entire hop sample population collected both in terms of spectral and analytical diversity. Table I describes the analytical constituent composition (α-acids, β-acids, and HSI values) of the FAR calibration and validation sets as determined by the reference laboratory method.

What is not apparent from Table I is the distribution of analytical values for each of the constituents. Figures 1–3 display the analytical distribution for the calibration set data found in Table I. Samples contained in the α-acids calibration set (ranging in α-acids content from 1.86 to 19.57%) form essentially three overlapping subpopulations (Fig. 1). Evident are "high-alpha" subpopulation with an α-acids range of approximately 9–20%, a "medium-alpha" subpopulation with a range of approximately 6–10%, and a large "low-alpha" subpopulation with a range of approximately 1–7%. The subpopulations of α-acids concentrations observed in the hop population may be accounted for by the nature of the hops themselves. Hops varieties tend to have narrow ranges of α-acids concentrations that are characteristic of the variety (10). Traditionally, hops have been classified according to whether they were "aroma type" or "bitter type". Varieties classified as aroma type typically have α-acids concentrations of less than 7% and in this study would be members of the low-alpha subpopulation. The bitter types typically have α-acid concentrations greater than 7%, and these varieties would be members of the medium- and high-alpha subpopulations. Having two subpopulations for the bitter-type hops is a result of a recent trend toward increased cultivation of hop varieties with very high (greater than 14%) α-acids concentrations.

The FAR β-acids calibration set (Fig. 2) was also composed of three overlapping subpopulations; the largest had a β-acids range between 1 and 7%. The two smaller subpopulations had β-acids ranges of approximately 6–10% and 9–12%. No subpopulations were observed in the analytical data of the HSI calibration set (Fig. 3). Rather, this data set had a skewed distribution.

Approaches to NIR Calibration Development and Evaluation

The objective of this study was to develop NIR calibrations for the prediction of α-acids, β-acids, and HSI values with precision and accuracy comparable to those of the UV spectrophotometric method. Of these three hop constituents, however, it is the α-acids concentration of hop samples that is of primary concern to hop

TABLE II
Composition of the High-Alpha-Range (HAR) Calibration and Validation Sets as Determined by UV Spectrophotometric Analysis[a]

	HAR Calibration Set (1,130 samples)			HAR Validation Set (220 samples)		
Constituent	**Range**	**Mean**	**SD[b]**	**Range**	**Mean**	**SD**
α-Acids/HAR (%)	9.04–19.57	13.51	1.74	9.35–18.79	13.49	1.69
β-Acids/HAR (%)	2.51–11.58	5.80	2.07	2.54–11.24	5.83	2.10
HSI[c]/FAR	0.220–0.480	0.279	0.0321	0.230–0.440	2.78	0.0320

[a] Hop samples having α-acids contents within the range of 9–20% were selected from the hop population for the calibration and validation sets.
[b] Standard deviation.
[c] Hop storage index.

TABLE III
Composition of the Medium-Alpha-Range (MAR) Calibration and Validation Sets as Determined by UV Spectrophotometric Analysis[a]

	MAR Calibration Set (285 samples)			MAR Validation Set (46 samples)		
Constituent	**Range**	**Mean**	**SD[b]**	**Range**	**Mean**	**SD**
α-Acids/MAR (%)	6.00–9.92	7.62	0.858	6.05–9.56	7.70	0.990
β-Acids/MAR (%)	2.51–8.04	5.13	0.807	2.54–6.98	5.10	0.741
HSI[c]/FAR	0.220–0.420	0.269	0.0257	0.230–0.400	0.273	0.0320

[a] Hop samples having α-acids contents within the range of 6–10% were selected from the hop population for the calibration and validation sets.
[b] Standard deviation.
[c] Hop storage index.

TABLE IV
Composition of the Low-Alpha-Range (LAR) Calibration and Validation Sets as Determined by UV Spectrophotometric Analysis[a]

	LAR Calibration Set (237 samples)			LAR Validation Set (42 samples)		
Constituent	**Range**	**Mean**	**SD[b]**	**Range**	**Mean**	**SD**
α-Acids/LAR (%)	1.86–7.00	5.14	1.15	3.01–6.96	5.28	1.09
β-Acids/LAR (%)	1.45–7.97	4.38	1.46	2.11–6.98	3.90	1.10
HSI[c]/FAR	0.220–0.450	0.293	0.0405	0.230–0.470	0.299	0.0430

[a] Hop samples having α-acids contents within the range of 1–7% were selected from the hop population for the calibration and validation sets.
[b] Standard deviation.
[c] Hop storage index.

producers and brewers alike. Hence, efforts were undertaken during this study to optimize calibration performance for the prediction of α-acids concentration if it could be accomplished without compromising the performance of the β-acids and HSI calibrations produced.

