Evaluation of the Pure Apple Juice Content in Commercial Apple Beverages Using FTMIR-ATR and Potential Curves

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ABSTRACT

An attenuated total reflectance Fourier-transform mid-infrared procedure (FTMIR-ATR, 1250 to 900 cm^{-1}) has been developed to evaluate the amount of pure apple juice employed to prepare commercial apple juice-based beverages. Samples were classified according to their percentage of pure apple juice using a multivariate technique called potential curves. The two classification patterns defined by the loadings are associated with the general sugar content and the sucrose vs. fructose-plus-glucose "ratio." In order to apply the methodology to some commercial beverages synthetic blanks were needed to correct for sugar added to

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the original juice. The procedure was validated using either laboratory prepared juices (2%, 4%, 6%, 8%, 10%, 16%, 20%, 25%, 50%, 70%, and 100% v/v apple juices), commercial 100% pure juices or commercial soft drinks. The contents of the three main sugars ranged from $0.03–4.65\,\text{g}\,100\,\text{mL}^{-1}$ for sucrose, $0.05–6.25\,\text{g}\,100\,\text{mL}^{-1}$ for glucose, and $0.13–6.5\,\text{g}\,100\,\text{mL}^{-1}$ for fructose. The methodology is fast, precise, and offers useful qualitative information.

Key Words: Apple; Juice; Potential curves; Soft-drink; Infrared-spectroscopy.

1. INTRODUCTION

Food authentication is an important issue for both consumers and food industries, throughout the food chain from raw materials to finished products.[1] The economic advances which have occurred in most countries during the period from 1950 to 1980, and the subsequent social changes that they induced, for example a consumer preference for ready-to-serve convenience foods, precipitated an explosive growth in fruit juice production and consumption and fuelled a financial incentive for its adulteration.[2] Unfortunately, food adulteration has significantly evolved from simple dilution with water, or the addition of sugars, acids and/or colourant agents, to such complex mixtures that authentic and fraudulent goods are hard if not impossible to differentiate by most analytical methods.

Orange and apple juices are widely consumed worldwide, and the latter are appreciated by their advantageous nutritional benefits. Around 21.5% of the worldwide apple production, ca. 44 million tons per year, is forwarded to beverage processing. Some apple juice health effects have been reviewed by several authors,[3–5] including antitumorigenic effects, reduced blood pressure, ingestion of important amounts of fibers, and protection from coronary heart disease, as well as antihemorragic, anti-inflamatory, and antiallergic properties.

Accordingly, the US Department of Agriculture stresses consumption of one or two apples (or their juice) per day. Interestingly, juice consumption is steadily increasing owing to "light diets," "ready-to-drink," and a general consiousness about healthy eating.

The most widely applied analytical techniques in food authentication are either gas or liquid chromatography. Typical applications comprise assessment of the authenticity of apple juice products based on sugar analyses,[6–9] nonvolatile acid analysis[10] and amino acids.[11] Despite this common

usage, chromatography is a slow and expensive technique. Therefore, some other techniques have also been employed, for instance, microbiological assays[12–14] and isotopic studies.[15,16] In contrast, spectroscopic techniques, which are rather inexpensive and quite fast, have not been broadly used. Two reasons may be the low resolution of the spectra when compared to chromatograms, and spectroscopic elucidation of what chemical species are present in the juice may be difficult.

However, there is considerable demand for simpler and straightforward spectroscopic procedures. In particular infrared spectroscopy is being increasingly applied in the food industry because of developments both in the near infrared (NIR) and classical mid IR (MIR) regions. Several authors have determined sugars in fruit juices using attenuated total reflectance (FTIR-ATR) and multivariate analyses.[17–22]

This work looks for a facile and fast infrared methodology to evaluate the percentage of juice in apple-based beverages, without sample processing. MIR spectroscopy is employed in the horizontal ATR format. Then, the chemometric technique "potential curves" is applied to, first, assign particular spatial locations to different classes, in particular samples with different percentages of juice and, second, to classify new samples according to the probability that they belong to different predefined classes.

The term "potential curves" refers to a maximum likelihood classification method, and it will be described briefly as it is a rather novel approach to classify samples. The algorithm typically uses the first and second principal component scores, while other possibilities are PC2–PC3, PC1–PC3, etc., to define groups of samples and, then, to independently model each one. The hypothesis made by the algorithm is that the samples in each group are homogeneously distributed into the PC1–PC2 subspace according to a bivariate Gaussian distribution. The two-variable Gaussian distribution can be described by Eq. (1):[23]

$$f(X, Y) = \frac{1}{2\pi\sigma_x\sigma_y\sqrt{1-\rho^2}} \exp\left[-\frac{A}{2(1-\rho^2)}\right] \qquad (1)$$

$$A = \left(\frac{X-\mu_x}{\sigma_x}\right)^2 + \left(\frac{Y-\mu_y}{\sigma_y}\right)^2 - 2\rho\left(\frac{X-\mu_x}{\sigma_x}\right)\left(\frac{Y-\mu_y}{\sigma_y}\right)$$

where X and Y are the PC1 and PC2 sample scores; μ_x and μ_y are the average scores for each group, and σ_x and σ_y are their standard deviations; ρ is the calculated correlation coefficient between the Xs and the Ys. Intercepting these curves with horizontal parallel planes yields elliptic sections which represent isoprobability sections. They are described by the general equation $a = (1/(1-\rho^2)) \times A$, where a is a positive constant.[24] The area of each ellipse

is related to the probability that a sample belongs to this group, Area $=$ $\exp(-a/2) = $ Prob[sample ε group].

