Improving reproducibility of refractometry measurements of fruit juices

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INTRODUCTION

The experiments detailed in this report suggest different methods for improving the reproducibility of natural strength fruit juice measurements on a refractometer and illustrate the improvements achievable by specific examples with various types of fruit juices.

BACKGROUND

Refractometers have been widely used for many years in the food industry, for measuring such products as sugar in refineries, jams, jellies and preserves, alcoholic beverages, soft drinks and fruit juices. A refractometer basically measures the refractive index of light, which is a measure of the speed of light through the substance, solid or liquid. This in turn is proportional to the total dissolved solids in for example a liquid. Hence measurement of refractive index can be used as a measure of product concentration. Refractometry is a technique of physical chemistry measuring one physical parameter of a product. Even when compared with chemical analysis techniques which give detailed information of product composition, refractometry still has an important role to play, especially in quality control because it is a very fast and easy to use method for checking product quality on a routine basis, often by relatively unskilled staff. This report describes techniques for obtaining good reproducibility of refractometer measurements of fruit juices.

THE BRIX SCALE

Traditionally in the fruit juice industry, most users have selected the Brix scale to express their refractometer readings. The term "Brix" comes from the sugar refining industry and means grams of sucrose per 100 grams of sucrose solution in water. Use of the Brix scale can be misleading if used for substances other than pure sucrose, especially when temperature corrections have to be made, however because it is so widely used, the following measurements have all been expressed in Brix units. All readings here were taken at 20.0°C, and so no temperature corrections needed to be made. For full details of the relationship between Brix and refractive index, see reference 1.

GENERAL COMMENTS AND EQUIPMENT USED

Long experience has shown that the most important factors affecting reproducibility when making refractometer measurements are good cleaning procedures and practices and consistency of sampling technique. This is illustrated by the following specific examples for various fruit juices. In all cases, except as otherwise stated, the refractometer used was a fully automatic instrument with digital read-out, accurate to ±0.05 Brix, with a resolution of 0.01 Brix. For these tests, readings were recorded manually, however for an automatic hard copy result, a printer could have been connected to the refractometer. The refractometer would also accept a bar code reader for automatic sample identification.

SAMPLES

As a representative selection of different types of natural strength fruit juices the following samples were used;

BRIEF NAME	DESCRIPTION ON CONTAINER
Clear apple	Pure apple juice, unsweetened, made with concentrated apple juice
Cloudy apple	Pressed apple juice made from freshly pressed apples

Apple and mango	Pressed apple and mango juice - a blend of freshly pressed apple juice and mango puree
Grapefruit	Pure Florida pink grapefruit juice made with concentrated grapefruit juice
Pineapple	Pure pineapple juice made with concentrated pineapple juice
Grape juice	American Concord grape juice made from concentrated Concord and other selected grape juices
Orange with bits	Florida pure premium original with juicy bits of orange, 100% squeezed, not from concentrate
Orange	Florida pure premium smooth style (no bits) 100% pure Florida orange juice not from concentrate

All samples were purchased from a local supermarket in ready to drink form and all were measured straight from a freshly opened bottle or carton. The container was thoroughly shaken before the juice was poured into a beaker for sampling. The fluid in the beaker was stirred or agitated before ingesting or pipetting the sample. All samples were measured without pre-treatment except for the orange juice with bits which was sieved through a 1mm mesh metal sieve to prevent clogging of the pipes.

All samples were at approximately room temperature, which was a comfortable 21 to 22°C before they were applied to the refractometer.