In an effort to optimize calibration performance for the prediction of α-acids concentration, two approaches for calibration development were explored. The first involved the preparation of three NIR calibrations for each of the three hop constituents, α-acids, β-acids, and HSI values. The three calibrations were prepared using samples representative of the full analytical range of values expected for each constituent. The description of the data sets used to prepare the three FAR calibrations (Table I) was discussed in previous text.

The second approach to calibration development took into consideration the presence of three analytical alpha subpopulations (high, medium, and low alpha ranges) within the hop sample population. This approach required the preparation of nine calibrations, three for each of the three hop constituents. Calibration and validation sets for each of the nine calibrations were prepared by segregating the hop sample population according to α-acids concentration, the main constituent of interest. HAR, MAR, and LAR calibration and validation set were prepared from hop samples in the three populations (Tables II–IV), as reported in previous text.

The performance statistics of each of the calibrations developed by each approach were compared to determine which calibration provided the highest degree of analytical performance for each constituent. In particular, the performance statistics of NIR calibrations produced using the FAR calibration sets were compared to those produced using HAR, MAR, and LAR calibrations sets. The performance statistics of all calibrations developed were compared to benchmark values that were determined by examining the analytical capabilities of the reference laboratory method for each constituent.

Benchmarking NIR Calibration Performance

When determining NIR calibration performance, the standard error values (SECVs and SEPs) and the degree of explainable variation (1–VR and r^2) are the calibration and validation statistics typically examined. In general, 1–VR and r^2 values should be close to 1, and standard error values should be as small as possible.

When determining a reasonable magnitude for standard error values, the analytical capability of the reference method must be ascertained. The performance of the ASBC UV spectrophotometric method has been reported in terms of standard deviations of laboratory data (2,3). Analytical data on constituent concentrations were collected from laboratories participating in industry collaboratives, and standard deviations of the data for each constituent were calculated. For α-acids analysis, standard deviations in analytical data showed ranges of 0.37–0.46% for fresh hops and 0.26–0.85% for stored hops (2). For beta acid analysis, standard deviations were 0.33–0.56% for fresh hops and 0.19–0.55% for stored hops (2). For HSI, standard deviations ranged from 0.00 to 0.0229 (3).

Although the standard deviation ranges provided an indication of expected analytical performance, it was desirable for this study to establish a single error value for each constituent for evaluation purposes. Using data obtained from the local (Yakima, WA) area check service hop analyses (1997–1999) and check service data reported in the *Journal of the ASBC* (1996 and 1998), the standard deviations of UV spectrophotometric results were calculated. The averages of the standard deviation values for α-acids analysis, β-acids analysis, and HSI analysis were 0.33%, 0.25%, and 0.017, respectively. It was not assumed that these values were the definitive measures of the reproducibility error of the UV spectrophotometric technique. However, they would serve as suitable benchmarks for NIR calibration performance for this study. It was hoped that the performance of NIR calibrations developed would equal or surpass the benchmark values.

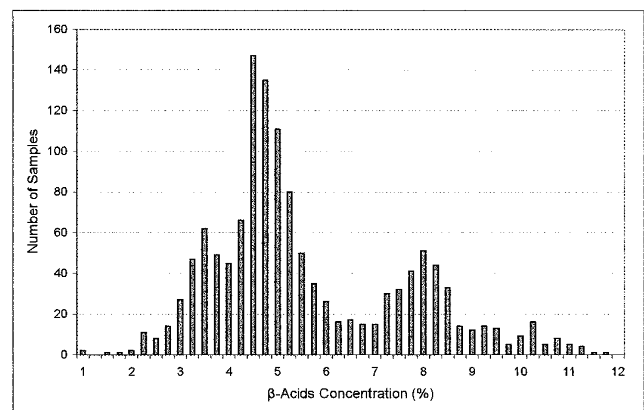

Fig. 2. Distribution of β-acids concentrations in hop samples for the full-analytical-range calibration set as determined by the UV spectrophotometric method.