Note that this is an objective criterion to classify new samples into a set of predefined groups, since the sample will be included into the group where the probability is highest. More details can be found elsewhere.[24,25] In addition, as the technique is based on PCA, a detailed study of the main loadings can reveal useful spectral regions to distinguish different classes.

Synthetic blanks were developed, because direct comparison between commercial beverages and laboratory standards is not possible, except for beverages declared as 100% pure juice. The reason is that, in general, commercial drinks have sugars added (glucose, fructose, and inverted cane sugar). Such addition is legal to soft drinks as long as the amount is less than 10% of the amount of added sucrose.[26] European companies are not legally obliged to declare the amount of added sugar(s) which, in turn, complicates the search for the amount of pure juice used to prepare the beverages. In the USA, many firms provide this information. Only those juices declared to be 100% cannot have added sugar.

To determine the amount of (pure) apple juice employed to prepare commercial soft drinks we must first determine the total amount of sugars present in the drink and then how much sugars were added. The spectrum of the latter is subtracted from the spectrum of the drink.

The contents of glucose, fructose, and sucrose in the three types of commercial soft drinks found on the market (10%, 16%, and 25% of apple juice) were assessed in five studies using different brands and presentations, bricks and glass bottles.

The process was performed also for several laboratory-prepared diluted juices (10%, 16%, and 25% of apple juice). The spectral difference between the total contents of glucose, fructose, and sucrose in commercial beverages and their corresponding quantities in laboratory-prepared juices are a reasonably good approach to the amounts of sugar(s) added to the (diluted) apple juice employed to prepare the commercial beverages. The point here is to use such values to prepare synthetic blanks, one for each type of beverage.

2. EXPERIMENTAL

2.1. Equipment

A Perkin Elmer 16PC FT-IR spectrometer operating in the mid-infrared region ($4000-400\,\text{cm}^{-1}$) with a germanium/KBr beamsplitter and a deuterated triglycine sulfate (DTGS) detector equipped with an horizontal ATR (ZnSe, trapezoidal, $45°$ incidence angle, 12 nominal internal reflections)

was used. Each final spectrum is the average of 50 interferograms, recorded with $4\,cm^{-1}$ resolution, background corrected and Fourier transformed (Beer-Norton strong apodization function). Reflectance spectra were baseline corrected ($1250-900\,cm^{-1}$) and transformed to absorbance spectra using the built-in algorithms. The useful working range was $1250-900\,cm^{-1}$; spectra were digitized (one $datum/2\,cm^{-1}$) and exported (LOTUS $123^{®}$) to MATLAB$^{®}$. The spectrometer was assessed by daily and weekly tests: laser energy, S/N ratio, detector gain, polystyrene bands—position and height ratios, etc. An AND electronic balance, mod ER-180A, precision $0.0001\,g$ was employed. It was checked weekly with certified standard weights [Physikalisch-Technische-Bundesanstalt (PTB) from the Deutscher Kalibrierdienst (DKD), Germany]. Other devices were: a Milli-Q ultrapure water system (Millipore, Madrid, Spain, $18\,M\Omega\,cm$ resistivity); a Heraeus Instruments centrifuge, Labofuge 400e; and a domestic Nevir blender, mod NVR 3285L. All glassware and plasticware were soaked with soap, rinsed with Milli-Q water and dried.

2.2. Reagents

$D(-)$-Fructose ($C_6H_{12}O_6$) $98-103\%$ purity, biochemistry grade, Panreac (Barcelona, Spain); $D(+)$-glucose ($C_6H_{12}O_6$) 99.991% purity, analytical grade, Panreac (Barcelona, Spain); sucrose ($C_{12}H_{22}O_{11}$) 99% purity, analytical grade, Panreac (Barcelona, Spain). $L(+)$-Ascorbic acid ($C_6H_8O_6$) 99.7% purity, analytical grade, Panreac (Barcelona, Spain); anhydro citric acid ($C_6H_8O_7$) 99.5% purity, analytical grade, Panreac (Barcelona, Spain); DL-malic acid ($C_4H_6O_5$) $99.0-100.5\%$ purity, analytical grade, Panreac (Barcelona, Spain).

2.3. Samples

Hereby, all percentages will be expressed as % (v/v), as is common.[27] Laboratory-prepared samples, synthetic samples, commercial juices, and soft drinks were centrifuged at 3000 rpm and filtered through a number 4 paper filter just before IR measurement. All measurements were carried out at room temperature ($20-23°C$).

2.3.1.   Commercial Samples

Commercial apple juice-based beverages ("100% apple juice," soft drinks, and "energy" drinks) were purchased in different shops. When possible,

packages of different sizes from the same firm were taken as it had been reported that smaller packages might affect sample composition because of their more extensive manipulation.[1] Samples were kept at 4°C until analysis. Before IR measurement, the samples were equilibrated to room temperature.

2.3.2. Laboratory-Made 100% Apple Juice

To obtain authenticated 100% juices, apple juices were prepared in the laboratory squeezing out different varieties of commercially-available apples (Gloster, Golden, Granny Smith, Reineta, Royal Gala, and Starking). They were measured by FTMIR-ATR against water as background (see Fig. 1).

