Anthocyanins and Assessment of Authenticity of Red/Black Juices

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Abstract

Although the majority of suppliers are honest, there are always a few that take a "flexible view" on what is allowed or are "duped" by their suppliers and do not have the appropriate "checks and balances" in place to detect any extensions. There is a requirement under FSMA[1] for all producers to have a quality assurance program in place and have evidence to verify that the materials they are using are of a suitable quality.

Any authenticity assessment of a red/black juice or purée should always include a screen of the anthocyanin pigments. These natural pigments provide a very useful fingerprinting tool for analysts to detect the extension of these types of products with cheaper juices and/or colorants. However, as with most methods it requires a reference or database for comparison. A recent example of a limitation of this type of approach occurred when a "new" raspberry variety, Driscoll's Maravilla, passed from use in the fresh fruit area into the processing sector. Although the fruit shows the "normal" raspberry pigments, the actual levels of the individual compounds are very different to that seen in other raspberry varieties. Presented in this poster are some data for this "new" variety that will be of use for anyone who buys or uses this type of fruit for processing.

Introduction

The economic adulteration of fruit juices is a problem that occurs from time to time, but is especially a problem when commodity prices are high or a juice is in short supply. Due to this risk there is a statutory requirement, under the food safety Modernisation act[1] for all producers to verify that the products that they pack are authentic. Many different approaches are used to detect the economic adulteration of fruit juices/purées, which include:- isotopic methods, conventional testing for bulk components (like sugars, acids and minerals) and fingerprinting methods (oligosaccharides and anthocyanins).

Stable isotopic methods have been found very useful to detect the addition of added sugar and/or acids, which are considerably cheaper than the fruit juice solids. An authoritative review of this area[2] is to be published shortly by the International Fruit Juice Union (IFU) which will provide an extensive overview of the application of isotopic methods. The use of polyphenols as a fingerprinting tool for fruit juices and purees has also recently be reviewed by IFU[3]. Ideally any analysis undertaken in this area should use validated methods. Although AOAC provides a number of validated procedures for the use on fruit juices, the most comprehensive list of methods is provided by the IFU[4].

The adulteration of most juices generally involves the substitution of fruit juice solids with cheaper sugars and/or acids. This addition may be detected using conventional methods, isotopic procedures and fingerprinting methods such as oligosaccharide and polyphenol profiling. The oligosaccharide method developed by Prof. Low (University of Saskatchewan)[7] still proves useful at detecting the addition of added sugar syrups, that share a common sugar profile (levels of the simple sugars) to fruit juices (IFU rec 4).

All assessments of red/black juices should include an analysis of the "colored" compounds, as any significant extension is likely to need the addition of a material to "top up" the color. This can take the form of; a synthetic colour such as FD&C red 2 (figure 4); a natural color extract from a fruit or vegetable (e.g. grape skin or black carrot extract) (figure 5) or a cheaper juice (e.g. red grape, elderberry). This addition can be detected using either thin layer chromatography[8] or ideally HPLC. IFU has a method for this analysis (IFU 71), which has recently been extensively updated. Peak resolutions have been improved, by the use of column with a smaller particle size Published as part of the method is a reference library which now details the individual anthocyanin pigments in each fruit, so it is ideal for the less experienced analyst.

Unfortunately the "analysis" of the product is only part of the story. Once the data have been collected it needs interpretation. Data may be compared with "your" own reference samples, if available, or with published data. However, the validity of published data may sometimes be suspect. Another, source, is available on the Technical Committee for Juice and Juice products website[9]. However, a more extensive source is contained in the AIJN Code of Practice Reference Guides where criteria have been drawn up for 26 fruits/vegetables juices[6].

Methodologies:

Key tests:- Brix (AOAC 983.17)[5], citric acid (IFU 22[4] or AOAC 986.13), the enzymic method for isocitric acid (IFU 54), sugars (IFU67 or 55 & 56), Na, K, Mg, Ca (IFU 33), oligosaccharides (IFU Rec. 4), ^{13}C-IRMS (AOAC 981.09) for C_4 sugar addition. Other useful tests are malic acid (IFU 65 or AOAC 986.13).

