

Quantification of Glucose, Fructose and Sucrose in Apple Juices Using ATR-MIR Spectroscopy Coupled with Chemometry

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Abstract. A combination of Fourier transform infrared spectroscopy (FTIR) and multivariate statistics (chemometry) was applied as a screening tool for the quantitative determination of carbohydrates, such as glucose, fructose and sucrose, in 11 processed commercial apple juices and 2 genuine juices obtained from squeezed apples. For calibration, a number of 24 mixtures of glucose, fructose and sucrose solutions (synthetic samples), at different concentrations were prepared and scanned in the 885 and 1500 cm^{-1} spectral range, using attenuated total reflectance (ATR) FTIR spectroscopy. Based on the mixtures spectra in the mid IR (MIR) region, we realized calibration models for each carbohydrate, using partial least squares (PLS) regression. The models were then used to predict the glucose, fructose and sucrose concentration in commercial apple juices, comparatively with concentrations in fresh, genuine juices, in order to assess the juice authenticity. The glucose concentrations (% w/w) predicted for commercial juices ranged from 1.664 to 3.133 versus 3.1 for genuine fresh juices. The fructose concentrations (% w/w) predicted for commercial juices ranged from 3.701 to 6.941 versus 9.2 for genuine fresh juices, while the sucrose concentrations (% w/w) predicted for commercial juices ranged from 0.746 to 5.795 versus 1.38 for genuine fresh juices. The standard deviations of most predicted values are below 10%. Most juices exhibited glucose, fructose and sucrose concentrations in the expected range. However, several samples showed discrepancies from average concentration values, thus the authenticity of these juices could not be confirmed. Also, high sucrose concentration can flag adulterated juices, or indicates sucrose addition to maintain the juice sweetness intensity. Our results indicate ATR-MIR spectroscopy to be a rapid, accurate, non-destructive and cost-effective tool for routine monitoring of multiple constituents in apple juices, as quality and safety indicators.

Keywords: glucose, fructose, sucrose, ATR-MIR, FTIR, chemometry.

INTRODUCTION

Fruit juice quality and authenticity determination is an important applied research area, with relevant impact in industry, food safety and consumer protection. Since decades, unscrupulous companies, manufacturers or traders seek substantial benefits using adulterated juices to gain a market advantage over honest competitors by using cheaper ingredients (sugar and syrups) or raw materials and false label indications for the consumers. Investigations on juice adulteration have shown that, as long as analytical capabilities have improved, the adulteration practices became more sophisticated (Brause *et al.* 1998).

Discrepancies in component ratios can be used to flag suspicious samples. Water and carbohydrates are the main components of apple juice, glucose, fructose, and sucrose being the main authenticity markers, with an approximate ratio of 3:6:2 and ranges from 1 to 4, 5 to

8 and 0 to 5% w/w, respectively (Irudayaraj and Tewari 2003). Downey et al indicate an average concentrations in apple juice of 2.5, 5.6 and 1.7 % w/w for glucose, fructose and sucrose, respectively (Downey and Kelly, 2006). Paty et al, indicate mean values for glucose, fructose and sucrose of 2.3, 6.7 and 1.9, respectively (Paty *et al.* 2006).

Various techniques such as gas chromatography (GC), high-performance liquid chromatography (HPLC), stable isotope analysis, electrophoresis, immunoassays, DNA analysis, and NIR spectroscopy have been developed and many different classes of compounds have been considered as tracers of juice adulteration (using proteins, carbohydrates, fatty acids, pigments and organic acids as markers) (Prodolliet, 1998).

However, the chromatographic techniques (GC, HPLC), successfully used to determine fruit juice authenticity by oligosaccharide profiling (Pan *et al.* 2002), although accurate, are time consuming, expensive and difficult to implement in an on-line setting.

Other spectroscopic approaches, such as nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy, considering the entire sample composition, were also applied for authenticity studies and food composition profiling (Kelly *et al.* 2004).

The absorption bands between 885 and 1500 cm^{-1} , in the mid infrared (MIR) region, are characteristic for carbohydrate specific bonds and functional groups. These bands correspond to stretching, bending and wagging molecular vibrations as well molecular rotations and may be used to fingerprint a given compound in a mixture. This type of FTIR spectroscopy is extremely fast (tests can be carried out in 1-2 min), simple to use and cheap. It is non-destructive, no sample preparation is required and no waste material is produced during a test (Kelly *et al.* 2005).

