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Simultaneous determination of alcohol and carbohydrate content in commercial beverages by ultrasound frequency analysis

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ABSTRACT

Controlling the composition of commercial beverages is critical for quality control. Rapid on-line measurements would allow optimization in real time. We have developed a methodology to monitor the volume fraction of ethanol and the carbohydrate concentrations in liquid mixtures using ultrasound frequency analysis. Characteristic distortion to ultrasound waves propagating through liquids is induced by the specific chemical composition of the mixture. The distortion induced by the hydrogen bonding between water, ethanol, and sucrose can be monitored in the frequency domain using 5 MHz wideband ultrasonic transducers. Multilinear regression was used to quantify both ethanol and sucrose over a wide range of concentrations with correlation coefficients (r^2) greater than 0.98. Calibrations based on prepared solutions were then used to estimate the ethanol volume and carbohydrate concentration in 22 commercial beverages ranging from sodas to distilled alcohols. Results indicate that the ethanol and carbohydrates could be estimated with a 3.18% and 0.032 g/mL error, respectively. Further, by focusing the analysis over a limited range, the error could be reduced to 0.81% ethanol. This technique demonstrates a strong potential for rapid, in situ monitoring of beverage production, which excludes sample extraction and pretreatment.

1. Introduction

Quality control is of utmost importance for the manufacture of commercial beverages ("beverages"). Alcoholic beverages are typically mixtures of several different constituents. Two dominant fractions in these beverages are ethanol and carbohydrates. A precise balance between the alcohol and carbohydrate content is required to maintain characteristic product qualities. Monitoring of these two constituents is a key step in optimizing the manufacturing process. Currently, sugars and alcohol are measured using separate methods such as refractometry and densitometry [1,2]. The requirement for separate measurements and off-line sample preparation is limiting for routine and high throughput analyses. Techniques such as near infrared spectroscopy offer promise for simultaneous quantification [2–4]. However, samples are not always readily analyzed by optical means. For the food and drink industries, where consistency must be balanced with high throughput, rapid, in situ analysis techniques would be useful.

In recent years, more attention has been paid to the use of ultrasound for the analysis of sample properties [1,5,6]. Unlike electromagnetic waves, acoustic waves are carried by oscillations in the material itself. As a result, propagation through opaque liquids or containers is possible. The speed of ultrasound is related to the viscoelastic properties of the medium. By measuring the velocity, it has been shown that concentrations of alcohols and sugars can be determined over limited ranges [1,7]. However, the relationship between sound velocity and chemical composition is not always linear or monotonic. Further, estimating concentrations in multicomponent mixtures is limited as the measured velocity may have a non-unique solution [8,9]. Due to the wide array of alcohol and sugar concentrations found in beverages, single ultrasound velocity measurements may provide insufficient information for component quantification.

A frequency analysis of the ultrasound wave provides a promising alternative to address the limitations of single-parameter velocity measurement. The viscoelastic properties of a medium also affect the frequency content of the wave. Small contributions to the velocity of the ultrasound due to particle motion in the medium result in the compressive phases travelling slightly faster than in rarefaction. As a result, the ultrasound takes on a sawtooth characteristic, which can be measured by an increase in harmonic frequency components [10,11]. The change in frequency content caused by this nonlinear distortion of the signal can be quantified by the dimensionless ratio

$$\frac{B}{A} = \frac{\rho}{{c_0}^2}\left(\frac{\partial^2 p}{\partial \rho^2}\right)_{\rho=0,s} \quad (1)$$

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where ρ is the mass density of the medium, c is the velocity of the ultrasonic wave, p is the pressure, and s is the entropy of the system [12,13]. The value of the B/A parameter is related to the viscoelastic properties of a medium with a given composition. For example, the value of B/A in water is 5.0, while in ethanol it is 10.52 [14]. As a result, the nonlinear distortion in these two liquids produces characteristically different frequency profiles [15]. In mixtures, the value of the nonlinear parameter is dependent on the ratio of the components [16]. The frequency profiles of mixtures are therefore dependent on the composition of the matrix, and a multivariate approach should allow the determination of volume ratios in mixtures.

Changes in the viscoelastic properties have been associated with the formation of molecular complexes and hydrogen bonding within a liquid [17,18]. While these changes are small, the contribution of molecular vibrations to the ultrasound wave is related to the volume fraction of a species [15,19]. Due to the amphoteric nature of water and ethanol, extensive hydrogen bonding between different conformers will be present in multicomponent mixtures [25]. Carbohydrates are larger molecules that must be extensively solvated in water or alcohol mixtures, producing characteristic hydrogen bonding structures or shells around the molecules [20]. The number of solvent molecules in this multiple-layer shell is dependent on the concentration of the specific carbohydrate in the solution [21,22]. Likewise, there is also a dependence on the ethanol volume fraction [23]. As a result, changes in the viscoelastic properties are expected with varying carbohydrate concentration [24]. The cumulative molecular interactions result in distinctive ultrasound profiles, where the magnitude of certain frequencies will depend on the concentrations of each component. An analysis of ultrasonic frequencies propagating through a medium would allow quantification of the components using a multivariate approach.

In this work, we demonstrate a simple methodology to simultaneously determine the volume fraction of ethanol and the concentration of the common carbohydrate sucrose in aqueous mixtures by the frequency analysis of ultrasound. Ultrasound frequency profiles were measured to illustrate the quantification of the two constituents over a wide range of concentrations that are found in beverages. Though the specific frequency changes may not be linear, multivariate analysis of the results allow analytical quantification in both model and commercial mixtures.

2. Materials and methods

2.1. Reagents

Calibration using the ultrasound frequency analysis was carried out using model mixtures prepared with distilled water, anhydrous ethanol, and sucrose. Although many types of sugars are common in beverages, sucrose was used due to its important role in human nutrition and solubility in ethanol mixtures [26]. Solutions were mixed for 2 h to ensure complete dissolution of sucrose. Ethanol and sucrose were obtained from Sigma–Aldrich (Oakville, CA).