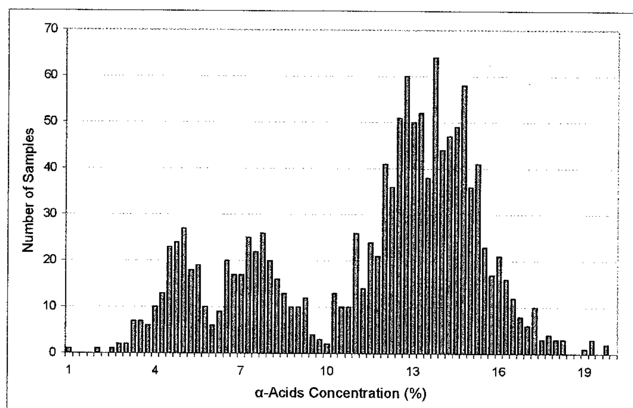

Fig. 1. Distribution of α-acids concentrations in hop samples for the full-analytical-range calibration set as determined by the UV spectrophotometric method.

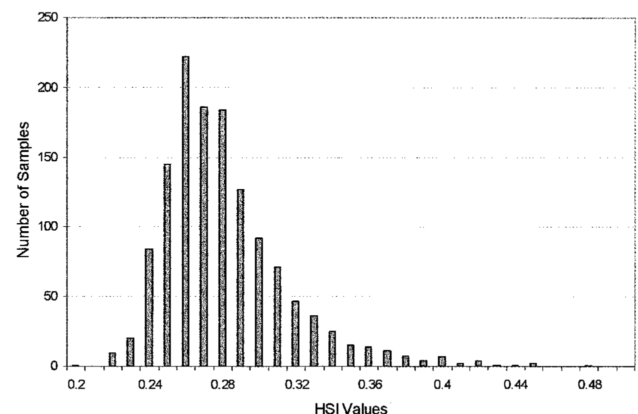

Fig. 3. Distribution of hop storage index (HSI) values in hop samples for the full-analytical-range calibration set as determined by the UV spectrophotometric method.

In most instances, analytically strong NIR calibrations have 1–VR and r^2 values greater than 0.95 and often close to 1. However, a calibration that has a 1–VR or r^2 value in the range of 0.90 can still have perfectly acceptable analytical performance (16,21). What makes this possible is the fact that the 1–VR and r^2 values are a function of both the standard error term and the standard deviation of analytical values in the data set (refer to the section under Experimental entitled "Statistical Evaluation of NIR Calibration Performance"). As the value of the standard error term decreases and/or the value of the standard deviation term increases, the value of 1–VR or r^2 approaches 1. It is beyond the scope of this article to discuss all the factors that have an effect on the standard error or standard deviation values. However, it is worth recognizing that, for some products, the natural analytical range (and hence the standard deviation) for a constituent may be small due to raw material selection and/or processing parameters. Having to produce a product to meet customer specification can work against having a broad analytical range of values for a constituent. Therefore, it is possible to have an NIR calibration that has standard error values (i.e., SEC, SECV, and/or SEP values) of a magnitude almost equal to those of the standard error of the reference method, but that has 1–VR and/or r^2 values of <0.95 as a result of a small standard deviation in the analytical data contained in the calibration and/or validation data set. This does not mean that the magnitude of the 1–VR and r^2 values should be entirely disregarded when evaluating NIR calibration performance. In fact, an examination of the analytical data should be undertaken to determine a probable cause for low (<0.90) 1–VR or r^2 values. What is implied is that the best gauges of calibration performance tend to be the standard error values (i.e., SEC, SECV, or SEP) and that not all analytically useful NIR calibrations can or will have 1–VR or r^2 values close to 1, or even >0.95.

TABLE V
Performance Statistics for Full-Analytical-Range (FAR) Calibrations[a]

	Calibration Set Statistics[b]		Validation Set Statistics[c]		
Calibration Set	**SECV**	**1–VR**	**SEP**	r^2	**Bias**
α-Acids/FAR	0.31%	0.99	0.34%	0.99	0.021%
β-Acids/FAR	0.20%	0.99	0.23%	0.99	–0.002%
HSI[d]/FAR	0.010	0.89	0.014	0.85	0.002

[a] Composition of the calibration and validation sets is described in Table I.
[b] SECV = standard error of cross validation, 1–VR = one minus the variance ratio.
[c] SEP = standard error of prediction, r^2 = coefficient of determination.
[d] Hop storage index.