2.3.3. Diluted Apple Juices

Different aliquots of laboratory-made pure apple juices were diluted with Milli-Q water to prepare the calibration (training) and validation sample sets, see Table 1 (the 100% samples corresponded to undiluted aliquots). Dilution was used to simulate pure beverages with different percentages of juice.

The 2–20% interval of apple juice was modeled using 134 samples; the model was validated with 39 samples. The 20–100% range of apple juices was modeled using 86 samples and 44 to validate the model (Table 1). More samples were included into the calibration sets to model as much variability in the apple juices as possible: apple variety, degree of ripening, climateric situation of the apple, etc.

2.3.4. Synthetic Blanks

If commercial beverages were made only from diluted apple juice, their classification would be straightforward using any model developed from laboratory-standards. Disappointingly, addition of sugars and/or inverted cane sugar[28–30] to beverages can modify the spectral profile (see Fig. 2).[9]

In a preliminary search it was found that both laboratory-prepared and commercial samples presented a quite similar profile (see Fig. 3) which can be described by the three most important sugars present in apple juices (glucose, $15-35 \, g \, L^{-1}$; fructose, $55-80 \, g \, L^{-1}$ and sucrose, $5-30 \, g \, L^{-1[9]}$). Their amounts can be evaluated by a matching procedure where the spectrum of the beverage or laboratory-diluted juice is compared against the spectra of standard solutions of the three sugars.

If a spectral subtraction is performed between the spectrum of a particular beverage and its corresponding synthetic blank it would be possible to classify the spectral difference in any of the classes described above (see Table 2; Fig. 1).
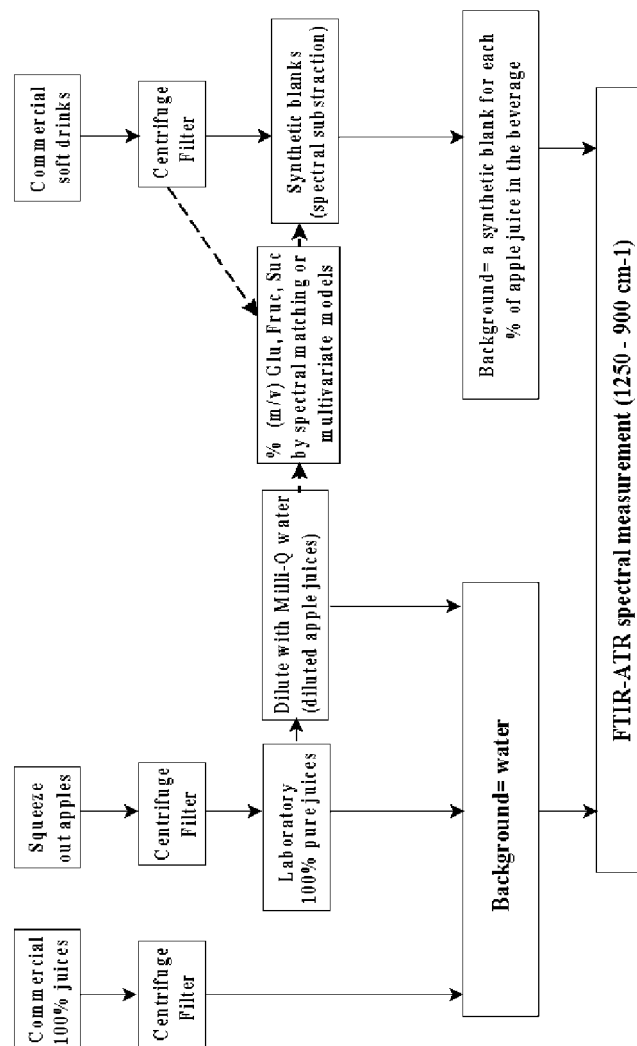
Figure 1. Scheme showing how samples were prepared.

Table 1. Percentages of diluted apple juices employed for training (calibration) and validation, and the number of samples used.

	%	Training set	Validation set
Low range	2	19	1
	4	17	1
	6	16	13
	8	22	6
	10	21	6
	16	20	6
	20	19	6
High range	25	20	18
	50	16	13
	70	14	1
	100	17	6

A drawback of this strategy is that the matching step assumes that producers indeed deliver the amount of pure apple juice as declared on the label (following legal requirements) and that they do not exceed the maximum allowed levels of added sugars. This is difficult to check as only isotopic and chromatographic methods can distinguish the sugar added by the maker from that in the original juice.[9,29]

Although the matching step against standards gave satisfactory results (see Fig. 4), a faster approach based on partial least squares (PLS) multivariate calibration, as presented elsewere[17,18] is still being implemented in our laboratory.

In this work other products, like high fructose syrup, were not tested. Regarding the influence of acids on the IR spectra, only high concentrations ($41\,g\,L^{-1}$ of ascorbic acid, $40\,g\,L^{-1}$ of malic acid and $40\,g\,L^{-1}$ of citric acid) affected the combined band at $1320-1211\,cm^{-1}$ (C–O stretching and O–H bending). These concentrations substantially exceed the maximum allowed limits: no limit, 5 and $10\,g\,L^{-1}$, respectively.[26]