METHOD 1

The refractometer was fitted with a hinged sample cover which, when closed pressed the sample onto the refractometer prism with a gap of less than 0.5mm. Temperature control was by flowing water from a thermocirculator to both the base of the refractometer prism and the hinged sample cover. Samples were applied using disposable plastic pipettes (1ml size). After drawing the sample up into the pipette, the bulb was squeezed gently to expel any air in the pipette so that no bubbles were dropped onto the prism surface. About 4 drops of sample were applied each time, equivalent to between 0.1 and 0.2ml. After every sample measurement, both the prism and the sample cover were wiped clean with a soft tissue. The prism was then washed by flooding with 1 or 2ml of pure water. This gave enough water, when wiped with a soft tissue, to also wash the cover. The prism was then polished with a clean dry soft tissue and the cover dried before applying the next sample. If a bubble was formed in the sample, or if the refractometer display indicated difficulty in reading any particular sample, that sample was wiped off, the prism cleaned again in the standard way and a fresh sample applied. It was found that, if an attempt was made to clear bubbles, or lift and replace the cover a few times to make the instrument read, spurious results often occurred.

The refractometer was set to read out after a fixed time delay. This stabilisation time was chosen to allow sufficient time for the sample to come to thermal stability with the prism but to keep the overall sampling time as short as possible. Checks were made with each type of sample to find the time needed so that any subsequent changes were less than 0.03 Brix. For most samples this was 15 seconds; some samples (pineapple juice and orange juice) required 30 seconds.

20 replicate samples of each juice were measured in this way and the results are given in Table 1. The average value for each juice is also given along with the computed standard deviation.

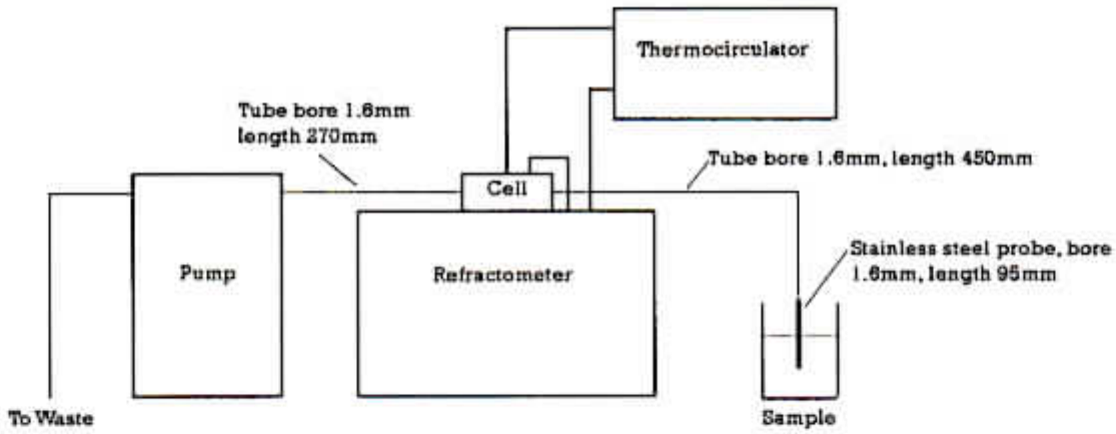
Method 1
SGP-1 Cell

	Clear Apple	Cloudy Apple	Apple & Mango	Grape-fruit	Pine-apple	Grape juice	Orange with bits
	11.68	11.48	12.73	10.30	13.14	15.89	11.97
	11.67	11.45	12.70	9.88	13.15	15.80	11.87
	11.66	11.48	12.72	10.27	13.08	15.95	11.91
	11.67	11.47	12.75	10.30	13.10	15.99	11.89
	11.67	11.48	12.69	10.31	13.10	15.88	11.98
	11.66	11.45	12.69	10.29	13.11	15.86	11.36
	11.66	11.47	12.72	10.29	13.11	15.88	11.88
	11.65	11.46	12.75	10.25	13.08	16.05	11.96
	11.66	11.47	12.61	10.30	13.10	15.87	11.85
	11.66	11.43	12.72	10.32	13.15	15.88	11.98
	11.66	11.50	12.70	10.26	13.12	15.22	11.92
	11.67	11.42	12.60	10.28	13.12	15.87	11.93
	11.79	11.49	12.75	10.21	13.16	15.88	11.85
	11.66	11.45	12.74	10.24	13.09	15.89	11.90
	11.74	11.44	12.69	10.30	13.07	15.87	11.95
	11.66	11.50	12.68	10.28	13.09	15.87	11.92
	11.66	11.49	12.67	10.26	13.13	15.88	11.88
	11.66	11.45	12.73	10.29	13.13	15.73	11.91
	11.65	11.46	12.72	10.46	13.10	15.88	11.86
	11.66	11.47	12.74	10.29	13.13	15.86	11.91
Average	11.67	11.47	12.71	10.27	13.11	15.88	11.88
Std Dev	0.033	0.022	0.041	0.101	0.025	0.062	0.127
Max	11.79	11.50	12.75	10.46	13.16	16.05	11.98
Min	11.65	11.42	12.60	9.88	13.07	15.73	11.36