When the absorption bands do not overlap, a simple mathematical calculation (using Beer-Lambert's calibration of peak heights or area) related to the single analyte concentration is possible. When multiple analytes are to be determined and when the absorption peaks overlap, one of several multivariate methods such as PLS or principal component regression (PCR) need to be used in conjunction with the spectra, developing multi-component calibration models (Irudayaraj and Tewari 2003). Such chemometry methods are applied to reduce the dimension of the collected data and to extract the useful quantitative information from the complex spectra. Such analysis of attenuated total reflectance (ATR) spectra in the MIR region have been used previously to determine sugar and/or ethanol concentrations in fermentation broths, as well as in sugar solutions (Irudayaraj and Tewari, 2003).

In this study, we used the ATR-MIR spectroscopy, in combination with chemometry in order to quantify simultaneously the glucose, fructose and sucrose content in 11 processed commercial apple juices, comparatively with freshly extracted juice, after a former calibration with different synthetic solutions of these three carbohydrates, used as authenticity biomarkers.

MATERIALS AND METHODS

Samples. A number of 11 apple juices were supplied from different supermarkets. Genuine (authentic), pure apple juices were obtained by squeezing a Golden Delicious apple and a Jonagold apple. The 13 samples are described in Tab. 1, accordingly to the package label.

Tab. 1.

Samples notation and short description

Nr.	Sample	Description
1	A1_100%	apple juice 100%, green apple
2	A2_100%	apple juice 100%
3	A3_100%	apple juice 100%
4	A4_100%	apple juice 100%
5	A5_100%	apple juice 100%
6	A6t_100%	apple juice 100%, turbid
7	A7t_100%	apple juice 100%, turbid
8	A8t_100%	apple juice 100%, turbid
9	A9c_100%	apple juice 100%, from concentrate
10	A10c_100%	apple juice 100%, from concentrate
11	A11_50%	apple juice 50%
12	Golden	Authentic juice from squeezed Golden apple
13	Jonagold	Authentic juice from squeezed Jonagold apple

Different concentrations (g / 100 ml) of standard solutions of glucose (g) (1.0, 1.25, 2.0, 2.5, 4.0 and 5), fructose (f) (3.25, 3.5, 6.0, 6.5, 7.0, 9.0 and 13.0) and sucrose (s) (0.75, 1.0, 1.5, 2.0, 3.0, 4.0) were used to prepare 24 synthetic sample mixtures (coded g_f_s). The prepared samples covered the minimum and maximum concentration ranges representative for natural samples and were used for calibration model development.

Instrumentation. The MIR spectra were recorded on a single reflection ATR unit at room temperature using a Tensor FTIR spectrometer (Bruker, Germany), equipped with a liquid nitrogen cooled highly sensitive mercury cadmium telluride (MCT) detector. The spectral resolution was 4 cm^{-1} and 32 scans were accumulated for each spectrum.

Quantitative Analysis. The Unscrambler software (CAMO, Norway) for quantitative analysis was applied for the PLS routine analysis. The calibration models were developed using the first-derivative (Savitsky-Golay) transformed spectra of the 24 synthetic solutions of glucose, fructose and sucrose. The spectra were smoothed, by averaging 4 point left side and right side. The carbohydrates fingerprint region in the spectral range $885\text{-}1500\text{ cm}^{-1}$ was used for calibration and prediction purposes. The developed calibration models were first cross-validated in all cases to minimize the risk of overfitting and then used for testing or validation with the juice sample FTIR data.

RESULTS AND DISCUSSION

1. Specific ATR-MIR fingerprint of main carbohydrates found in apple juices. In order to fingerprint the carbohydrate region and specific glucose, fructose and sucrose absorptions we recorded the ATR-MIR absorption spectra of individual solutions at different concentrations, e.g. glucose (at 5%), fructose (at 13%) and sucrose (at 3%), as presented in Fig.1.