3. RESULTS AND DISCUSSION

3.1. Spectral Information

The ATR spectral profiles observed for laboratory-made apple juices were very similar regardless of the apple variety (see Figs. 3 and 5). They differed only in minor details that do not differentiate between groups.
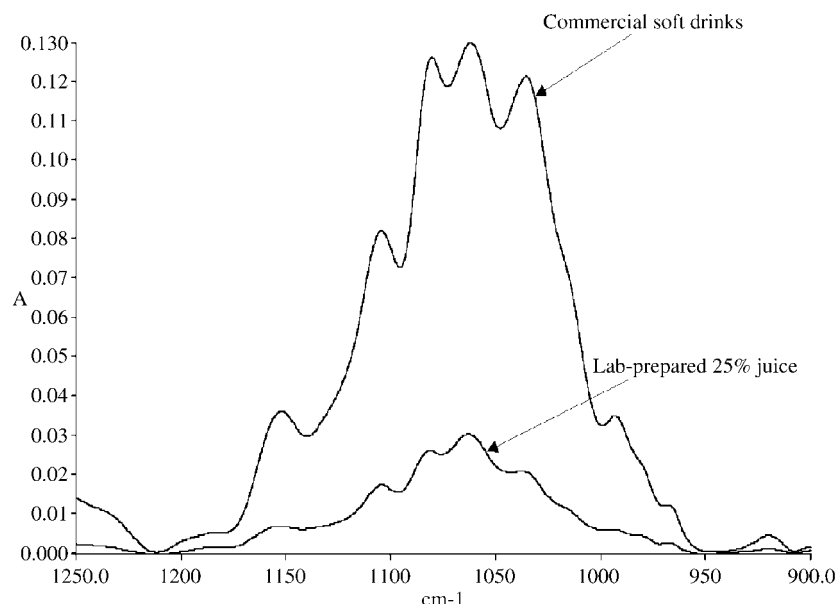
Figure 2. Spectral differences between a laboratory-prepared and a commercial beverage (25% of apple juice). (*View this art in color at www.dekker.com.*)

The multivariate plots are not shown here. Also, they were similar to the spectra of commercial samples, but for soft drinks, where absorbances were higher and spectra were somewhat changed due to the addition of sugars. We could not observe differences between the IR spectra of aliquots withdrawn from different packages (330 mL, 1 L, cans, bottles, etc.).

The most interesting spectral region is 900 to $1250\,cm^{-1}$. It is free from water and CO_2 spectral interferences, and combines spectral bands for glucose (1151, 1106, 1080, 1034, 922, and $917\,cm^{-1}$), fructose (1156, 1102, 1084, 1063, 1033, 980, 967, and $920\,cm^{-1}$) and sucrose (1138, 1056, 999, and $927\,cm^{-1}$). The intensities were confirmed by comparison with spectra of individual sugars measured here, and in previous reports.[19,30–32] The IR qualitative information gathered from this spectral region allows for a simple and fast detection of added sugars, adulteration, and for the detection of anomalous ratios of the sugars. For instance, Fig. 6 shows the spectra of a laboratory-100% apple juice and a 100% commercial juice. The commercial juice has higher absorbance at $1076–1080\,cm^{-1}$ (glucose and/or fructose), $1033\,cm^{-1}$ (fructose), and $992\,cm^{-1}$ (sucrose), which is not seen in any other 100% commercial juice.
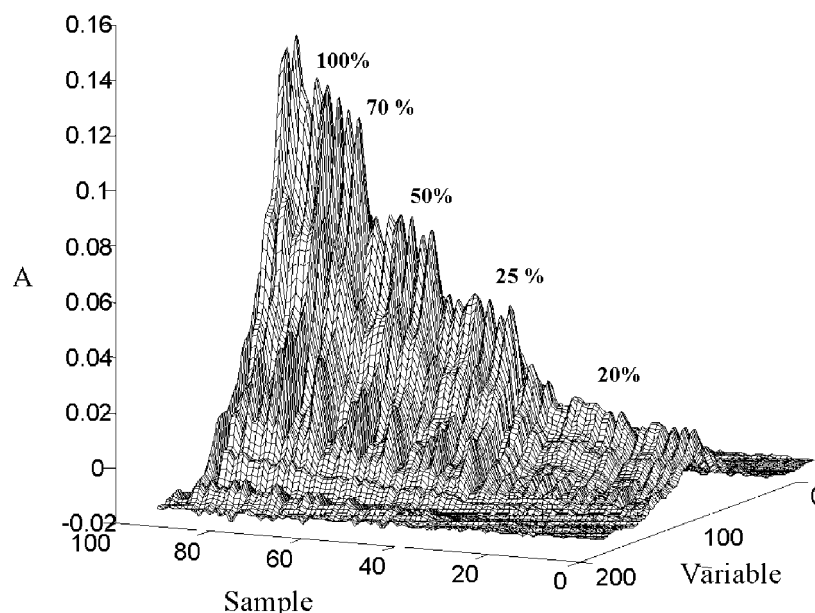
Figure 3. Spectral profiles of different laboratory-diluted apple juices. Each calibration group in the 20–100% range is indicated.

As laboratory-prepared 100% juices were the starting point for this work, their stability was studied to assess whether apple storage might affect the spectra. Eighteen apples of the same variety were purchased on the market all at once and stored at 4°C. None showed any physical damage at the acquisition time nor did they become damaged during storage. Approximately every fortnight, from January to June, an apple was squeezed out and measured by FTIR-ATR. At the end of the study, all the spectra were compared. It was found that absorbances decrease with storage time (up to 20% from January to June). The main reason is the climateric process suffered by apples during storage, which transforms sugars to ethylene.[1] The effect was negligible during the first month, and, does not affect normal testing, which is readily performed within this period.