Performance of the FAR Calibrations

The performance statistics for NIR calibrations produced using the FAR α-acids, β-acids, and HSI calibration sets are displayed in Table V. For each constituent, the standard errors (SEPs and SECVs) were of approximately equal magnitude. For α-acids, the values of SECV and SEP were 0.31 and 0.34%, respectively. For β-acids they were 0.20 and 0.23%, respectively, and for HSI they were 0.10 and 0.14, respectively. The standard error values obtained for these constituents were comparable to the benchmark standard deviations calculated for the reference laboratory test (0.33% for α-acids, 0.25% for β-acids, and 0.017 for HSI).

Also found in Table V are the 1–VR and r^2 values for the α-acids, β-acids, and HSI calibration and validation sets. The values for 1–VR and r^2 for the FAR α- and β-acids calibration sets were of equal magnitude (0.99) and were very close to being equal to 1. Lower 1–VR and r^2 values were obtained for the HSI calibrations (0.89 and 0.85, respectively). Further information on calibration performance for each constituent was gathered by examining the validation set data presented in graphical form.

Graphs of the validation set results are presented in Figures 4–6. A line displaying the "ideal" relationship (slope = 1) between reference laboratory and NIR predicted values has been added to each graph. The NIR predictions were in good agreement with the reference laboratory results for the FAR α-acids validation set (Fig. 4) and for the FAR β-acids validation set (Fig. 5) over the entire analytical range of the validation sets. For the HSI validation set (Fig. 6), however, the relationship between NIR predicted values and reference laboratory values deviates from the ideal relationship when HSI values are greater than approximately 0.3 (as determined by the reference method). When HSI values are >0.3, the NIR calibration generally underestimates the HSI values as they would be reported by the reference laboratory method. The

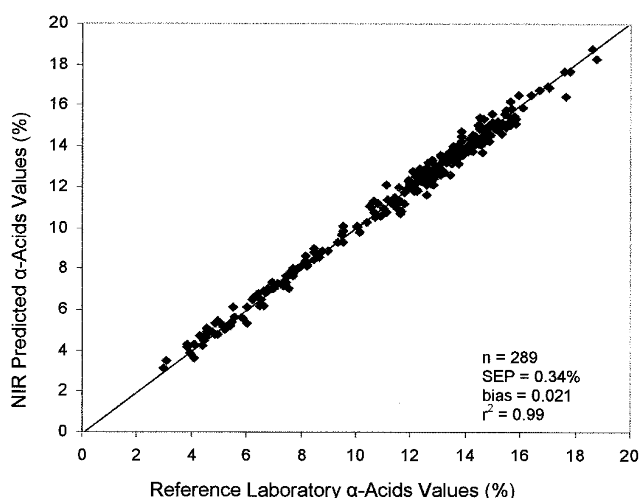

Fig. 4. Scatter plot displaying the relationship between near-infrared (NIR) predicted values and reference laboratory values for the α-acids content of hop samples found in the full-analytical-range validation set. The full-analytical-range α-acids NIR calibration was used to make predictions. SEP = standard error of prediction, r^2 = coefficient of determination.

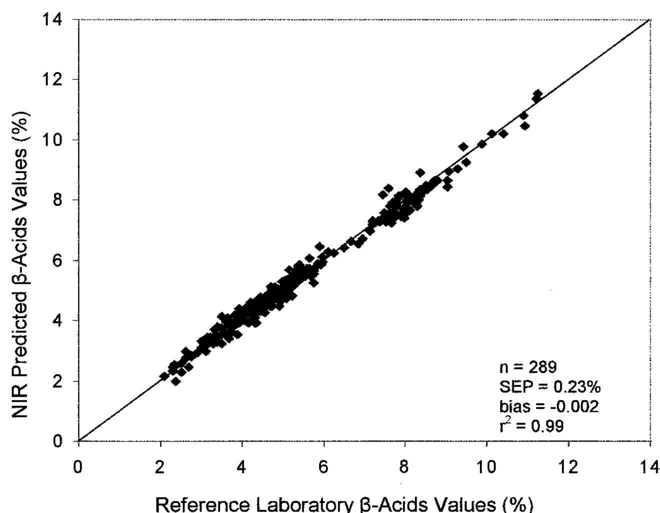

Fig. 5. Scatter plot displaying the relationship between near-infrared (NIR) predicted values and reference laboratory values for β-acids content of hop samples found in the full-analytical-range validation set. The full-analytical-range β-acids NIR calibration was used to make predictions. SEP = standard error of prediction, r^2 = coefficient of determination.

poor performance of the HSI calibration with high HSI hop samples was a contributing factor to the low 1–VR and r^2 values reported for the calibration and validation sets. In terms of practical application, these results indicate that the NIR predictions of HSI values for fresh hops (HSI values <0.30) will be more accurate than those for old hops (HSI values >0.30). It is foreseen that when NIR calibrations are implemented for routine analysis, the majority of the hop samples analyzed will be fresh hops with HSI values <0.30. Although of lesser analytical value, NIR predictions of HSI values in old hop samples (HSI values >0.30) may still serve to provide approximate values or rapid HSI screening.