Anthocyanin profile (IFU 71 adapted)

UPLC system: Waters ACQUITY H Class
Column: 15 cm C_{18} column 1.7 µm (BEH or equivalent)
Solvent A = 10% formic acid in H_2O: Solvent B = 50% acetonitrile in H_2O

Flow rate:	0.35 ml/min
Injection volume:	2 µl
Detection:	518 nm UV or DAD detector
Analysis time:	12 minutes

Gradient conditions

Time	%B
0.00	12.0
8.50	30.0
9.00	100
10.00	100
11.00	12.0
12.00	12.0

Photo 1:Size comparison between Maravilla & Heritage varieties

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Table 1: Chemical data for the Maravilla Raspberry variety

		AIJN Reference Guide (RG) for Raspberry		FC Normalised to AIJN minimum	NFC Unnormalised	NFC Unnormalised	FC Normalised (10.6 Brix)
		Column A	Column B	Column C	Column D	Column E	Column F
F		Minimum	Maximum			Average data	
Brix (corr)	(%)	7		7	11.1	10.6	10.6
Sucrose	(g/l)	Typically	< 1	nd	nd	nd	nd
Glucose	(g/l)		38	21.6	29.8	29.1	32.7
Fructose	(g/l)		45	25.1	36.3	35.5	38.0
G/F ratio			0.95	0.86	0.82	0.82	0.86
SFE	(g/l)	23		23.3	44.9	41.4	35.3
Sum of sugars	(g/l)		83	46.7	66.1	64.6	70.7
Sorbitol	(mg/l)		< 150	< 5	21	15	nd
Tit acid (pH 8.1)	mmol/l		280	177.1	313.8	325.2	268.2
Citric acid	(g/l)		18	12.8	21.8	19.7	19.4
Isocitric acid (Total)	(mg/l)	60		53.6	104	100	81.2
Cit/Iso			240	239	210	197	239
Tartaric acid	(mg/l)	Nd		nd	nd	nd	nd
L-malic acid	(g/l)	0.2		0.29	0.64	0.55	0.4
D-Malic acid	(mg/l)	Nd		nd	nd	nd	nd
Formol Index	ml 0.1 M NaOH/100 ml	10		12.9	27.3	26.8	19.5
Na	(mg/l)		< 40	5.2	4.9	5.1	8
K	(mg/l)	1300		1025	1792	1805	1552
Mg	(mg/l)	110		95	147	140	144
Ca	(mg/l)	110		54	97	88	82
P	(mg/l)	100		85	152	148	129
NO_3^-	(mg/l)		< 10	1	6	5	2
Anthocyanin profile		Cyanidin-3-O-sophoroside; Cyanidin-3-O-glucoside; Cyanidin-3-O-glucosyl-rutinoside and Cyanidin-3-O-rutinoside.		Atypical pattern	Atypical pattern	Atypical pattern	Atypical pattern

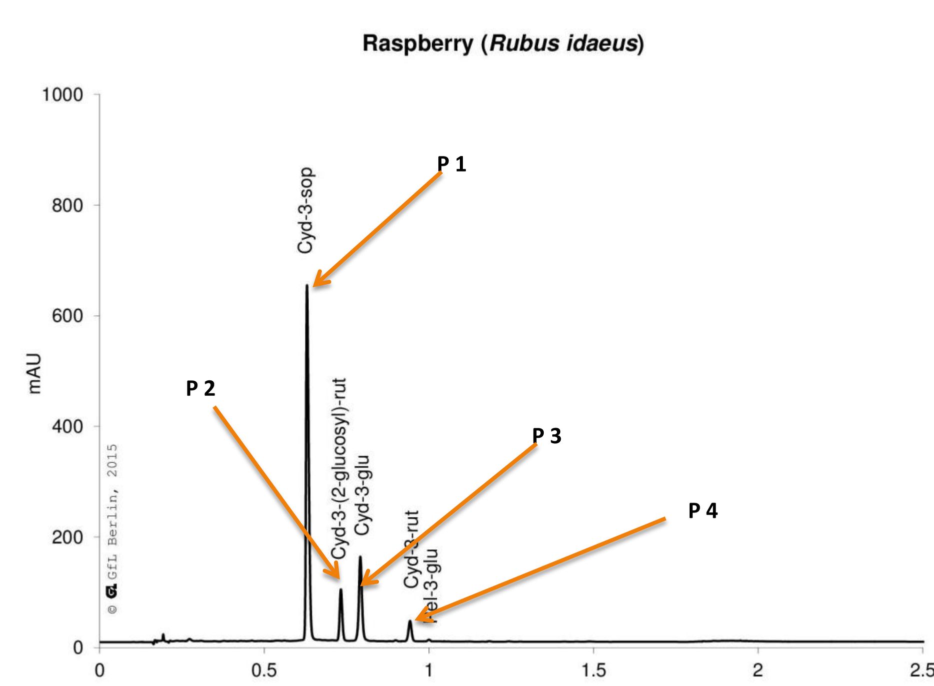

Figure 1: "Typical" anthocyanin profile for raspberries © IFU 2015

References:

(1) 2011 Food Safety Modernisation Act. http://www.fda.gov/Food/GuidanceRegulation/FSMA/
(2) International Fruit Juice Union Recommendation # 3 "The Use of Isotopic Procedures in the Analysis of Fruit Juices (updated)" http://www.ifu-fruitjuice.com
(3) International Fruit Juice Union Recommendation # 12 "The Use of polyphenols in the analysis of fruit juices" http://www.ifu-fruitjuice.com
(4) Validated analytical methods for fruit juices available as downloads (for a fee, free to members). http://www.ifu-fruitjuice.com
(5) AOAC methods available as downloads (for a fee, free to members). www.aoac.org
(6) AIJN code of practice reference guides www.aijn.org
(7) Low. N.H. (1995) Fruit Processing 11, 362-367
(8) Rapid TLC method for screening juice samples for synthetic colors (in house method)
(9) US Technical Committee for Juice and Juice Products. www.tcjjp.org
(10) Driscoll's Patent application for Maravilla fruit http://www.google.com/patents/USPP14804
(11) Brix levels for concentrate reconstitution. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=101.30

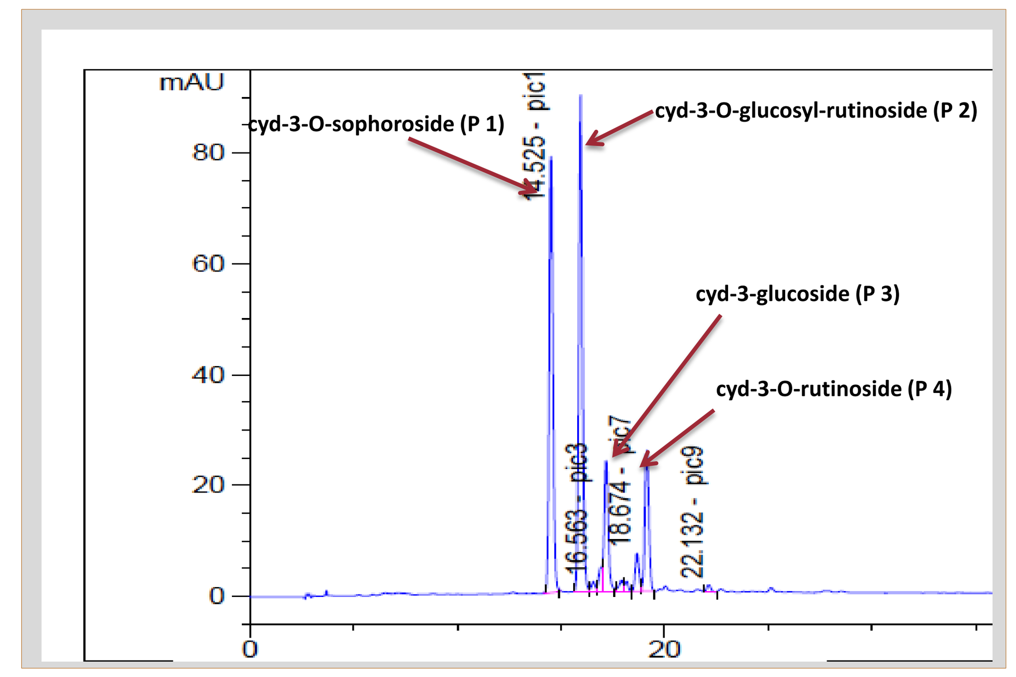

Figure 2: Anthocyanin profile for Maravilla showing higher levels of peaks 2 and 4 than "normal" for raspberries

Results & discussion

During the routine analysis of two raspberry juice samples it was found that both products produced unusual data, which were not consistent with the AIJN Reference guide (RG) for raspberries[6] & suggested that they were not 100% raspberry juices.