The repeatability (r) and reproducibility (R) of the overall analytical procedure were assessed using one apple variety during 2 weeks. Ten independent replicates were made to derive the short-term and long-term standard deviations. Then, current calculations for r ($2\sqrt{2} \times S_{\text{short term}}$) and R ($2\sqrt{2} \times S_{\text{long term}}$) were made. It was found that $r = 6.4 \times 10^{-4}$ A.u (=0.53% of peak height at $1076\,\text{cm}^{-1}$) and $R = 7.4 \times 10^{-4}$ A.u (=0.62% of peak height at

Table 2. Sugar contents ($g\,100\,mL^{-1}$) obtained after a matching step for commercial soft drinks and laboratory-diluted juices and estimation of the sugars added to the soft drinks (=synthetic blank).

	Sample code			
	SF0	SF00	E0	E00
% Juice declared	10	16	25	25
		Total sugar		
Soft drink				
Fructose	3.580	3.648	2.345	4.940
Glucose	3.505	3.785	4.323	6.250
Sucrose	4.650	3.545	3.375	0.800
Laboratory-diluted juice				
Fructose	0.650	1.000	1.625	1.625
Glucose	0.250	0.385	0.625	0.625
Sucrose	0.150	0.231	0.375	0.375
		Estimated added sugar		
Synthetic blank				
Fructose	2.930	2.648	0.720	3.315
Glucose	3.255	3.400	3.698	5.625
Sucrose	4.500	3.314	3.000	0.425

$1076\,cm^{-1}$), which are good values for ATR mid-IR spectroscopy. The sensitivity of the technique was evaluated using the slope of aqueous calibration lines for each of the three sugars with water as the background. They were $0.023\,uA\,(g\,100\,mL^{-1})^{-1}$ for fructose, $0.024\,uA\,(g\,100\,mL^{-1})^{-1}$ for glucose and $0.008\,uA\,(g\,100\,mL^{-1})^{-1}$ for sucrose. The methodology was found to be robust and sensitive, and could also be applied as a quality control tool to monitor sugars in soft drinks. Here, the procedure would be simpler for the producer since the exact amounts of sugars are known.

3.2. Classification

Two classification models were developed to classify both commercial 100% apple juices and soft drinks while considering all commercial percentages. The first considered were low percentages of apple juice (from 2% to 20%) whereas the second were high percentages (from 20% to 100%). Besides
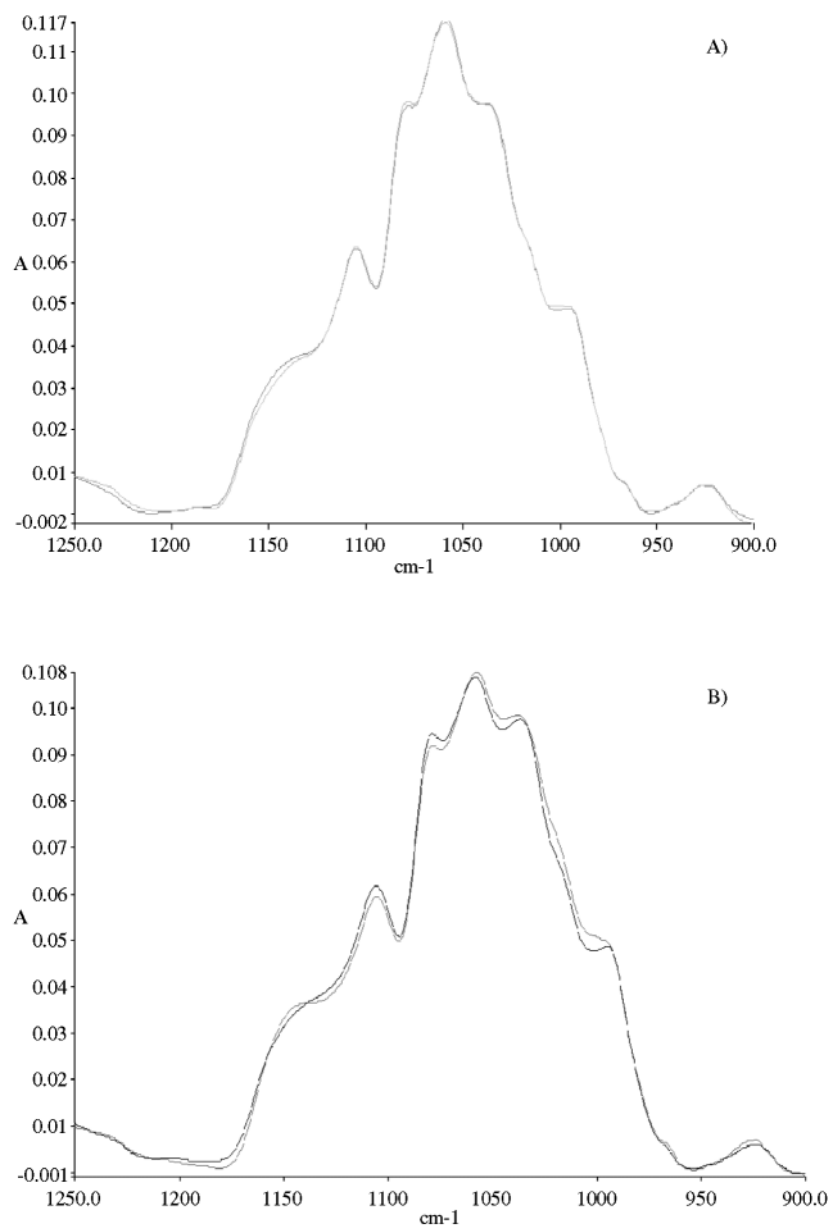
Figure 4. Overlaid IR spectra for: (A) 16% commercial soft drink and a solution of 3.785% glucose, 3.648% fructose, and 3.545% sucrose, and (B) 25% commercial soft drink and a solution of 4.323% glucose, 2.345% fructose, and 3.375% sucrose. (*View this art in color at www.dekker.com.*)
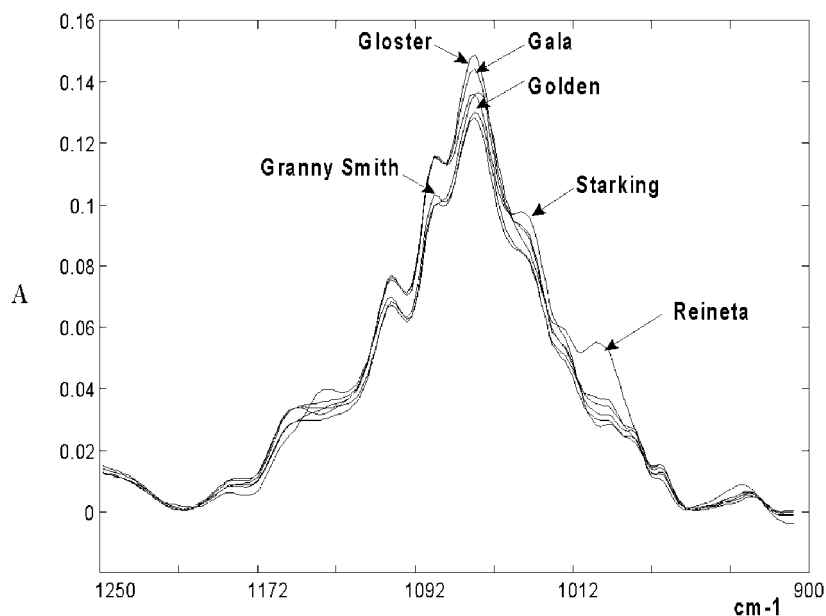
Figure 5.   Spectral profile for 100% pure juices from different apple varieties.