In summary, from their calibration statistics and their agreement with the benchmark values, the FAR NIR calibrations were strong predictors of hop α-acids and β-acids concentrations over the full analytical range and of HSI values when less than approximately 0.30. Research efforts undertaken during this study did not discover why the NIR calibration for HSI could not accurately predict HSI values for hop samples when those hop samples had HSI values >0.30.

Performance of the FAR Calibrations vs. the HAR, MAR, and LAR Calibrations

Although analytically sound calibrations for each of the constituents had been developed using the FAR calibration sets, efforts were made to determine whether calibrations of superior performance could be developed using individual calibration sets containing hop samples with only high, medium, or low α-acids concentrations (i.e., HAR, MAR, and LAR calibration sets). From the HAR, MAR, and LAR calibration sets, nine calibrations were developed; three for each of the three constituents (α-acids, β-acids, and HSI values). The calibration statistics for the HAR, MAR, and LAR calibrations are shown in Tables VI–VIII. For comparison purposes, the statistics for the FAR calibrations for each constituent (from Table V) have been included in these tables.

For the α-acids calibration results (Table VI), the SECV and SEP values for the MAR calibration (SECV = 0.21%, SEP = 0.22%) and the LAR calibration (SECV = 0.22%, SEP = 0.19%) were superior to those of the FAR calibration (SECV = 0.31%, SEP = 0.34%) and the HAR calibration (SECV = 0.32%, SEP = 0.34%). Also, with the exception of the SEP values for the FAR and HAR calibrations (0.34%), all other SECV and SEP values for the individual calibrations were lower than our α-acids benchmark value of 0.33%. In the case of the MAR and LAR calibrations, the SECV and SEP values were substantially lower than the benchmark value (approximately a third lower). Associated with the standard error values were 1–VR and r^2 values for each of the calibration sets. Except for the 1–VR value for the MAR calibration (0.94), all of the 1–VR and r^2 values were ≥0.96. The 1–VR value for the MAR calibration was not substantially

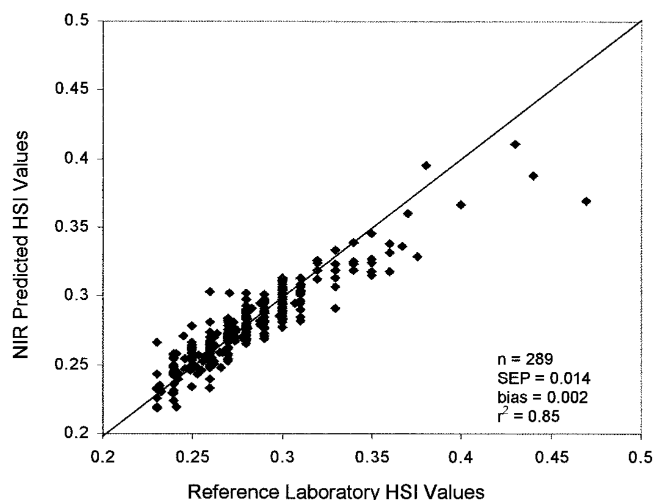

Fig. 6. Scatter plot displaying the relationship between near-infrared (NIR) predicted values and reference laboratory values for hop storage index (HSI) for hop samples found in the full-analytical-range validation set. The full-analytical-range HSI NIR calibration was used to make predictions. SEP = standard error of prediction, r^2 = coefficient of determination.

TABLE VI
Performance Statistics for α-Acids Calibrations[a]

	Calibration Set Statistics[b] (α-acids predictions)		Validation Set Statistics[c] (α-acids predictions)		
Calibration Set	SECV (%)	1–VR	SEP (%)	r^2	Bias (%)
α-Acids/FAR[d]	0.31	0.99	0.34	0.99	0.021
α-Acids/HAR	0.32	0.97	0.34	0.96	0.018
α-Acids/MAR	0.21	0.94	0.22	0.96	–0.005
α-Acids/LAR	0.22	0.97	0.19	0.97	–0.030

[a] For composition of the respective calibration and validation sets, see Tables I–IV.
[b] SECV = standard error of cross validation, 1–VR = one minus the variance ratio.
[c] SEP = standard error of prediction, r^2 = coefficient of determination.
[d] Calibration and validation set statistics for this set were reproduced from Table V.