The regression calibrations based on the model mixtures were then applied to a series of commercial beverages. These beverages were aliquoted and mixed as in the standard samples. All beverages were purchased from the Société des alcools du Québec. Relevant properties of these beverages are listed in Table 1. Beverages such as Coca Cola, which contain carbon dioxide, were degassed by agitation to ensure that the ultrasound would not be scattered by bubbling in the cell.

2.2. Measurement apparatus

Ultrasound frequency measurements were made using the instrument configuration shown in Fig. 1. Frequency profiles were measured by a transmission configuration using a pair of piezoelectric ultrasonic transducers with 5.0 MHz center frequencies (100% bandwidth) from Technisonic (Fairfield, US). One transducer was used to generate ultrasonic pulses while the other was used to receive the waveform that propagated though the samples. The two transducers were attached to opposing faces of a 1.8 mL aluminum sample reservoir with a 2.2 cm path length. The reservoir was kept at $21 \pm 0.25\,^{\circ}C$ with a thermoelectric cooler from AMS Technologies. This was done to minimize variations in the acoustic properties of the samples due to temperature changes.

Table 1
Ethanol and carbohydrate content of commercial beverages used in the study.

Beverage (*manufacturer*)	Ethanol (%)	Carbohydrates (g/mL)
Diet Coke (*The Coca-Cola Company*)	0.0	0.000
Gatorade (*PepsiCo*)	0.0	0.063
Coca Cola (*The Coca-Cola Company*)	0.0	0.109
Budweiser (*Anheuser-Busch InBev*)	5.0	0.031
Breezer (*Bacardi*)	5.0	0.098
Rockstar Vodka (*Rockstar Inc.*)	6.9	0.127
Bleue Dry (*Anheuser-Busch InBev*)	7.1	0.030
Bleue Dry (*Anheuser-Busch InBev*)	8.1	0.030
Bleue Dry (*Anheuser-Busch InBev*)	10.1	0.030
Late autumn Riesling (*Inniskillin*)	10.5	0.033
Arte Nova White wine (*Vins Arista*)	11.5	0.026
Arte Nova Red wine (*Vins Arista*)	11.5	0.026
Sake (*Gekkeikan*)	15.5	0.049
Muscat de Rivesaltes (*André et Bernard Cazes*)	15.5	0.117
Tia Maria (*Allied Distillers Limited*)	20.0	0.338
Blue Curaçao (*Marie Brizard*)	23.0	0.350
Amaretto (*McGuinness Distillers Limited*)	23.0	0.386
Triple Sec (*La Distillerie Meaghers Ltée*)	35.0	0.257
Brandy (*Maison Chemineaud Ltée*)	40.0	0.000
Troika Vodka (*United Distillers Canada Inc.*)	40.0	0.000
Goldschlager (*Diageo*)	40.0	0.202
Grand Marnier (*Marnier-Lapostolle SA*)	40.0	0.220

Acoustic matching across interfaces is important to minimize reflective losses [27]. Cellulose acetate (CA) windows were used to better match the impedance of the liquid mixtures examined. A layer of petroleum jelly between the transducer faces and the windows was used to further improve the impedance matching. This ensured that no air bubbles were trapped between the transducers and the CA as this would highly attenuate the signal intensity. The attenuation due to the window material was minimal.

The emitting transducer (ET) was powered by a 500PR Transmitter/Receiver from Olympus NDT (Waltham, US). A <20 ns, 250 V negative impulse was generated with a <0.01% duty cycle. The low duty cycle did not induce any heating in the sample or reservoir. Due to reflection at the transducer faces, the ultrasound pulses reverberate through the sample reservoir. Pulses were generated at a frequency of 1 kHz, allowing the reverberations to be attenuated to baseline noise levels by the media before the subsequent pulses were generated. The ultrasound pulse waveform was received by the second transducer (RT). The signal was digitized by a Tiepie (Sneek, NL) Handyscope HS3 computer controlled oscilloscope sampling at 50 MHz with a 12-bit dynamic range. For each sample, 600 waveforms were measured, for a total acquisition time of 30 s.

2.3. Methods of analysis

All samples were prepared in triplicate and were measured in random order to minimize any correlation with fluctuations in ambient conditions or instrumental response. Small changes in temperature or other ambient conditions can change the velocity of the ultrasound. To compensate for any potential phase offsets, signals were aligned to the highest positive signal intensity in the ultrasonic waveform. These waveforms were then averaged to

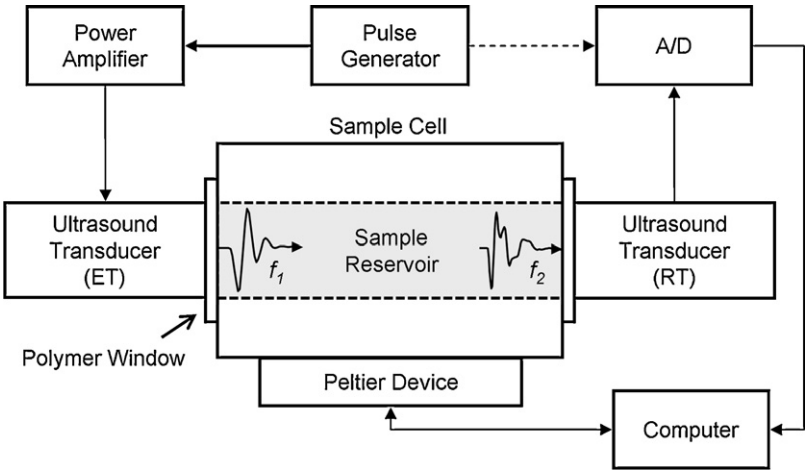

Fig. 1. Schematic diagram of the instrumentation used for multi-frequency ultrasound measurements. Samples are held in a channel between two ultrasound transducers. The emitting transducer generates a pulse waveform (f_1), which propagates through the media and undergoes specific absorption and nonlinear processes. The receiving ultrasound transducer records the waveform that is modified by the transit (f_2). The temperature in the aluminum sample cell is regulated by a Peltier device.

increase the signal-to-noise ratio. Although the CA windows and coupling fluid improve impedance matching, a fraction of the waveform is reflected at each interface. As a result, the ultrasound pulse train reverberates in the sample reservoir. However, the pulse duration is low and the length of the ultrasound waveform generated by the transducer is short (1.1 μs) relative to the pathlength of the reservoir (15 μs). As a result, the reverberations are well separated temporally, and only the first pulse transient is retained for further processing.