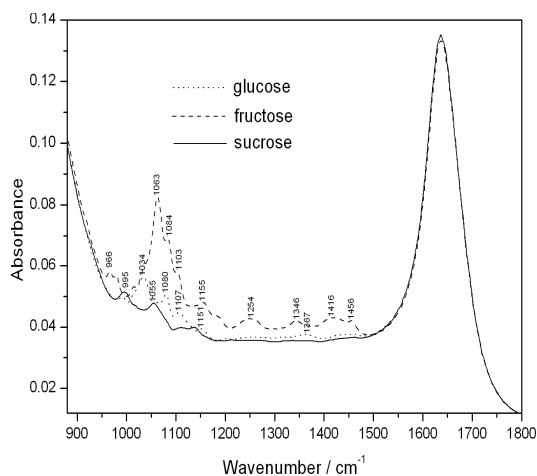


Fig. 1. Specific ATR-MIR spectra of aqueous solutions of glucose (5%), fructose (13%) and sucrose (3%)

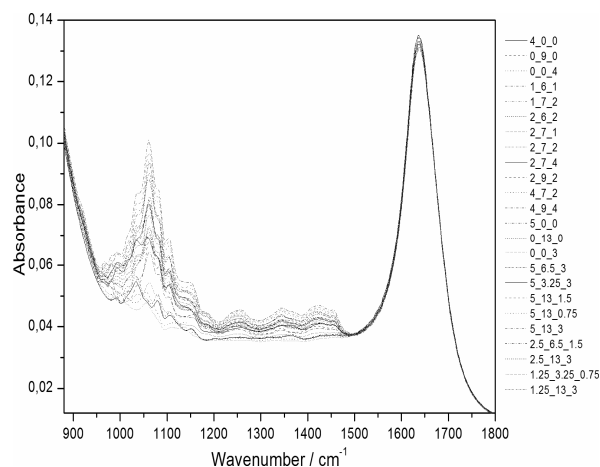


Fig. 2. Specific fingerprint of the carbohydrate synthetic solutions, coded according to Tab. 2

All MIR spectra of these solutions are dominated by a strong water absorption band, centered at 1650 cm^{-1} . However, a spectral window between 885 and 1500 cm^{-1} remains available for observing absorptions of carbohydrates characteristic bands. In this region we identified the characteristic bands of glucose (specific maxima at 991 , 1034 , 1080 , 1107 , 1151 , 1367 and 1460 cm^{-1}), fructose (specific maxima at 966 , 1063 , 1155 , 1254 , 1346 , 1416 and 1456 cm^{-1}) and sucrose (specific maxima at 995 , 1055 , 1113 , 1138 , 1338 and 1464 cm^{-1}). These data may be useful to recognize in apple juice extremely high concentrations of sucrose as adulteration marker. The bands in the region 904 – 1153 cm^{-1} are assigned to C–O and C–C stretching modes, while those in the 1474 – 1199 cm^{-1} region are due to O–C–H, C–C–H and C–O–H bending vibrational modes of the carbohydrates (Irudayaraj and Tewari, 2003).

2. ATR-MIR spectra and quantitative predictions of carbohydrate concentrations in synthetic mixtures of glucose, fructose and sucrose solutions. The ATR-MIR spectra recorded for the 24 synthetic mixtures of glucose, fructose and sucrose solutions are shown in Fig.2. Based on these spectra, we applied the multivariate statistics which facilitates the simultaneous inclusion of multiple spectral intensities and improves the precision and predictive ability of the analysis. Thus, the PLS method was applied for the multivariate calibration models (synthetic samples with predicted carbohydrate content). Table 2 includes the reference and predicted concentrations (% , w/w) of the synthetic solutions of glucose (glu), fructose (fru) and sucrose (suc) (coded as g_f_s) and their corresponding standard errors (SE). The three calibration models obtained for the carbohydrates, glucose, fructose and sucrose were further used to predict the concentration of these components in the samples (commercial and genuine ones). Thus, the models were sequentially loaded in the Unscrambler program and applied to the spectra of the 13 samples.

Tab. 2.

Reference and predicted concentrations (% , w/w) of the synthetic solutions of glucose (glu), fructose (fru) and sucrose (suc) (coded as g_f_s) and their corresponding standard errors (SE)

Sample type g_f_s	glu %	Predicted glu	SE glu	fru %	Predicted fru	SE fru	suc %	Predicted suc	SE suc
4_0_0	4	3.996	0.367	0	-0.492	0.471	0	0.0286	0.228
0_9_0	0	-0.318	0.356	9	9.017	0.487	0	-0.0908	0.223