Table 1.

METHOD 2

The refractometer was fitted with a small volume flow cell, approximate static volume 0.2ml, inlet and outlet tubes 1.6mm bore. A programmable peristaltic pump was used to ingest a pre-set volume of sample and to signal the refractometer to begin a measuring cycle once the sample was ingested. The temperature was controlled by flowing water from a thermocirculator to both the base of the prism and the flow cell. The apparatus is illustrated in Figure 1.



To eliminate the possibility of cross contamination, the pump was started just before the probe was put into the sample or water and the probe was wiped with a soft tissue between each sample. The pump was set to take 5ml of sample. This was found to be sufficient for a sample to displace water in the cell. This was checked by increasing the volume to confirm no further increase in reading. After every 10 samples, two 5ml ingestions of pure water were taken to check that the cell was being thoroughly flushed by checking that the refractometer reading returned to 0.00 ±0.01 Brix. A juice sample was never left standing in the cell; the system was always flushed with pure water after a batch of samples before leaving the equipment for any length of time. The refractometer was set to read 30 seconds after the sample had been ingested. This delay time was selected in a similar way as for Method 1 to allow for temperature stability. Checks were made with each type of sample and no changes greater than 0.02 Brix were observed within a minute. Longer stabilisation times would probably be necessary if the samples were much cooler or warmer before ingestion.

This method proved satisfactory for all the juice samples tested with the exception of pineapple juice. In this case the cell had to be flushed with water after every 5 samples to prevent build up of particles and sugars in the cell.

20 replicate samples of the same juices as those measured by Method 1 were measured by Method 2 and the results, along with the averages and computed standard deviations, are presented in Table 2.

Method 2 SGP4 Cell	Clear Apple	Cloudy Apple	Apple & Mango	Grape-fruit	Pine-apple	Grape Juice	Orange with bits
	11.65	11.42	12.63	10.24	13.05	15.86	11.76
	11.66	11.41	12.62	10.24	13.01	15.88	11.78
	11.66	11.41	12.66	10.26	13.01	15.87	11.80
	11.66	11.41	12.67	10.25	13.04	15.88	11.77
	11.66	11.42	12.63	10.25	13.02	15.88	11.77
	11.66	11.42	12.62	10.26	13.01	15.87	11.78
	11.65	11.42	12.61	10.26	13.06	15.87	11.76
	11.65	11.42	12.62	10.26	13.07	15.88	11.79
	11.65	11.42	12.62	10.26	13.09	15.88	11.76
	11.66	11.41	12.61	10.25	13.10	15.87	11.76
	11.66	11.42	12.64	10.24	13.02	15.88	11.77
	11.66	11.42	12.61	10.25	13.08	15.87	11.80
	11.65	11.41	12.63	10.25	13.16	15.88	11.83
	11.66	11.42	12.62	10.25	13.16	15.87	11.80
	11.65	11.41	12.61	10.24	13.10	15.87	11.77
	11.66	11.41	12.61	10.25	13.07	15.87	11.81
	11.66	11.41	12.63	10.26	13.12	15.88	11.80
	11.65	11.42	12.63	10.25	13.14	15.86	11.81
	11.66	11.41	12.62	10.26	13.07	15.87	11.78
	11.66	11.42	12.62	10.25	13.07	15.87	11.81
Average	11.66	11.42	12.63	10.25	13.07	15.87	11.79
Std Dev	0.005	0.005	0.016	0.007	0.046	0.006	0.020
Max	11.66	11.42	12.67	10.26	13.16	15.88	11.83
Min	11.65	11.41	12.61	10.24	13.01	15.86	11.76