To examine the frequency content of the signal, a fast Fourier transform was used to decompose the waveform into its component frequency spectrum. Prior to the transform, the waveform was zero padded to improve frequency domain interpolation [28]. Frequencies in the magnitude spectra outside of the 0.5–10 MHz frequency window were excluded from further processing. The data were then smoothed by a 0.1 MHz moving average to reduce the noise in the spectra and the spectra were normalized to the total area to account for potential deviations in the instrumental response over the course of the data acquisition.

For the analysis of model mixtures, data were divided into independent calibration and test data sets. Two of the three replicates for each sample were used to generate a calibration using stagewise multilinear regression (MLR). The linear combination of a subset of frequencies to best describe the data in the form

$$Y = b_0 + b_1X_1 + b_2X_2 + \ldots + b_nX_n \quad (2)$$

were determined, where Y is the dependent variable (the volume fraction of ethanol or concentration of sucrose), $\{X\}$ are independent variables (the magnitude at a given ultrasound frequency), and $\{b\}$ are the weighting coefficients. The algorithm computed the regression of the dependent variable with the magnitude of each frequency in the ultrasound spectrum. The residual values were then calculated. This was iteratively repeated with the subset of frequencies that were not yet incorporated into the regression model. The most parsimonious model with the fewest frequencies included was selected where an F-test between regression models ($\alpha = 0.05$) showed no difference with additional parameters. This regression equation was then used to estimate the dependent variable in the independent test sample set. These consisted of the independent third replicate of each sample that was not included in the calibration set. The quality of each model was measured by the correlation coefficient (r^2) and standard error (SE). Frequency selection in the MLR model is further described in Draper and Smith [29]. Separate MLR analyses were performed for the two components of interest, sucrose and ethanol.

For the analysis of beverages, the same MLR procedure was used. The model mixture data were used as the calibration data set. Here, however, all replicates were included in the so that greater variability can be accounted for by the model. As above, the most parsimonious regression models for ethanol and sucrose were selected using an F-test ($\alpha = 0.05$) between calibrations. The concentrations of ethanol and sucrose in the beverages were then estimated using these regression models and the correlation coefficient (r^2) and standard error (SE) are reported. All signal processing and analysis of the ultrasonic data was done in Matlab (2009b, The MathWorks Inc., Natick, US).

3. Results and discussion

3.1. Ultrasound measurements of two-component mixtures

For comparison with the multi-frequency analysis, the velocity of two-component water/ethanol and water/sucrose mixtures was first measured. Changes to the viscoelastic properties of a solution produce measurable changes to the velocity of propagating waves. However, nonlinearities in propagation velocity are limiting in mixtures that contain water, ethanol, and sucrose as major components [9]. To illustrate this, mixtures were prepared with ethanol volume fractions in water ranging between 0 and 40%. As shown in Fig. 2(a), the initial increase in the measured ultrasound velocity reaches an inflection point and begins to decrease above approximately 30% ethanol. As the velocity change is non-monotonic, it is not possible to determine the volume fraction of ethanol over the entire range using a single measurement of velocity. In contrast, the relationship between sucrose content and ultrasound velocity is monotonic over a wide concentration range. As shown in Fig. 2(b), as sucrose concentration increases from 0 to 0.5 g/mL, ultrasonic velocity also increases. However, despite the absence of an inflection point, the velocity change is not linear, as shown by the deviation from the linear trace between the low and high concentrations. These examples demonstrate that the velocity of ultrasound propagation can be useful for component determination over limited ranges in systems with closure. However, nonlinearities over wide ranges and non-monotonic behavior limit applicability to wider concentration ranges. Likewise, as the velocity in both systems spans a similar range. As a result, mixtures in which both components vary would result in overlapping velocities at certain mixture ratios.

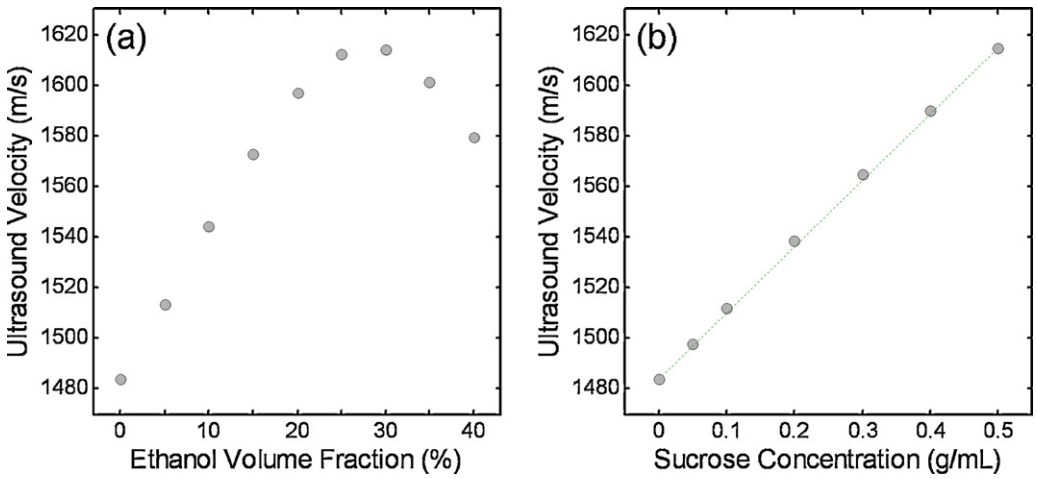

Fig. 2. Change in ultrasound velocity in water with increasing levels of (a) ethanol and (b) sucrose.