TABLE VII
Performance Statistics for β-Acids Calibrations[a]

	Calibration Set Statistics[b] (β-acids predictions)		Validation Set Statistics[c] (β-acids predictions)		
Calibration Set	SECV (%)	1–VR	SEP (%)	r^2	Bias (%)
β-Acids/FAR[d]	0.20	0.99	0.23	0.99	–0.002
β-Acids/HAR	0.22	0.99	0.23	0.99	0.000
β-Acids/MAR	0.18	0.94	0.18	0.95	–0.006
β-Acids/LAR	0.17	0.99	0.22	0.97	–0.043

[a] For composition of the respective calibration and validation sets, see Tables I–IV.
[b] SECV = standard error of cross validation, 1–VR = one minus the variance ratio.
[c] SEP = standard error of prediction, r^2 = coefficient of determination.
[d] Calibration and validation set statistics for this set were reproduced from Table V.

TABLE VIII
Performance Statistics for Hop Storage Index (HSI) Calibrations[a]

	Calibration Set Statistics[b] (HSI predictions)		Validation Set Statistics[c] (HSI predictions)		
Calibration Set	SECV	1–VR	SEP	r^2	Bias
HSI/FAR[d]	0.010	0.89	0.014	0.85	0.002
HSI/HAR	0.009	0.90	0.011	0.89	0.001
HSI/MAR	0.010	0.83	0.013	0.85	0.000
HSI/LAR	0.014	0.85	0.018	0.83	0.002

[a] For composition of the respective calibration and validation sets, see Tables I–IV.
[b] SECV = standard error of cross validation, 1–VR = one minus the variance ratio.
[c] SEP = standard error of prediction, r^2 = coefficient of determination.
[d] Calibration and validation set statistics for this set were reproduced from Table V.

lower than the rest of the 1–VR values reported and was not low enough to downgrade the performance of the calibration.

Overall, no substantial differences were noted in the performance statistics for the individual β-acids calibrations (Table VII). The SECVs and SEPs for the FAR, HAR, MAR, and LAR β-acids calibrations ranged from 0.17 to 0.23%. The associated 1–VR and r^2 values ranged from 0.94 to 0.99. The SECV and SEP values for all the calibrations exceeded the β-acids benchmark value of 0.25%.

The performance statistics for the HSI calibrations are presented in Table VIII. With the exception of the SEP value of 0.018% for the LAR calibration, the SECV and SEP values fell within a narrow range (0.009–0.014). For all calibrations, the 1–VR and r^2 values ranged from 0.83 to 0.90. In general, the LAR HSI calibration had the weakest performance statistics of the HSI calibrations developed. It was the only calibration whose SEP value did not exceed the benchmark HSI value of 0.017. The statistical performances of the rest of the HSI calibrations were of similar magnitude. No substantial improvements in 1–VR or r^2 values were noted when HSI calibrations were prepared from HAR, MAR, and LAR calibration sets. Also, the problems with predicting samples with high HSI values (>0.3) were not alleviated.

To summarize the results presented in Tables VI–VIII, improvements in the statistical performance of NIR calibrations for α-acids predictions were noted when calibrations were produced for individual α-acids ranges. Illustrating this point were the superior performances of the MAR and LAR α-acids calibrations when compared to those of the FAR and HAR α-acids calibrations. It was not expected that either β-acids or HSI predictions would be improved by preparing calibrations from data sets selected according to α-acids concentration. However, it was necessary to verify that the analytical performances of the β-acids and HSI calibrations were not seriously compromised, which they were not.

TABLE IX
Performance Statistics for α-Acids Validation Sets Predicted Using the Full-Analytical-Range (FAR) α-Acids Calibration[a]

Validation Set[b]	Statistics[c] for Validation Sets Predicted Using FAR α-Acids Calibration: SEP (%)	r^2	Bias (%)
α-Acids/FAR[d]	0.34	0.99	0.021
α-Acids/HAR	0.36 (0.34)[e]	0.95 (0.96)	0.053 (0.018)
α-Acids/MAR	0.24 (0.22)	0.95 (0.96)	–0.088 (–0.005)
α-Acids/LAR	0.29 (0.19)	0.94 (0.97)	–0.107 (–0.030)

[a] Calibration statistics are found in Table V.
[b] For composition of α-acids validation sets, see Tables I–IV.
[c] SEP = standard error of prediction; r^2 = coefficient of determination.
[d] Validation set statistics for this set are reproduced from Table V.
[e] Number in parenthesis is the statistic observed when the validation set was predicted by its respective calibration, i.e., the α-acids/high-alpha-range (HAR) validation set predicted by the HAR α-acids calibration.