Table 2

DISCUSSION OF RESULTS

It can be seen by comparing Tables 1 and 2 that, except for pineapple juice, Method 2 gave a considerable improvement in standard deviations over Method 1.

The differences in absolute values found with the two different sampling methods for cloudy juices is the subject of a separate report. (Ref. 2)

ADVANTAGES AND DISADVANTAGES OF METHODS 1 AND 2

Method 1: results subject to skill of operator

requires more effort and concentration from operator

lower initial cost of equipment

higher cost of consumables

easier to see if prism is clean

typical spread of readings (by one skilled operator): ±0.05 for clear samples up to ±0.3 for difficult samples.

Method 2: results not subject to operator

easy to obtain good results

higher initial cost of equipment

more difficult to see if cell is clean

easy to clean provided a standard cleaning procedure is adhered to

typical spread of reading (independent of operator):

±0.01 for clear samples up to ±0.15 for difficult samples (but see Method 5).

potential to further automate analysis using an autosampler

The overall time to complete the measurements was very similar for both methods. The sampling rate for methods 1 and 2 was approximately 55 samples per hour, athough it was much more tiring for the operator to keep up this sampling rate using method 1.

For labelling and packaging purposes, an accuracy of ±0.3 Brix may be acceptable. However, for better monitoring of production and quality control, a change of sampling method can improve the reproducibility or readings. As the fruit juices are all natural products, containing a complex mixture of substances with different contributions to the refractive index of the mixture, it is unlikely to be meaningful to measure Brix to better than ±0.05.

WAYS OF FURTHER IMPROVING REPRODUCIBILTIY

METHOD 3

As a low equipment cost variant on Method 1, individual sample reproducibility can be improved by taking at least 2, but preferably 3 or 5 separate readings of every sample and then taking the average as the best measurement of the sample. With 2 or 3 measurements, a simple average was used. With 5 readings, the largest and smallest readings were discarded and the average of the remaining 3 calculated. Table 3 shows the result of applying this method to the same grape juice results reported in Method 1. It can be seen that a significant reduction in standard deviation is achieved, but not quite as good as method 2.

The disadvantage of this method is the increase in overall sampling time to make multiple measurements and the possibility of arithmetic error, unless the calculations are computerised, but this would again increase the equipment cost.

Continued...

Method 3 SGP1 Cell	Grape Juice	2 average	3 average	5 average
	15.89			
	15.80	15.85		
	15.95		15.88	
	15.99	15.97		
	15.88			15.91
	15.86	15.87	15.91	
	15.88			
	16.05	15.97		
	15.87		15.93	
	15.88	15.88		15.88
	15.88			
	15.87	15.88	15.88	
	15.88			
	15.89	15.89		
	15.87		15.88	15.88
	15.87	15.87		
	15.88			
	15.73	15.81	15.83	
	15.88			
	15.86	15.87		15.87
Average	15.88	15.88	15.88	15.89
Std Dev	0.062	0.047	0.033	0.015
Max	16.05	15.97	15.93	15.91
Min	15.73	15.81	15.83	15.87

Table 3

METHOD 4

For the following tests, we used a similar automatic refractometer but with an accuracy of ±0.1 Brix and a resolution 0.1 Brix. The refractometer was fitted with a funnel cell with a static volume of 3.5ml. Approximately 45ml of sample was poured into the funnel for each measurement. Temperature control was by flowing water from a thermocirculator to both the base of the prism and the funnel cell. The refractometer delay time was set to 60 seconds. 10 replicate readings of an orange juice were taken. These are given in Table 4. For comparison Table 4 also gives results on the same orange juice using Methods 1 and 2.