To address these limitations in nonlinear systems, multiple-measurement approaches have been taken, such as measuring the velocity at several temperatures [8]. However, the need to precisely ramp the temperature of the system increases the cost, complexity, and time of the analysis. Likewise, this is not desirable for in situ analysis. It has also been shown that a frequency analysis of ultrasound waves can allow quantification in non-monotonic systems such as alcohols [15]. To demonstrate the applicability of this methodology to the components in beverages, a series of water/ethanol and water/sucrose mixtures were prepared. The changes in the measured frequency spectra for these mixtures were examined to illustrate the effect of viscoelastic changes on the nonlinear propagation.

As shown in Fig. 3(a), when ethanol is added to water, there is a change in the frequency profile. To better illustrate the changes in the frequency domain, the mean spectral profile was subtracted from the spectra. These mean-centered frequency profiles for water/ethanol mixtures are shown in Fig. 3(b). As ethanol increases from 0 to 40%, changes in the spectrum can be divided into two major regions. First, the magnitude of frequencies below 3 MHz changes in a manner similar to the velocity fluctuation. With increasing ethanol, the magnitude in this lower frequency range initially decreases. There is a minimum at approximately 25% ethanol, after which the magnitude begins to increase. As in the velocity measurements, the rate of change in the ultrasound signal is nonlinear with increasing ethanol. Different behavior is seen in frequencies above 4 MHz. In this range, both magnitude changes and frequency shifts are present. The magnitude of the ultrasound frequencies increase with rising ethanol content until an inflection at 25%, after which a decrease is measured. The frequency envelope in this range also shifts monotonically to lower frequencies as the concentration of ethanol increases. These changes in the frequency profile should allow quantification of ethanol volume fractions in mixtures.

Water/sucrose mixtures were also prepared to demonstrate the frequency changes in this system. Concentrations of 0–0.5 g/mL were prepared, representing a range which is common in beverages. As shown in Fig. 3(c), the effect of increasing the concentration of sucrose is an exchange in magnitude between the high and low frequency range. Fig. 3(d) shows the mean-centered frequency profiles of the water/sucrose samples, illustrating the exchange in frequencies. However, at the maximum concentration, different behavior is seen as the frequencies above 5 MHz begin to decrease. As with ethanol, the characteristic changes in the frequency domain are expected to allow multivariate determination of sucrose concentrations in mixtures.

A multilinear regression analysis was done to determine if the magnitude of the frequencies in the ultrasound spectrum could be used to estimate the composition of a mixture. The frequency spectra were first divided into two data sets. A calibration set composed of two replicates at each concentration was used to generate a regression. This regression was then used to estimate the sample concentrations in the test set, which was made up of an independent replicate of each concentration. For water/ethanol mixtures, multilinear analysis revealed a close correlation between the magnitude of a subset of the ultrasound frequencies and the volume fraction of ethanol. Using two ultrasonic frequencies, ethanol volumes could be estimated an r^2 greater than 0.99 and a standard error of 0.95%. Fig. 4(a) illustrates the estimates of ethanol volume fractions relative to the known values. This shows that the results are linear over the entire range examined, which is not the case when using a single velocity measurement. The volume fractions were estimated using frequencies from both the low frequency range (1.06 MHz) and the high frequency range (3.29 MHz). This suggests that both velocity of propagation and the nonlinear propagation distortions are useful for estimating the mixture composition. As this is a two-component mixture with system closure, the second component (water) can be determined by subtraction with the same error.

The multilinear regression algorithm was also used to estimate concentrations of sucrose in water ranging between 0 and 0.5 g/mL. A close correlation was found between the magnitude of a single ultrasound frequency and the concentration of sucrose, which could be estimated with an r^2 greater than 0.99 and a standard error of 0.004 g/mL. These data are illustrated in Fig. 4(b). The determination of sucrose required one parameter from the low frequency range (1.39 MHz). As with the water/ethanol mixtures, this indicates that the region of the frequency spectrum showing partial correlation with the velocity is useful for component determination. To determine if the correlation was due to the velocity bias in this range, a separate calibration was made using only frequencies above 3 MHz. Similar sensitivity could be obtained by focusing on this range, suggesting that the velocity only adds an offset in the low frequency. The use of fewer frequencies relative to the water/ethanol system is attributed to the monotonic change of the physical properties in water/sucrose mixtures.

It is important to note that the configuration of the instrument influences the measured frequency spectrum. Transmission of ultrasound through a thin window creates a secondary wave due to partial reflection at the interface. This secondary waveform is slightly out of phase with the primary wave. The convolution of the two waveforms produces subharmonic frequencies that are