"target" classes (10%, 16%, 25%, and 100%) several percentages were considered as well to avoid the possible yes/no dichotomy and try to approximate the content of pure apple juice in the beverage.

3.2.1.   Low Percentage of Apple Juice

After analysing the 134 training samples by PCA, using mean centred data, with no further normalization, the PC1–PC2 and PC1–PC3 score subspaces were studied. As the PC1–PC2 subspace collected about 99.8% of the total variability (PC1 and PC2 explain ca. 99% and 0.8% of the variance, respectively) and the sample groups appeared quite well defined their scores will be used throughout. Then, potential curves were developed to model each class (% of juice).

The factors loadings are shown in Fig. 7. The first factor resembles the average spectrum of the three sugars, whose concentrations can explain most of the variability of the data set. Hence, the first component distinguishes samples according to their total sugar content [Fig. 7(a)]. The second factor [Fig. 7(b)] distributes the samples according to the "ratio" of fructose plus glucose,with positive loadings, vs. sucrose, negative loadings.
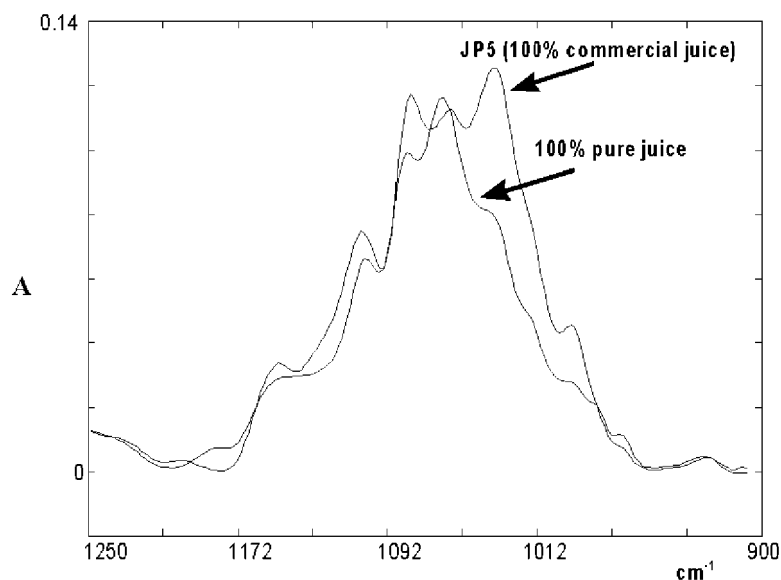
Figure 6. Spectral differences between 100% pure apple juice made in the laboratory and a commercial juice declared to be 100% pure. See text for details.

Figure 8(a) presents the potential curves in the PC1–PC2 scores subspace (the *z*-dimension is the probability of belonging). Figure 8(b) projects the samples on the isoprobability ellipses. The separation between the groups is not perfect, which can be attributed to minor differences in apple varieties, harvesting and ripening, random errors, and, mainly, to the fact that spectral differences between some groups are very small.

Three statistical parameters were calculated for each class to evaluate the goodness of the model:[33] model-efficiency, which is the percentage of juices correctly classified in a class; model-sensitivity, which is the percentage of juices belonging to a class and correctly classified within that class, and model-specificity which is the percentage of juices that do not belong to a class and correctly classified outside that class. These are presented in Table 3. Note that they were calculated for the validation group and not for the training group to avoid too many positive results. The worst situation was for the 8% group, which overlaps extensively with the 6% and 10% classes.