0_0_4	0	0.00224	0.362	0	0.184	0.467	4	3.943	0.232
1_6_1	1	0.896	0.344	6	5.958	0.456	1	0.92	0.209
1_7_2	1	0.958	0.343	7	7.642	0.446	2	2.347	0.208
2_6_2	2	2.493	0.338	6	5.969	0.445	2	2.333	0.204
2_7_1	2	2.459	0.337	7	7.401	0.447	1	0.895	0.206
2_7_2	2	2.646	0.338	7	7.489	0.444	2	2.321	0.204
2_7_4	2	2.694	0.338	7	7.559	0.452	4	3.729	0.212
2_9_2	2	2.55	0.339	9	9.008	0.446	2	2.354	0.205
4_7_2	4	4.056	0.342	7	7.508	0.45	2	2.436	0.207
4_9_4	4	4.306	0.345	9	8.902	0.467	4	3.7	0.214
5_0_0	5	4.683	0.374	0	-0.607	0.472	0	-0.127	0.234
0_13_0	0	-0.303	0.366	13	13.004	0.502	0	-0.0313	0.23
0_0_3	0	-0.0823	0.365	0	0.0452	0.468	3	2.974	0.225
5_6.5_3	5	4.744	0.348	6.5	6.548	0.46	3	2.91	0.212
5_3.25_3	5	4.819	0.354	3.25	3.29	0.47	3	2.968	0.216
5_13_1.5	5	4.67	0.356	13	12.958	0.462	1.5	1.528	0.216
5_13_0.75	5	4.58	0.354	13	12.726	0.459	0.75	0.756	0.219
5_13_3	5	4.862	0.359	13	12.86	0.473	3	2.881	0.217
2.5_6.5_1.5	2.5	2.233	0.338	6.5	6.59	0.446	1.5	1.34	0.205
2.5_13_3	2.5	2.369	0.345	13	11.473	0.454	3	2.872	0.21
1.25_3.25_0.75	1.25	0.995	0.348	3.25	3.224	0.464	0.75	0.64	0.213
1.25_13_3	1.25	1.192	0.357	13	13.242	0.462	3	2.87	0.217

3. ATR-MIR spectra and quantitative predictions of carbohydrate concentrations in commercial and authentic juices. Fig.3 represents the ATR-MIR spectra of the 13 apple juice samples (commercial and genuine ones), presented in Tab. 1.

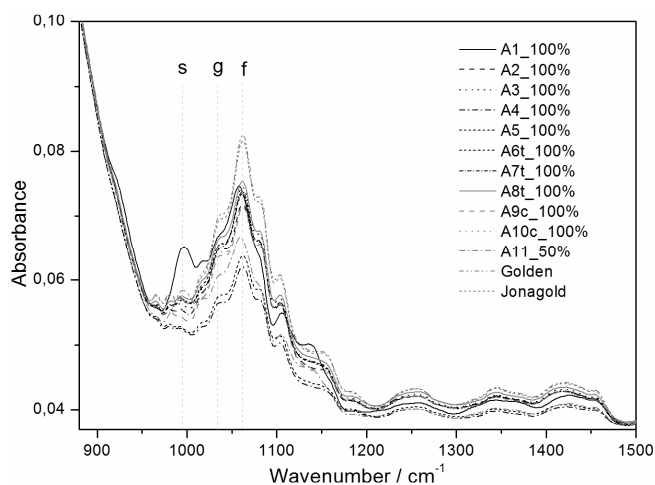


Fig. 3. ATR-MIR spectra of the commercial and genuine apple juices (presented and coded in Tab. 1). The fingerprint regions of glucose (g), fructose (f) and sucrose (s) are marked by dotted lines

The characteristic bands of the carbohydrates in apple juice appear predominantly in the 885 – 1500 cm^{-1} spectral range. Qualitatively one can identify the peaks characteristic to glucose, fructose and sucrose at 1034, 1063 and 995 cm^{-1} , respectively, in accordance with the peaks observed in Fig.1. By using only these marker bands, a quantitative evaluation of the carbohydrates in juices is extremely difficult or even speculative, considering the overlapping

of these peaks with different absorption intensities. Using the data obtained from the synthetic solutions (Tab. 2), by means of chemometric methods, we were able to predict the glucose, fructose and sucrose concentrations in the commercial and genuine juice samples, as presented in Fig.4.

Tab. 3 includes the predicted values of glucose, fructose and sucrose in the analyzed juice samples. The maximum values for glucose (3.166%) and fructose (9.344%) were obtained for the Golden Delicious squeezed apple juice, the Jonagold squeezed apple juice showing also similar values. The sucrose concentrations in the Golden Delicious and Jonagold samples were 1.393% and 1.381%, respectively.