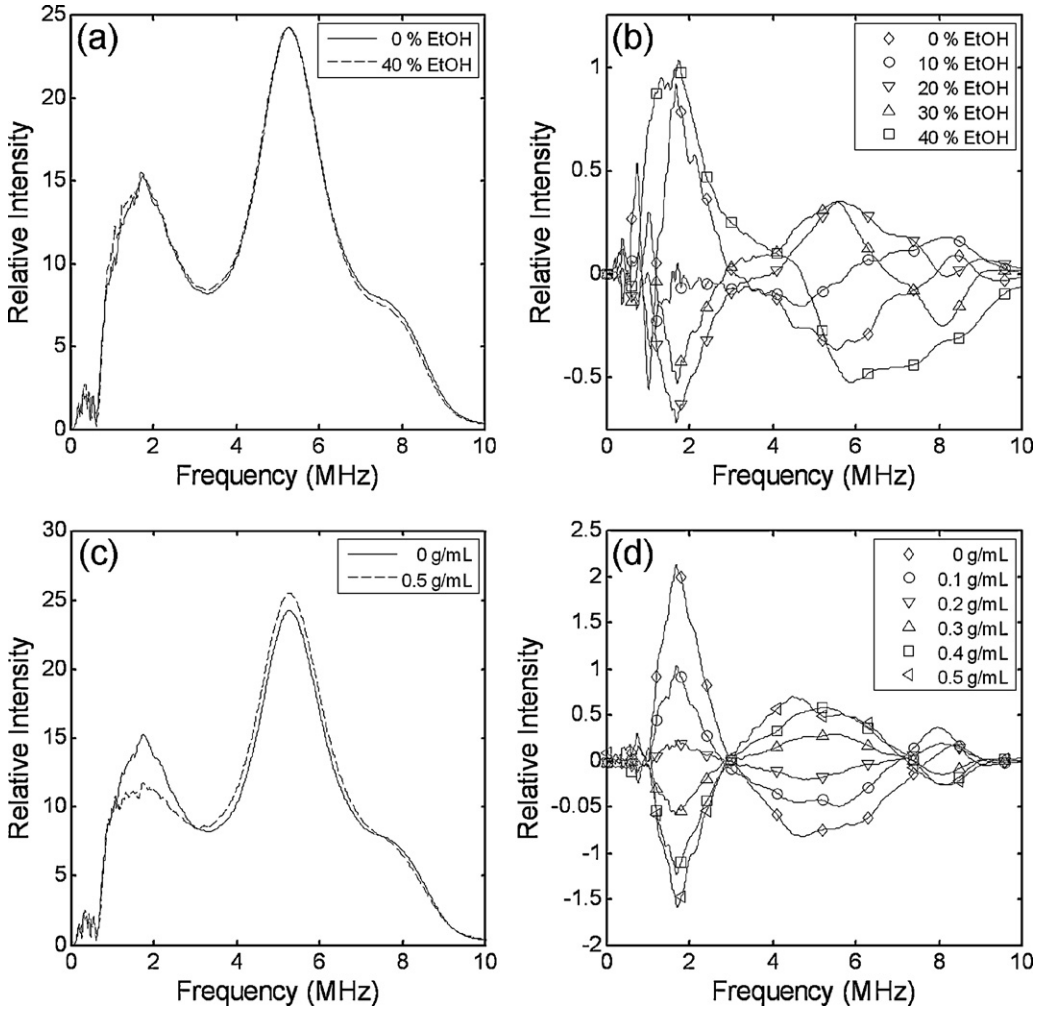

Fig. 3. Frequency profiles of binary liquid mixtures showing changes with (a) ethanol volume fraction, (b) ethanol volume fraction where the mean spectral profile has been subtracted, (c) sucrose concentration and (d) sucrose concentration where the mean spectral profile has been subtracted.

dependent on the ultrasound velocity [15]. This may account for correlation between the velocity and the magnitude of the frequencies in the <3 MHz region. To again verify that the velocity is not the only parameter being measured, the transducers were placed in direct contact with the liquid and frequency profiles for a series of mixtures were measured. Changes in the frequency distribution were seen using this configuration. The magnitude of frequencies in the 1–3 MHz region decreased by approximately 50%. This

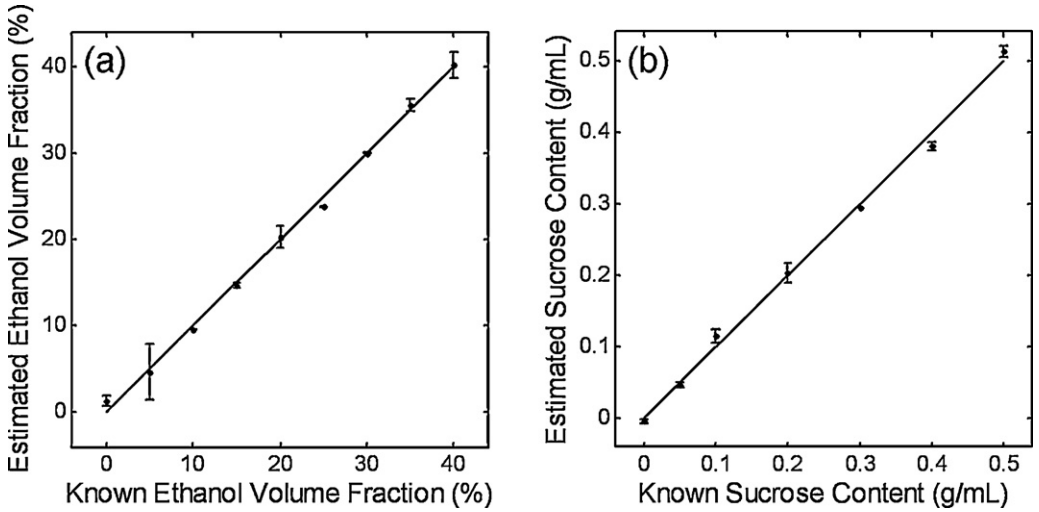

Fig. 4. Determination of components in binary liquid mixtures. Known concentration values correlated with the values estimated by multilinear regression for (a) ethanol in water/ethanol mixtures and (b) sucrose in water/sucrose mixtures.

Table 2
Figures of merit for the determination of fractional components in three-component mixtures.

Component	r^2	SE	Model frequencies
Ethanol	0.98	2.07%	1.09, 5.62, 1.72, 2.01
Sucrose	0.98	0.027 g/mL	4.18, 7.63

was accompanied by a similar increase in magnitude of the higher range surrounding the fundamental frequency. Variations in the frequency distribution with mixture composition and comparable sensitivities for both ethanol and sucrose estimation were still observed. Therefore, the coupling between the liquid sample and the transducers only affects the relative frequency distribution and not quantification.

Although the thin windows are responsible for the convolution in the data, contact between the ultrasound transducers and the mixtures is not always practical. For routine analyses, contamination or degradation of the transducer surface would change the frequency profile of the ultrasound pulse and reduce the sensitivity. Additionally, it would be preferable to monitor the composition of beverages inside a process container or in a bottled product rather than extracting a sample. In analyzing data where the transducers are not in direct contact with the solution, the frequency spectra are modified in a characteristic manner, which can be examined using multivariate methods.

