

AN INVESTIGATION OF POTENTIAL FRAUD IN COMMERCIAL ORANGE JUICE PRODUCTS IN MALAYSIAN MARKET BY CLUSTER ANALYSIS AND PRINCIPAL COMPONENT ANALYSIS

(Satu Siasatan Tentang Penipuan Berpotensi Dalam Produk Komersil Jus Oren Di Pasaran Malaysia Dengan Analisis Kelompok Dan Analisis Komponen Utama)

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Abstract

This study was triggered by Malaysia Ministry of Health to monitor quality of commercial orange juice products sold in Malaysia market. A total of 19 orange juice samples from 14 different brands of packed orange juice products and 5 different brands of fresh orange fruit juices were analyzed for total soluble solids content, total titratable acidity, sugar composition and amino acid profiles. Hierarchical Cluster analysis (HCA) and Principal component analysis (PCA) on amino acid composition alone allowed visual discrimination between fresh squeezed orange juices and commercial packed orange juices. Suspicion of mislabel was raised in cases of miss-classification.

Keywords: food authentication, orange juice, principal component analysis, hierarchical cluster analysis

Abstrak

Kajian ini dicetuskan oleh Kementerian Kesihatan Malaysia demi memantau kualiti produk komersil jus oren yang dijual dalam pasaran Malaysia. Sejumlah 19 sampel jus oren terbungkus dari pelbagai jenama dan 5 jenama jus oren buah segar dari jenama berbeza telah dianalisa dari segi kandungan zat terlarut, asiditi tertitrat, komposisi gula dan profil asid amino. Analisis Kelompok Hierarki (HCA) dan Analisis Komponen Utama (PCA) ke atas komposisi asid amino sahaja telah membenarkan pembezaan yang jelas antara jus oren diperah segar dan jus oren komersil terbungkus. Kesangsian silap-label telah dibangkitkan dalam kes-kes di mana kesilapan-klasifikasi dapat dikesan

Kata kunci: Ketulenan makanan, jus oren, analisis komponen utama, analisis kelompok hierarki

Introduction

Fruit juice refers to fluid extracted from fruits and is not fermented. Fruit juice products are a big business because consumers have taken it as a healthy option to junk food, that is nutritious and fast absorbed by the body. Owing to the diverse form of juice products available in the market, regulatory and market standards are set to control the quality of juice products, however these standards vary from country to country.

Fruit juice adulteration, particularly orange juices, has always been prime targets for economic gain [1]. This falsification act not only affects the quality of the products, it deceives the consumers who spend more for a product with false claim in its ingredient statement. Fruit juice can be adulterated by simple addition of water, sugar, acids and/or colouring. Quantification of these major components present in fruit juices is only useful for quality control but not for authenticity assessment, because the adulterators can simply manipulate the composition so that the ratios are consistent with those found in the pure counterpart. Thus, determination of the minor components such as free amino acids, minor organic acids and phenolic compounds could be a better way to authenticate fruit juice, since it is not economically feasible to adjust the levels of these components [2,3,4]

For the past two decades, many works have been reported on detecting adulteration of orange juice and comprehensive review can be found in Simpkins et al. [1] and Robards and Antolovic [5]. In 1997, Goodacre et al. [6] presented a work focused on using pyrolysis mass spectroscopy and chemometrics to detect and quantitatively assess the adulteration of orange juice with beet sucrose. On the other hand, Simpkin et al. [7] has established a statistical database of authentic values for adulteration testing of orange juices using stable carbon isotope ratio analysis. Besides, others proposed using organic acid profiles, anionic profiles and amino acid profiles as fingerprints for authenticity testing of orange juice [3, 8, 9].

Knowledge on the quality of commercial fruit juice products is not well established in Malaysia. Most studies have just focused on the chemical composition of fresh juice, and the study was still not comprehensive enough as to provide data to support authenticity assessment. The purpose of the current study was to assess quality of orange juice products found in the Malaysian market and identify potential mislabeled samples. Both the major and minor constituents were quantified and the ability to detect adulteration was reported. Hierarchical cluster analysis (HCA) and Principal component analysis (PCA) were applied to explore the clustering tendency among freshly prepared and commercial orange juice samples.