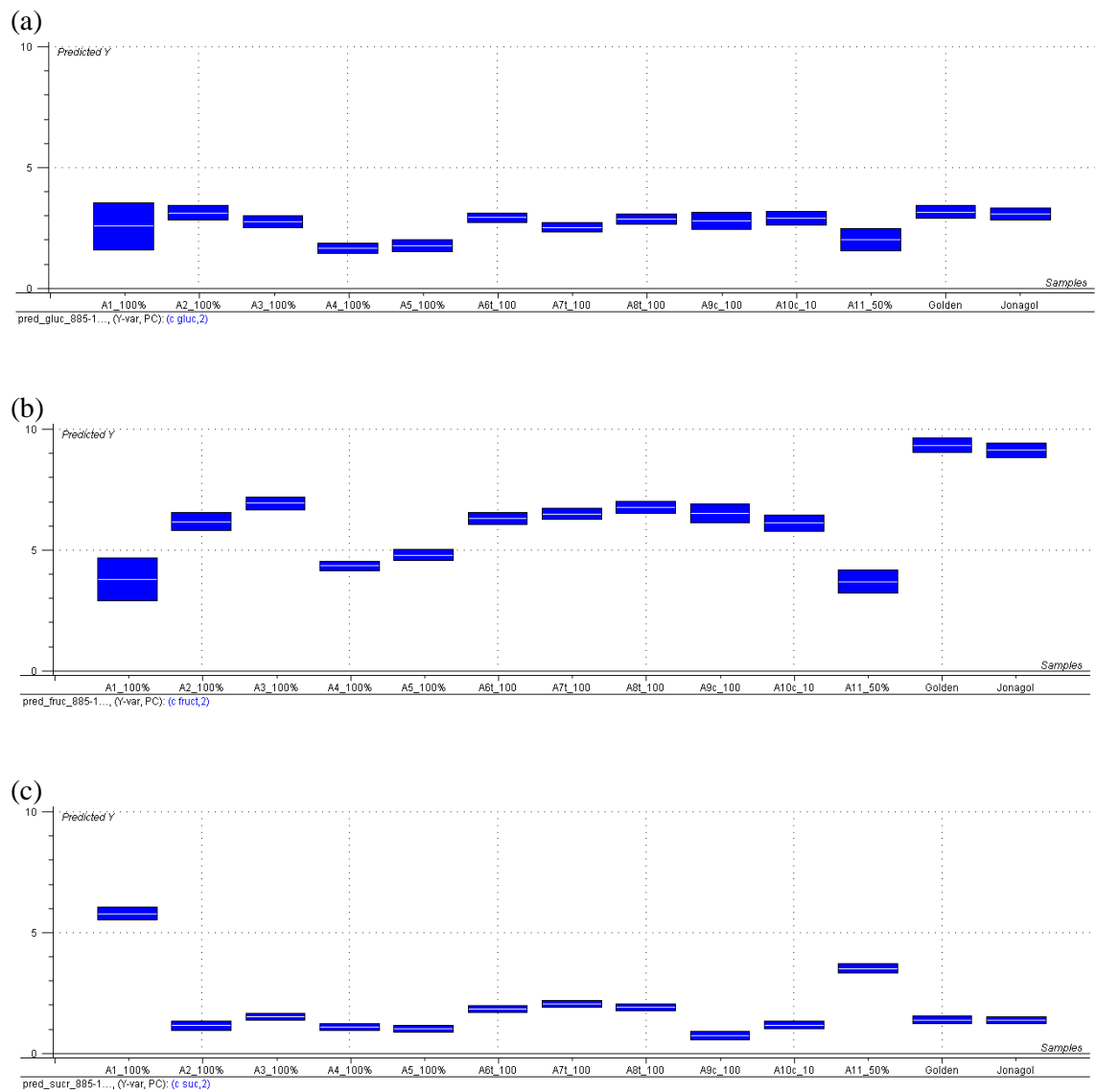


Fig. 4. Predicted values for glucose, fructose and sucrose concentrations in the commercial and genuine apple juices using the PLS regression models

Tab. 3

Predicted glucose, fructose and sucrose values and standard deviations (SD) for the juice samples described in Tab. 1