3.2. Ultrasound measurement of three-component mixtures

Even in ideal conditions and with system closure, a single velocity measurement is insufficient to quantify more than two components. Velocity measurements can be enhanced by repeated measurement over varying temperatures [8] or supplemented with secondary techniques such as density measurement [9]. Over large ranges, however, the overlap in the measured velocities remains limiting. Further, multiple measurements are time consuming, and in many practical applications changing the conditions the reaction vessel is not possible. To address these concerns, an experiment was designed to determine whether the frequency analyses could simultaneously determine the concentrations of ethanol and sucrose in water. Mixtures were prepared with ethanol volume fractions between 0 and 40% and sucrose concentrations between 0 and 0.5 g/mL in a series of combinations.

The ultrasound frequency profiles of the three component mixtures show similar characteristic changes as seen in the two-component data. Changes occur in the two primary regions: the 1–3 MHz range and the 3–9 MHz range. The intensities of the ultrasound frequencies in the 1–3 MHz region change in a manner similar to the propagation velocity in the mixture. In the higher frequency range, nonlinear changes with varying mixture composition are more pronounced, including shifts in peak maxima and changes in the rate of signal magnitude variation with composition. A multilinear regression analysis was done to determine if the concentrations of ethanol and sucrose could be simultaneously determined in three-component mixtures based on the observed changes in the spectra. Samples were divided into independent calibration and test sets. The calibration set was used to develop a multilinear regression model. This regression was then used to estimate the property of interest in the test data set. As shown in Fig. 5(a), estimates of ethanol volume fractions relative to the known values are linear over the entire range examined. The volume fractions could be estimated with an r^2 of 0.98 and a SE of 2.07% ethanol using four frequencies as shown in Table 2. As with the two-component ethanol determination, both the 1–3 and 3–9 MHz regions in the frequency spectrum are used by the MLR regression model. The small increase in estimation error is attributed to the added viscoelastic changes caused by the varying sucrose concentrations. Despite the increase in error, this analysis shows that the volume fraction of ethanol in water can be estimated in the presence of varying sucrose concentrations.

Table 3
Figures of merit for the determination of fractional components in beverages.

Component	r^2	SE	Model frequencies
Ethanol	0.95	3.18%	2.76, 1.13, 6.94, 1.07
Sucrose	0.98	0.032 g/mL	1.33, 7.57, 4.04

A separate multilinear regression model was made to estimate the concentration of sucrose in the mixtures. It was found that the magnitude of two ultrasound frequencies was well correlated with the concentration of sucrose. As shown in Table 2, the volume fraction of ethanol could be estimated with an r^2 of 0.98 and a SE of 0.027 g/mL. The two frequencies used for this determination were 4.18 and 7.63 MHz. Unlike the two-component mixtures of water/sucrose, the higher frequency range was shown to be of greater predictive value. As with the determination of ethanol, the viscoelastic properties of the media are also affected by the presence of other species in the solution, resulting in a small increase in estimation error. However, as with ethanol, this analysis demonstrates that estimation of sucrose content over a wide range is possible using ultrasound frequency analysis.

Sucrose–solvent complexes and hydrogen bonding between water and ethanol were shown above to influence the nonlinear distortion of the ultrasound wave. In three-component mixtures, the viscoelastic properties of the solution will depend on both of these factors. The interrelationship between these parameters is likely the cause of the reduced sensitivity as compared to the 2-component mixture determination. However, with the incorporation of additional frequencies in the multilinear model, ethanol and sucrose concentrations are both well estimated. Both components can be measured simultaneously rather than using separate methods. Further, the non-invasive ultrasound methodology removes the need for extensive sample preparation. As a result, this methodology would provide substantial savings in time and cost of analysis.

3.3. Ultrasound measurement of commercial beverages

To determine the applicability of the ultrasound frequency analysis to commercial product testing, samples of 22 beverages were examined. These ranged from 0 to 0.386 g/mL of carbohydrates and had volume fractions of ethanol between 0 and 40%. The beverages were used without any pretreatment, meaning that a wide variety of other chemical constituents were also present. Ultrasonic frequency spectra of these beverages were measured and the sucrose and ethanol concentrations were then estimated using multilinear regressions trained on the model water/ethanol/sucrose model mixtures.

The ethanol content of the beverages was estimated using frequencies determined by MLR analysis of the model solutions. With four frequencies, the volume fractions of ethanol were estimate with an r^2 of 0.95 and a standard error of 3.18% ethanol. The frequencies incorporated into this model are found in Table 3. As in the regression for the three-component model system, the velocity information in the low frequency (1–3 MHz) region was found to be important. Likewise, frequencies above 3 MHz were also used for ethanol estimation, demonstrating the value of the nonlinearities that have been associated with the viscoelastic changes. The mean estimated values for the ethanol volume fractions of the calibration data (circles) and beverages (squares) are plotted against the known values in Fig. 6(a). Although there is higher error in estimating certain beverages, the volume fraction estimates were generally

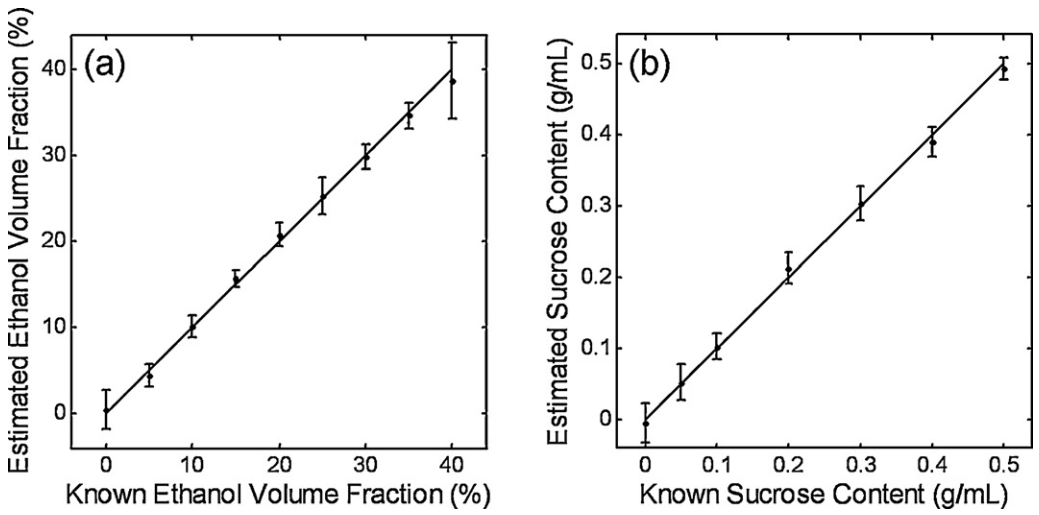

Fig. 5. Determination of components in three-component mixtures of water/ethanol/sucrose. Known concentration values correlated with the values estimated by the multilinear regression for (a) ethanol and (b) sucrose.