The commercial soft drinks were classified using the spectra that resulted from measuring the commercial soft drinks against their "associated" synthetic blanks. Table 4 shows that the two available commercial soft drinks were classified correctly.
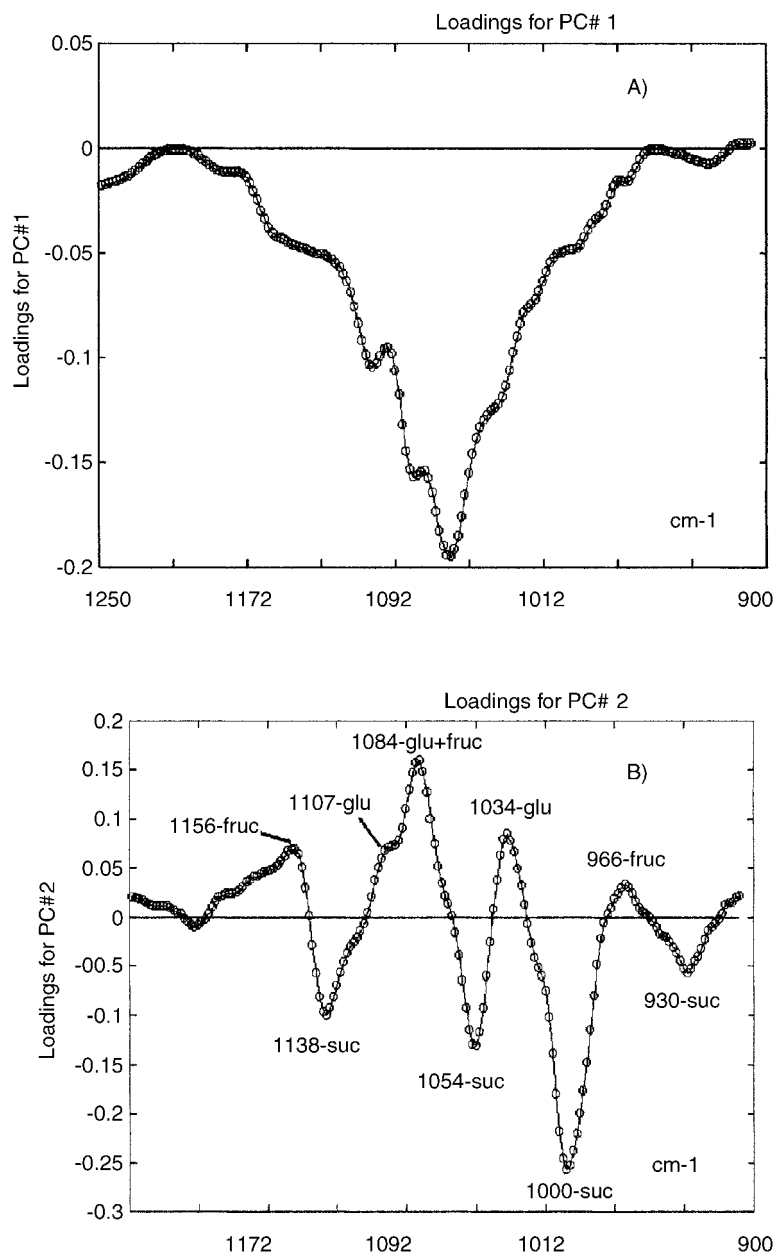
Figure 7. Loading profiles for: (A) PC1 and (B) PC2. Fruc, fructose; glu, glucose; and suc, sucrose.
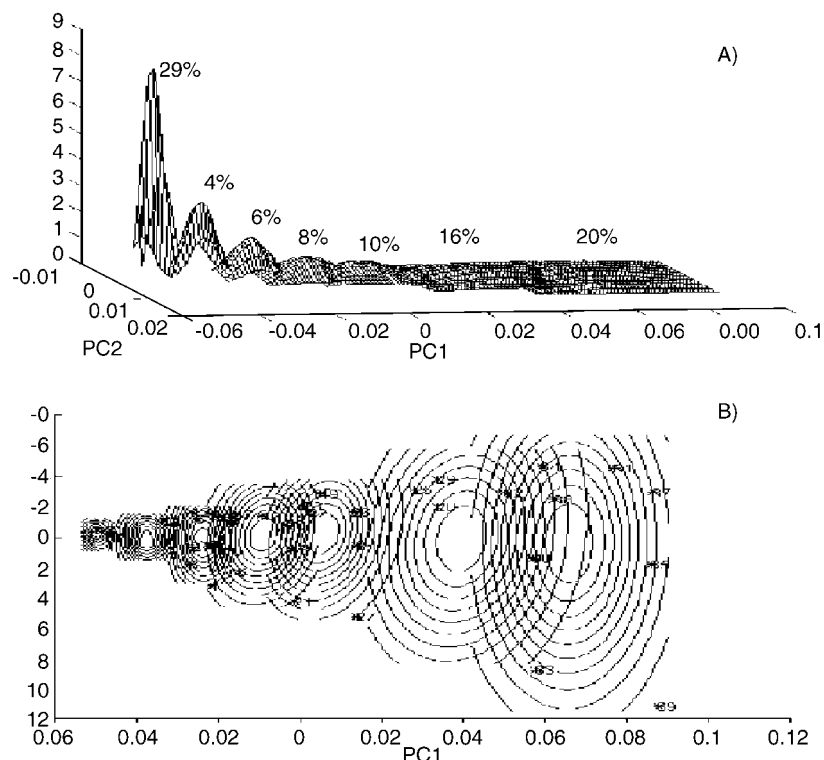
Figure 8. (A) Potential curves obtained for different sample groups in the PC1-PC2 scores subspace (low percentages of juice) and (B) isoprobability ellipses. The (*) represent validation samples.