	Orange Method 4	Orange Method 1	Orange Method 2
	11.9	12.2	12.0
	12.0	12.2	12.0
	12.0	12.2	12.1
	12.0	12.3	12.0
	12.0	12.2	12.0
	12.0	12.2	12.0
	12.0	12.4	12.0
	12.0	12.9	12.0
	11.9	12.4	12.0
	11.9	12.3	12.0
Average	11.97	12.33	12.01
Std Dev	0.05	0.21	0.03
Max	12.0	12.9	12.1
Min	11.9	12.2	12.0

Table 4

The results achieved using the funnel cell were again much more consistent than those using Method 1. The lowest standard deviation is still generally achieved with Method 2.

Method 4 proved to be an alternative way of improving reproducibility without the expense of using a programmable peristaltic pump.

METHOD 5

Because of the difficulties found when measuring pineapple juice, a further experiment was carried out to improve these results. The cell selected was a small volume flow cell, but one that had a static volume of about 0.5ml and inlet and outlet pipes of 3.2mm bore. The method was very similar to Method 2, however optimum settings were found to be a pumped sample volume of 10ml and a refractometer delay time of 60seconds. The cell was flushed with 3 ingestions of 10ml of pure water after every 10 samples. The reading for water returned to 0.00 ±0.01 Brix. The pineapple juice tested was the same type as reported in Table 1, however a fresh container was opened. For comparison, a single result using Method 3 is included in Table 5 along with 20 replicate readings using the larger bore flow cell.

	Pineapple Method 5	Pineapple Method 3	(5 average)
	13.04	13.05	
	13.03	13.13	
	13.06	13.03	
	13.06	13.06	
	13.05	13.05	13.06
	13.01		
	13.04		
	13.04		
	13.07		
	13.03		
	13.01		
	13.06		
	13.03		
	13.03		
	13.04		
	13.03		
	13.04		
	13.07		
	13.09		
	13.04		
Average	13.04		
Std Dev	0.020		
Max	13.09		
Min	13.01		

Table 5

It can be seen from Table 5 that very good reproducibilty was obtained for the difficult pineapple juice sample using Method 5.

CONCLUSION

The most reliable way of improving the reproducibility of the refractometric measurement of fruit juices is to use a consistent sampling method which does not rely on individual operators. A spread of readings of up to 0.3 Brix for juices with suspended particles can be reduced to a maximum spread of ±0.04 Brix even for the most difficult juices.

The refractometers used to carry out the experiments in this report were, for comparison purposes, all from the Index Instruments general purpose (GPR) range of refractometers. The conclusion indicates that the most important consideration for improving reproducibility of fruit juice measurements is the sampling technique.

Index Instruments manufacture three ranges of refractometers (TMR, TCR and GPR), all of which are sutable for measuring fruit juices, each with their own advantages.

The most sensitive and accurate range is the TMR. These instruments have a built in programmable pump for sampling, ensuring the best reproducibility. The maximum Brix measureable is 25%.

The TCR range is available with a selection of optional sample cells to ensure good reproducibiility. The TCR has the added advantage of internal electronic temperature control, eliminating the need for a thermocirculator. This instrument will measure Brix up to 95%.

All GPR instruments, as illustrated by this report, also have a range of optional sample cells to cover most requirements. As with the TCR, samples may be poured through a funnel, or a separate sampling pump is available (Indexpump 300) for improved reproducibility.

REFERENCES

1. "ICUMSA Specification and Standard, SPS-3, Refractometry and Tables - Official", published by the International Commission for Uniform Methods of Sugar Analysis (I.C.U.M.S.A.).
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