Performance of the FAR Calibrations When Analyzing the HAR, MAR, and LAR Validation Sets

Thus far, evaluations of calibration performance have been based on calibration statistics produced by predicting validation sets with calibrations of the same analytical range (i.e., the HAR validation set predicted by the HAR calibrations). As an additional check of calibration performance, the FAR calibration for each constituent was used to predict each of the HAR, MAR, and LAR validation sets. The reverse operation could not be performed (for example, the FAR validation set predicted by the HAR calibration) because predicting constituent values outside the range of the calibration set is not a reasonable test of a calibration's performance.

The validation set statistics for all four validation sets predicted by the FAR calibrations are displayed in Tables IX–XI. The validation set statistics for each of the validations sets predicted by their respective calibrations have also been added to the tables for reference (i.e., the statistics for the HAR validation set predicted by the HAR calibrations, etc.). Table IX shows that the SEP and r^2 values for the HAR validation set predicted by the FAR α-acids calibration (SEP = 0.36%, r^2 = 0.95) were comparable to the values reported for the HAR validation sets predicted by the HAR α-acids calibration (SEP = 0.34%, r^2 = 0.96). Also, and unexpectedly, the performance statistics for the FAR α-acids calibration (SEP = 0.24% and r^2 = 0.95) were very similar to those of the MAR α-acids calibration (SEP = 0.22% and r^2 = 0.96) when predicting the MAR validation set. This was an unexpected finding since the calibration statistics for the MAR α-acids calibration were found to be superior to those of the FAR α-acids calibration (Table VI). In light of this finding it was concluded that the analytical performance of the MAR α-acids calibration with future hop samples would be comparable, rather than superior, to the FAR α-acids calibration. These results also demonstrate the sensitivity of FAR α-acids calibration performance to the selection of validation set samples.

Substantial gains in calibration performance were not observed when the FAR α-acids calibration was used to predict the LAR validation set (Table IX). The performance statistics for the FAR α-acids calibration (SEP = 0.29%, r^2 = 0.94) were still inferior to

TABLE X
Performance Statistics for β-Acids Validation Sets Predicted Using the Full-Analytical-Range (FAR) β-Acids Calibration[a]

Validation Set[b]	Statistics[c] for Validation Sets Predicted Using FAR β-Acids Calibration: SEP (%)	r^2	Bias (%)
β-Acids/FAR[d]	0.23	0.99	–0.002
β-Acids/HAR	0.24 (0.23)[e]	0.99 (0.99)	–0.001 (0.000)
β-Acids/MAR	0.22 (0.18)	0.91 (0.95)	–0.022 (–0.006)
β-Acids/LAR	0.22 (0.22)	0.96 (0.97)	–0.021 (–0.043)

[a] Calibration statistics are found in Table V.
[b] For composition of β-acids validation sets, see Tables I–IV.
[c] SEP = standard error of prediction; r^2 = coefficient of determination.
[d] Validation set statistics for this set are reproduced from Table V.
[e] Number in parenthesis is the statistic observed when the validation set was predicted by its respective calibration, i.e., the β-acids/high-alpha-range (HAR) validation set predicted by the HAR β-acids calibration.

TABLE XI
Performance Statistics for Hop Storage Index (HSI) Validation Sets Predicted Using the Full-Analytical-Range (FAR) HSI Calibration[a]

Validation Set[b]	Statistics[c] for Validation Sets Predicted Using FAR HSI Calibration: SEP	r^2	Bias
HSI/FAR[d]	0.014	0.85	0.002
HSI/HAR	0.011 (0.011)[e]	0.89 (0.89)	0.002 (0.001)
HSI/MAR	0.015 (0.013)	0.82 (0.85)	0.003 (0.000)
HSI/LAR	0.024 (0.018)	0.80 (0.83)	0.005 (0.002)

[a] Calibration statistics are found in Table V.
[b] For composition of HSI validation sets, see Tables I–IV.
[c] SEP = standard error of prediction; r^2 = coefficient of determination.
[d] Validation set statistics for this set are reproduced from Table V.
[e] Number in parenthesis is the statistic observed when the validation set was predicted by its respective calibration, i.e., the HSI/high-alpha-range (HAR) validation set predicted by the HAR HSI calibration.

those observed for the LAR α-acids calibration set (SEP = 0.19%, r^2 = 0.97).