well correlated to the model system trend. It is likely that the beverages with larger prediction errors contained other constituents that have a significant impact on the viscoelastic properties. Although sucrose was used to generate the model solutions making up the calibration set, different carbohydrates present in certain beverages may have been less well approximated by these solutions.

Separate estimation of sucrose in the beverages also showed a close correlation between carbohydrate concentration and a subset of frequencies. Sucrose concentration in the beverages was estimated with an r^2 of 0.97 and a SE of 0.032 g/mL using the frequencies found in Table 3. The similarity between these frequencies and those used for the two and three-component model mixtures suggests that the frequencies associated with the nonlinear distortion caused by sucrose in the solution are minimally affected by the presence of other components in the beverage matrices. Fig. 6(b) shows the mean estimated values for the calibration solutions (circles) and beverage estimates (squares) plotted against the known values. This illustrates that the concentration estimates are well correlated to the model system trend. The varying carbohydrate composition may account for the larger estimation errors in beverages with carbohydrate concentrations above 0.3 g/mL. In contrast, the error of this model when estimating the carbohydrate content of the 4 beer samples was found to be only 0.017 g/mL. This factor of 2 improvements by isolating to just the one matrix suggests that the regression model is better calibrated for the carbohydrates found in this beverage class. These results indicate that a regression trained on model mixtures with the appropriate carbohydrates found in a given beverage should improve the sensitivity of the method further.

The beverages examined also contain several other components in varying concentrations. These include different sugars such as glucose, fructose, and polysaccharides, as well as caffeine, proteins, and salts. These compounds would be expected to also induce characteristic nonlinear distortions in the propagating ultrasound wave that would be different than those of ethanol and sucrose. Additionally, while the compressibility of small molecules can be considered to be negligible, it may be significant in larger polymers [23]. To reduce offsets resulting from other components, more specific multilinear regression models could be made by using model solutions that better mimic the beverage being investigated.

Component estimation in the beverages can also be improved by narrowing the range of samples analyzed. As a demonstration,

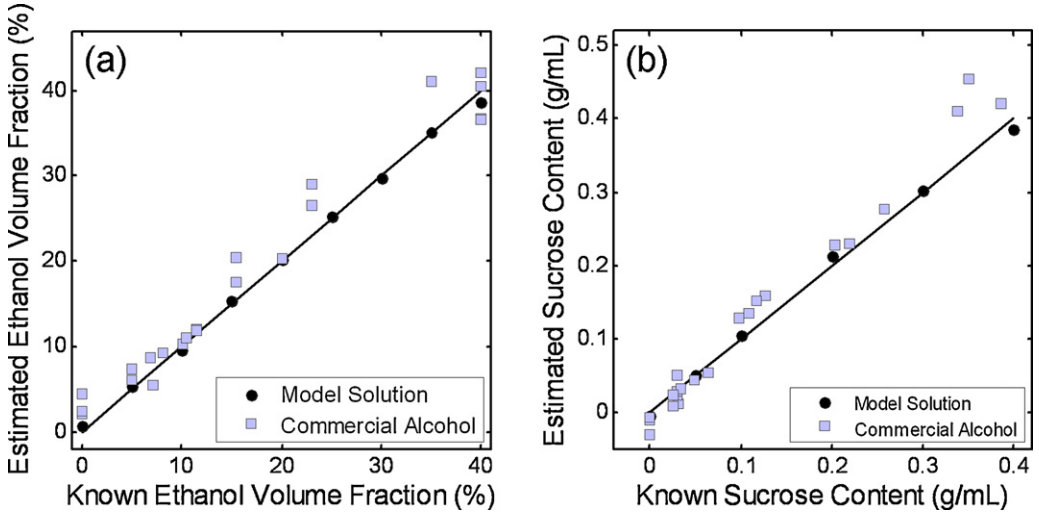

Fig. 6. Determination of components in commercial beverages. Known concentration values correlated with the values estimated by the multilinear regression for (a) ethanol and (b) sucrose.

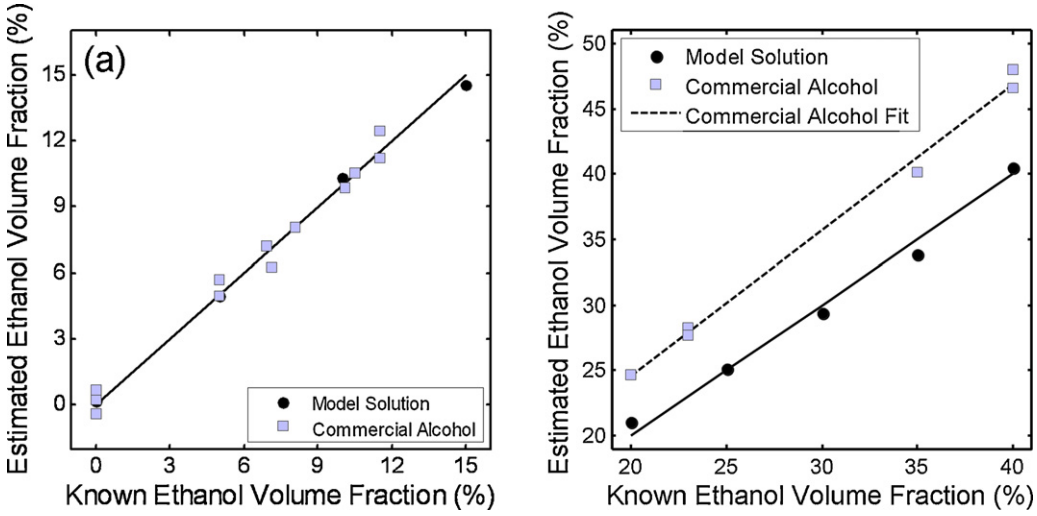

Fig. 7. Determination of components in commercial beverages over narrow concentration ranges. Known concentration values correlated with the values estimated by the multilinear regression for (a) 0–15% ethanol and (b) 20–40% ethanol.