Both samples had an unusual anthocyanin profile. Raspberries normally contain four major cyanidins (cyd):- cyd-3-O-sophoroside (p 1); cyd-3-glucoside (p 3), cyd-3-O-glucosyl-rutinoside (p 2) & cyd-3-O-rutinoside (p 4). In a recent revision of the AIJN code of practice a new section has been added to the reference guides (RG) for all "red/black" fruits, which now details which anthocyanins each fruit contains in a decreasing order of concentrations. This is illustrated in the chromatographic trace in Figure 1, which has been taken from IFU 71 (revised 2015). The Maravilla variety contains the "normal" raspberry anthocyanins, but they are present at different levels so the pattern looks very different (Figure 2). This variety has higher levels of both the cyd-3-O-glucosyl-rutinoside and cyd-3-O-rutinoside to that seen in a "normal" raspberry as illustrated in Figure 1.

Data for the juice concentrate, normalised to the AIJN minimum (7 Brix), showed many parameters below the minimum values given in the RG, see columns C & A in Table 1. These features suggested that the concentrate had been diluted. The NFC product also showed values "out of range" (acidity & citric acid), see column D Table 1. Subsequent discussions with the supplier & examination of their processing records clearly showed that both these products were prepared from only "Maravilla".

Maravilla is a relatively new fruit variety that had been introduced by Driscoll's[10] to the fresh fruit market because of some specific beneficial properties (high fruit yield, large fruit size (see photo 1), firm texture and a longer post-harvest shelf-life). This variety has recently been making a move from the fresh to processed sectors.

Normally juices are prepared using a range of different varieties, but a growing number of small producers are starting to manufacture "single variety products" as they provide a unique taste/aroma, but this can itself introduce problems. Nearly all "juice" reference data are produced on blended varieties and very few, if any, are available for "single variety "materials. With blended materials any peculiarities seen in a single variety will be "smoothed out" in the final product. Whereas in single variety products these peculiarities may show through which can make interpretation difficult. The unusual features seen in this fruit prompted this small study to provide some relevant analytical data specific to this variety for analysts.

Sample preparation

Samples of whole fresh fruits, labelled as Maravilla, were purchased at local supermarkets and squeezed in the laboratory. The seeds were removed by sieving & the samples were heated to inactive enzymes. The juices were subsequently analysed for the normal parameters to confirm if the anomalies, seen in the commercial products, were characteristic of this particular variety of fruit.

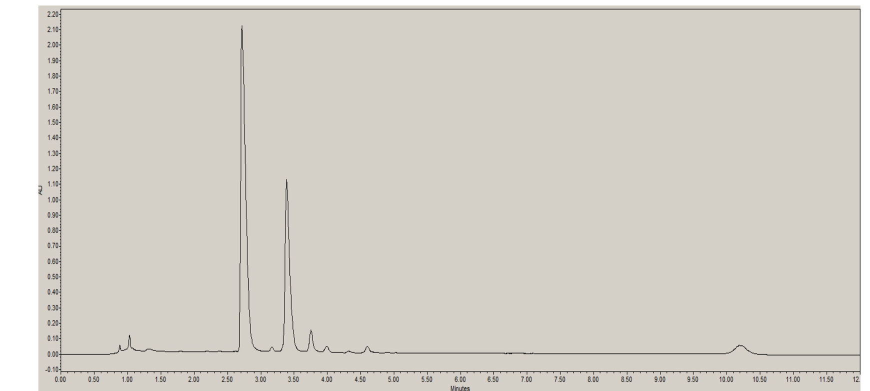

Figure 3: Anthocyanin profile for Chilean raspberry using UPLC

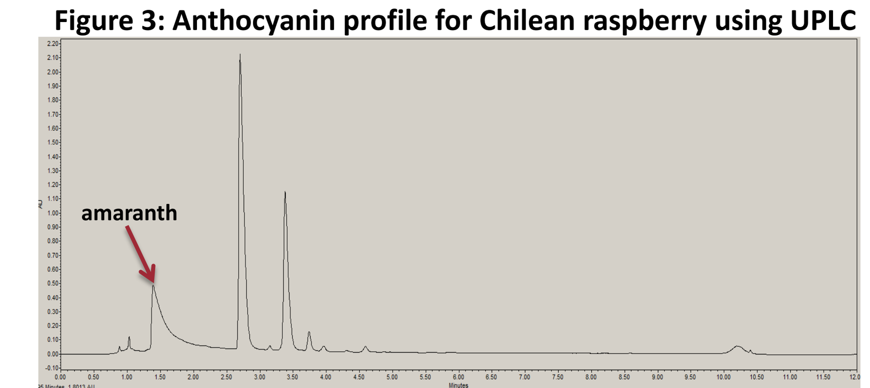

Figure 4: Anthocyanin profile for Chilean raspberry using UPLC showing the presence of amaranth