3.2.2. High Percentage of Apple Juice

The second range (20–100%) comprises soft drinks with high apple juice content such as energy drinks, nectars, when available, and 100% pure apple juices. Often beverages with 25% apple juice—plus other components—are advertised as "energy" drinks. The PC1–PC2 scores subspace explained about 99.9% of the variability, 99.1% and 0.8% for PC1 and PC2, respectively, and showed a quite good separation between groups. The loadings are the same as for the low apple content samples.

In this range the classes need to be more separated than in the lower range since they are less differentiated. There is a important overlap between the 20% and 25% groups, but we decided to keep the 20% group. Table 3

Table 3. Parameters to evaluate the goodness of the model for each concentration range.

	2%	4%	6%	8%	10%	16%	20%
Efficiency (%)	100	100	100	75	71.4	100	71.4
Sensibility (%)	100	100	92.3	60	100	60	100
Specificity (%)	100	100	100	96.7	93.3	100	93.3

	20%	25%	50%	70%	100%
Efficiency (%)	66.7	88.2	92.9	100	100
Sensibility (%)	40	83.3	100	100	100
Specificity (%)	97.4	88.5	96.6	100	100

shows efficiency, sensitivity, and specificity for the validation set, which are fairly good, except for the 20–25% overlap mentioned.

Up to 20 commercial beverages out of 21 were classified correctly (Table 4). The only exception was the "PJ19" juice, which presented anomalous spectral behavior with very low absorbances. This suggests it may contain less than the declared 100% juice. Note that the two different brands of soft drinks with high apple juice (25%) were correctly classified, with low probability of being included into the 20% class.

4. CONCLUSIONS

Inspection of the FTMIR-ATR spectral profiles allows detection of sugars added to commercial juices and soft drinks. The methodology has high precision and is useful to detect changes in sugar contents.

A combination of FTIR-ATR as the measuring technique and potential curves as a classification tool was successful to classify commercial samples. The main limitation is that the percentage of apple juice declared on European soft drinks must be assumed correct to prepare synthetic blanks. Although they are likely to be correct in most cases analysis would be easiest if percentages of sugars added by the maker were declared as in products in the USA. Inspection of loadings revealed that samples are distributed according to (i) the contents of sugars and (ii) the sucrose vs. fructose–glucose "ratio."

The initial implementation of the methodology is a bit slow due to the calibration stage required, as for any multivariate-based procedure. Nevertheless, routine use and classification of commercial beverages is faster, at less

Table 4. Classification of commercial beverages using potential curves. The assignments are italicized.

Sample	Container	2%	4%	6%	8%	10%	16%	20%	% Juice declared
SF-1	Can 33cL	0.00	0.00	0.18	11.46	*32.15*	2.56	0.17	10
SF-2	Can 33cL	0.00	0.00	0.00	0.01	1.45	*79.35*	18.12	16

Sample	Container	20%	25%	50%	70%	100%	% Juice declared
PJ1	Brick 1L	0.00	0.00	0.00	5.53	*76.60*	100
PJ2	Brick 1L	0.00	0.00	0.00	3.44	*68.81*	100
PJ3	Brick 1L	0.00	0.00	0.00	0.00	*55.85*	100
PJ4	Brick 1L	0.00	0.00	0.00	6.99	*91.70*	100
PJ5	Brick 1L	0.00	0.00	0.00	0.09	*83.51*	100
PJ6	Brick 1L	0.00	0.00	0.00	3.18	*94.24*	100
PJ7	Glass 1L	0.00	0.00	0.00	0.27	*64.17*	100
PJ8	Glass 1L	0.00	0.00	0.00	2.60	*80.36*	100
PJ9	Brick 1L	0.00	0.00	0.00	4.07	*95.42*	100
PJ10	Brick 1L	0.00	0.00	0.00	0.02	*33.21*	100
PJ11	Brick 1L	0.00	0.00	0.00	0.69	*98.38*	100
PJ12	Brick 1L	0.00	0.00	0.00	0.24	*66.31*	100
PJ13	Brick 1L	0.00	0.00	0.00	0.90	*98.96*	100
PJ14	Brick 1L	0.00	0.00	0.00	4.64	*95.05*	100
PJ15	Brick 1L	0.00	0.00	0.00	4.69	*94.94*	100
PJ16	Brick 1L	0.00	0.00	0.00	0.22	*92.82*	100
PJ17	Brick 1L	0.00	0.00	0.00	2.12	*98.60*	100
PJ18	Brick 1L	0.00	0.00	0.00	2.84	*92.93*	100
PJ19	Brick 1L	0.00	0.00	0.09	*62.47*	39.71	100
E1	Brick 1L	24.06	*83.05*	1.05	0.03	0.00	25
E2	Glass 500 mL	0.23	*14.66*	2.53	0.12	0.01	25

than 10 min per sample. It may be possible, using multivariate regression methods instead of the matching step, to speed up the calibration steps and this will be tested.

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