Finally, the bias values for the FAR α-acids calibration predictions of the validation sets were higher than the bias values for the validation sets predicted by their respective calibrations (Table IX). In the case of the FAR α-acids calibration, even when the SEP value of 0.107% was corrected for bias (calculations not shown), the SEP (corrected) value of 0.28% was still substantially higher than the LAR α-acids calibration SEP value of 0.19%.

In general, the performances of the FAR β-acids calibration (Table X) and FAR HSI calibration (Table XI) were comparable to those of the corresponding HAR, MAR, and LAR β-acids and HSI calibrations when predicting the HAR, MAR, and LAR validation sets. The only notable exceptions were the SEP value of 0.024 and the r^2 value of 0.80 reported for the prediction of the LAR validation set by the FAR HSI calibration (Table XI). The SEP value of 0.024 was highest of the HSI SEP values reported, and 0.80 was the lowest of the HSI r^2 values reported for the prediction of the validation sets.

To summarize the results presented in Tables IX–XI, the performance statistics (SEP and r^2 values) for the FAR calibrations were comparable to those reported for the HAR and MAR calibrations when predicting the α-acids, β-acids, and HSI values for the HAR and MAR validation sets. However, the LAR α-acids and HSI calibrations out-performed the respective FAR α-acids and HSI calibrations in predictions of α-acids and HSI values for samples contained in the LAR validation set. For α-acids, the SEP of the LAR calibration was only 66% of the value of the SEP of the FAR calibration. For HSI, the SEP of the LAR calibration was only 75% of the value of the SEP of the FAR calibration. The performance of the FAR and LAR β-acids calibrations were comparable when predicting β-acids concentrations in LAR validation set samples.

CONCLUSIONS

NIR analysis is an important analytical technique for providing rapid and cost-effective analyses for a wide range of agricultural products. This study demonstrated how NIR calibrations of considerable analytical performance can be developed for the analysis of three important hop constituents, α-acids, β-acids, and HSI. The results of this study stemmed from an extensive hop sample database in which NIR spectral data (wavelength range of 1,100–2,498 nm) and reference laboratory data (ASBC UV spectrophotometric analyses) were collected for 3,014 samples. Due to its spectral and analytical diversity, it was expected that this database would be representative of the majority of spectral and analytical variation that would be encountered when examining hop samples in the future.

Although NIR calibrations were developed for each of the hop constituents, this study focused on the optimization of NIR calibrations for α-acids determinations, as this hop constituent is of greatest concern to the hop and brewing industries. NIR calibrations were developed using calibration sets comprised of samples covering the full analytical range (FAR) for each of the constituents (α-acids values: 1.86–19.57%, β-acids values: 1.45–11.58%, and HSI values: 0.220–0.480). NIR calibrations were also developed using calibration sets produced by segregating the hop sample population according to α-acid content (high alpha range: 9–20%, medium alpha range: 6–10%, and low alpha range: 1–7%). Standard error values of 0.33% for α-acids, 0.25% for β-acids, and 0.017 for HSI were established as benchmark values for calibration performance, and in general, all calibrations developed had performance statistics that were comparable to, or exceeded, the benchmark values. However, all calibrations were analytically weak when predicting HSI values for hop samples that exceeded 0.30.

When examining calibration performance over a given α-acids content range, the performance of the FAR calibration was comparable to calibrations produced using calibration sets composed only of samples with high and medium α-acids content. However, the performance of the low-α-acids calibration was superior to the FAR calibration with hop samples that had α-acids contents less than 7%. The analytical performance of β-acids or HSI calibrations was not seriously compromised when NIR calibrations were developed for these constituents using calibration sets defined according to α-acids content.

The next phase of hop NIR research is to implement the NIR calibrations developed during this study for routine analytical purposes. Because hops can be roughly categorized into ranges of α-acids concentrations according to variety, it is recommended that for hop samples with an α-acids concentration of >7% the FAR calibration be used to predict α-acids, β-acids, and HSI values. For hop samples with α-acids <7%, the low-α-acids-range calibration developed during this study should be used. For both calibrations, predicted values for HSI >0.30 should be considered as suitable for screening purposes only.

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