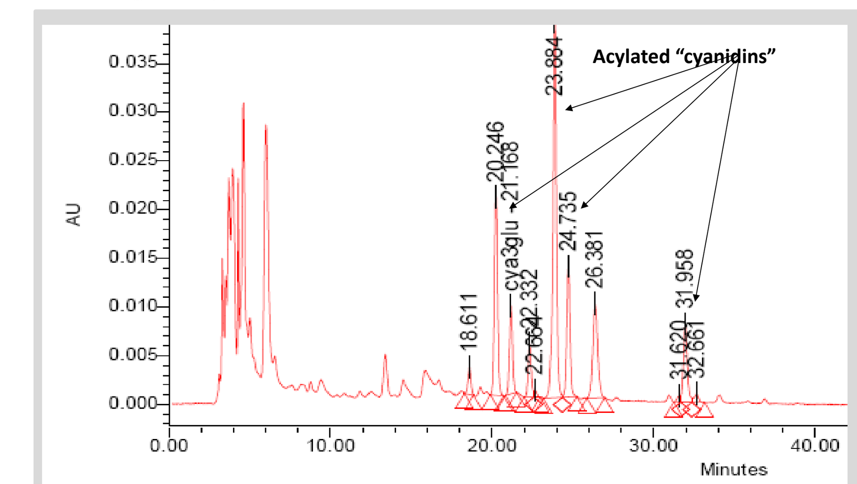

Figure 5: HPLC profile of pomegranate juice with added black carrot

The usefulness of HPLC screening of red/black juices for added colours is illustrated in figures 3, 4 & 5 using a UPLC & the original IFU 71 method. Figure 3 is a UPLC trace for pure raspberry, which shows the much shorter analysis time (12 mins) which is possible using this approach. Figure 4 shows an additional peak, for amaranth, at the start of the run. Although the UPLC conditions are not optimized for this separation, due to the poor peak shape of the added colour, it was easily detected. It is also detectable using a TLC method[8]. Figure 5 shows an HPLC trace for a pomegranate juice with added black carrot color from the late running acylated cyanidin peaks.

Conclusions

This study illustrates one important point that it is important to provide as much information as possible about samples submitted for testing so that the best possible interpretation can be made. This is particularly important if dealing with "single variety" products.

Analysis of fresh fruit samples clearly showed that these had the same "atypical" anthocyanin profiles to that seen in the commercial products. This indicates that Maravilla shows higher levels of cyn-3-O-glucosyl-rutinoside and the cyn-3-rutinoside, to what is normally seen in other raspberries varieties used for processing. There is no clear reason why this has arisen as in the Driscoll's patent[10] they do not clearly identify the parent stock used for its development & further DNA analysis of the product may provide useful information here.

Examination of the processing records for this variety showed that the average single strength corrected Brix was 10.57 %. This is *ca* 50% higher than the AIJN minimum and explains why the concentrate "appeared" to have been diluted. Normalisation of the conc. data to this Brix gives data that were well within the RG values with the exception of the citric acid level. Reconstitution of the conc. to the US minimum Brix level (9.2%)[11] also gives much more acceptable values.

"Take away points"

This study clearly shows that Maravilla produces a different anthocyanin profile to other raspberry varieties normally used for processing. The chemical data for the samples in this study also suggest that this variety may have a higher citric acid level than other processing varieties. However, as this is parameter that is very susceptible to season changes, more data will be needed from subsequent seasons to see if this is a consistent trend or purely a seasonal deviation.

IFU or AOAC methods provides an analyst with tried and tested methods for the analysis of fruit juices that maybe adopted quickly and easily. They list expected repeatability (r) and reproducibility (R) values so an analyst can verify their own performance of the methodology. The AIJN code of Practice can greatly assist an analyst in their interpretation of the chemical data for fruit juices and assist in their assessment of the quality and authenticity of fruit juices and purées.

Finally this study clearly demonstrates that databases need to be kept up to date with values generated for new varieties as they are developed and introduced, so that "safe and meaningful" conclusions can be drawn about commercial products.