a subset of the beverages with a maximum ethanol volume fraction of 15% and a maximum sucrose concentration of 0.3 g/mL were examined. A new, independent multilinear regression analysis was carried out using model solutions that were likewise limited to these concentration ranges. This analysis revealed that an improved estimation was possible, showing errors in ethanol volume of only 0.81%. Fig. 7(a) illustrates that the linearity was also improved and showed minimal bias. As shown in Table 4, the MLR model incorporated frequencies in similar ranges to those in the above models (1.74 and 6.97 MHz). This suggests that these frequencies show a strong correlation with ethanol content at low volume fractions independent of other matrix components. The incorporation of additional frequencies is then likely due to the variety of other beverage constituents.

The estimation of ethanol content in beverages with higher volume fractions can also be improved. An independent regression model was generated using model solutions containing 20–40% ethanol. In this case, the error in the ethanol estimation was reduced to 1.64% with the frequencies listed in Table 4. The frequencies determined by this multilinear regression differed from those used for the model solutions or the full range beverage analysis. However, two frequencies (4.70 and 4.69 MHz) were similar to ones used in the analysis of 0–15% ethanol beverages. This may indicate that certain frequencies are more correlated with ethanol when the number of other constituents in the complex beverage matrices is reduced. As shown in Fig. 7(b), however, there is an offset in the ethanol estimation in these distilled beverages. This may be due to common components that these beverages share. By using one of the beverages as a control point, this offset can be subtracted, greatly reducing the error. Although adding more parameters to the multilinear model could account for bias, using model solutions that better mimic the beverages being studied would allow for more robust estimation and reduce overfitting. Likewise, by examining beverages over more targeted ranges, the error would be expected to decrease further.

Table 4
Figures of merit for the determination of fractional components in beverages over targeted ranges.

Component	r^2	SE (%)	Model frequencies
Ethanol, 0–15%	0.95	0.81	1.74, 6.97, 2.18, 4.84, 3.07
Ethanol, 20–40%	0.97	1.64	5.57, 4.70, 5.64, 4.69

Although the estimation error is somewhat higher when measuring a wide range of concentrations, many commercial analyses are performed over a narrow range of concentrations. For routine analysis, broad calibrations could provide initial estimates of sample properties that would then be refined using separate multilinear models calibrated to appropriate matrices. This would improve the sensitivity of the method, while having little impact on the analysis time. The overall sensitivity of the ultrasonic ethanol estimation is comparable to the <0.20% errors expected using current reference methods [30]. Though invasive carbohydrate measurements can be made with an error of <0.002 g/mL [31], using a targeted carbohydrate mixture may provide improve the sensitivity of the ultrasound technique to similar levels. While reference methods can potentially provide more sensitive detection of ethanol and carbohydrates, the immediacy of the measurement using the ultrasound frequency analysis is a key advantage. Likewise, the low cost and lack of sample pretreatment are advantageous over current measurement techniques for routine analysis.

4. Conclusions

This work has demonstrated that frequency analysis of ultrasound can provide quantitative information about the composition of mixtures containing water, ethanol, and sucrose. Nonlinear distortions of the frequency spectrum arise due to inter-molecular complex formation. Using a multilinear approach it was shown that these complexes could be probed to determine the concentrations of sucrose and ethanol simultaneously using different subsets of frequencies. The analysis was further extended to look at a selection of 22 commercial beverages ranging from non-alcoholic sodas to distilled alcohols. By measuring the frequency components, the volume fraction of ethanol was determined over a range of 0–40% by volume. Likewise, the carbohydrate contents ranging between 0 and 0.386 g/mL in these beverages could be approximated using a multilinear model.

This methodology demonstrates a distinct advantage over conventional analyses used for quality control in food and drink production. Although highly sensitive, conventional methods measure ethanol and carbohydrate content separately. Further, these techniques typically require sample extraction and preparation prior to analysis. Other ultrasound approaches have been suggested to address these issues using multiple measurements at different temperatures [8]; however, this is not always practical. The

methodology outlined in this work could be used to quickly measure multiple components in situ, which would provide substantial savings in time and cost of analysis.

There are practical concerns which must be addressed to allow use of this methodology for routine food and drink analysis. Although measurement of the ultrasound frequencies is rapid, the initial development of the calibration model is critical and more complex. The viscoelastic properties of the solution are temperature dependent. It is therefore important that the temperature at which the measurements are made be kept constant. As the temperature is typically well controlled during manufacturing of commercial beverages, it should not prove difficult to regulate to less than $\pm 0.25\,^{\circ}C$ as was done in this study. If the conditions cannot be controlled, separate regression models could also be made at different temperatures. A simple measurement of temperature would then allow the appropriate calibration model to be used for rapid sample analysis.

As discussed in previous sections, the low frequency range of the ultrasound spectrum is also sensitive to waveform reflections at the coupling interfaces. It is therefore important that the regression model is trained on the appropriate container. In case of any changes to the container caused by corrosion or coating by the beverage, a second reference measurement across a separate path could be used to correct for changes over time. In combination with a temperature measurement, this would allow continuous monitoring of beverages, facilitating process optimization and quality control. For industries such as the food and drink production where consistency must be maintained, the methodology outlined would be a useful tool if these practical concerns could be addressed as indicated.

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