Sample	Predicted glu %	SD glu	Predicted fru %	SD fru	Predicted suc %	SD suc
A1_100%	2.576	0.974	3.796	0.885	5.795	0.281
A2_100%	3.133	0.297	6.184	0.366	1.155	0.187
A3_100%	2.758	0.235	6.941	0.267	1.512	0.149
A4_100%	1.664	0.227	4.347	0.207	1.1	0.146
A5_100%	1.779	0.251	4.796	0.227	1.03	0.146
A6t_100%	2.925	0.198	6.31	0.236	1.843	0.147
A7t_100%	2.52	0.201	6.507	0.224	2.041	0.137
A8t_100%	2.865	0.206	6.775	0.238	1.901	0.146
A9c_100%	2.804	0.363	6.519	0.394	0.746	0.179
A10c_100%	2.911	0.286	6.128	0.334	1.176	0.171
A11_50%	1.999	0.462	3.701	0.478	3.518	0.188
Golden	3.166	0.255	9.344	0.303	1.393	0.158
Jonagold	3.087	0.253	9.141	0.295	1.381	0.154

The “100% natural” labeled juices A2_100% and A3_100% present comparable carbohydrate concentrations with the genuine, squeezed apple juices indicating their authenticity. However, we observed higher fructose content in the squeezed juice, most probably because of the extra quality of these apples.

The authenticity of the samples A4_100% and A5_100% could not be confirmed by ATR-MIR analysis. The concentrations of glucose (1.664%) and fructose (4.374%) are much lower than for the above mentioned “100% natural” labeled juices. The sample A1_100% shows high SD in the determination of glucose and fructose, but the results show clearly a high sucrose concentration. The high sucrose concentration can be observed in the fingerprint region in Fig.3 and is also confirmed by the predicted value 5.795% in Table 3. The high sucrose content indicates an adulteration of this juice.

The processed commercial apple juices obtained from concentrate (A9c_100% and A10c_100%), as well as the turbid juices A6t_100%, A7t_100% and A8t_100% present good quality in terms of glucose, fructose and sucrose content, due to close carbohydrate concentration values to the other pure juices. The juice turbidity did not influence the IR absorption, thus the turbid juices could be also successfully classified.

The apple juice A11_50% (50% fruit juice content) shows approx. 60% of the glucose (1.999%) and fructose (3.701%) content of the genuine natural apple juice, whereas the sucrose (3.518%) concentration is almost two times higher than in pure apple juice, probably due to the addition of supplementary sucrose, necessary to maintain the sweetness intensity.

CONCLUSIONS

We applied here a combination of Fourier transform infrared spectroscopy (ATR-MIR) and multivariate statistics (chemometry) as a screening tool for apple juice authenticity, by determination of glucose, fructose and sucrose concentrations in 11 processed commercial apple juices and comparing the values with 2 genuine juices from squeezed apples. To

calibrate and predict correctly the carbohydrate concentrations in the apple juice samples, a number of 24 mixtures of glucose, fructose and sucrose solutions (synthetic samples), at different concentrations were prepared and the predicted values were obtained based on absorptions recorded between 885 and 1500 cm^{-1} and partial least squares (PLS) regression. The glucose concentrations predicted for commercial juices ranged from 1.664 to 3.133 versus 3.1% w/w for genuine fresh juices. The fructose concentrations predicted for commercial juices ranged from 3.701 to 6.941 versus 9.2% w/w for genuine fresh juices, while the sucrose concentrations predicted for commercial juices ranged from 0.746 to 5.795 versus 1.38% w/w for genuine fresh juices.

Most juices exhibited carbohydrate concentrations in the expected range, with an average ratio of 2.8, 6.5 and 1.5% w/w for glucose, fructose and sucrose, respectively. However, several samples showed discrepancies from average values, thus the authenticity of these juices could not be confirmed. Also, high sucrose concentration can flag adulterated juices, as observed for sample A1_100%, or indicates sucrose addition to maintain the sweetness intensity, as observed for sample A11_50%.

Analyzing the prediction results, it can be observed that for most juice samples the standard deviation of the predicted values is below 10%. The turbidity of several juices did not induced perturbations in the IR analysis.

Overall, our results suggest that ATR-MIR is a, rapid, accurate, non-destructive and cost-effective tool for routine monitoring of multiple constituents in a apple juices, as quality and safety indicators.

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