Materials and Methods

Juice sample preparation

A total of 14 different brands of packed orange juice products (Table 1) and 5 different brands of fresh oranges (Table 2) were sampled from local market. Packed juice samples were categorized into two main groups as 100% juice and non-100% juice as tabulated in Table 1, based on respective label description. Single strength just pressed orange juice samples were manually squeezed using a reamer. All samples were stored at -20 °C. Before analysis, samples were thawed at 4 °C overnight, filtered with muslin cloth followed by polyvinyl difluoride (PVDF) syringe filter with a pore size of 0.45 micron. Analyses were replicated with independent individual orange fruit or carton of orange juice.

Determination of total soluble solids content

Total soluble solids content was determined with a hand-held 0-32 ° Brix refractometer (Thermo Fisher Scientific Inc., 13-946-21, Waltham, MA, USA).

Determination of total titratable acidity

An aliquot of 10 g sample was titrated with 0.1 N sodium hydroxide (NaOH) to an end-point of pH 8.2. Total titratable acidity was calculated with the following equation 1, with reference to anhydrous citric acid [10]:

$$\% \text{ acid} = \frac{(\text{vol of NaOH used in mL}) \times (0.1 \text{ N NaOH}) \times 0.064 \times 100}{\text{grammes of sample}} \quad (1)$$

Table 1. Commercial juice samples received and used for analysis

No.	Brands	Label description
<u>Claiming 100% juice</u>		
1.	A	100% Unsweetened Orange Juice
2.	B	100% Unsweetened Orange Juice
3.	C*	100% Unsweetened Orange Juice
4.	D	100% Orange Juice
5.	E*	100% Orange Juice
6.	F*	100% Unsweetened Orange Juice
7.	G*	100% Chilled Orange Juice
8.	H	100% Unsweetened Orange Juice
9.	I	100% Unsweetened Orange Juice
<u>Not Claiming 100% juice</u>		
10.	J	Orange Fruit Drink
11.	K	Orange Fruit Drink
12.	L	Orange Fruit Drink
13.	M	Orange Fruit Juice Drink
14.	N	Reconstituted orange juice

Note: * Water is listed in the ingredients list

Table 2. Fresh oranges used in this study

Brands of orange fruits	Genus & species
Australian Citrus	<i>Citrus sinensis</i>
Kangara	<i>Citrus sinensis</i>
MFC	<i>Citrus sinensis</i>
Unifrutti	<i>Citrus sinensis</i>
Vitor	<i>Citrus sinensis</i>

Determination of major sugars composition

Sugars analysis was performed using the HPLC system (Waters 2690 Separations Module, Milford, MA, USA) equipped with a RI detector (Waters 2410 Refractive Index Detector, Milford, MA, USA) and Millennium³² Version 3.20 software (Waters, Milford, MA, USA), following the standard analysis conditions as described in Zorbax Carbohydrate Analysis Column 4.6mm ID x 150mm (5µm) (Agilent, Santa Clara, CA, USA) Datasheet. The LC settings were: 75/25 Acetonitrile/Water mobile phase at a flow rate of 1.4mL/min, at 30 °C; refractive index detector was set at 30 °C and sample volume was 3µL in 50/50 Acetonitrile/Water.

Calibration curves were constructed using pure sugars standards at concentrations of 2, 4, 6, 8 and 10 mg/mL for fructose and glucose, and at 4, 8, 12, 16 and 20 mg/mL for sucrose. The linear equations obtained were used to calculate the concentration of sugars in samples.

Determination of amino acids profile

Amino acid profile was determined using commercial test kit, EZ:faast™ Physiological Amino Acid Kit KG0-7166 for GC-MS (Phenomenex, Torrance, CA, USA). Sample cleanup, derivatization and analysis were performed following steps listed in the test kit manual. All analyses were performed using Shimadzu GCMS-QP 2010 equipped with AOC-20S autosampler and LabSolutions GCMS solution Version 2.70 software (Shimadzu, Nakagyo-ku, Kyoto, Japan). The GC column used was Zebron EZ-AAA Amino-Acid GC column 10m x 0.25mm provided in the test kit. The injection volume was 2.0µL at a Helium carrier gas flow of 1.1 mL/min with a split ratio of 1:15. The initial oven temperature was at 110 °C and then raised to 320 °C at 30 °C/min. The conditions for mass spectrometer were 240 °C MS source temperature and 180 °C MS Quad temperature. Linear calibration curves were constructed using pure amino acids standards provided by the test-kit at concentrations of 50, 100 and 200 nmoles/mL.

Statistical analysis

The SPSS v20 for Windows (SPSS Inc., Chicago, IL) was used in this study. Hierarchical cluster analysis (HCA) and Principal component analysis (PCA) were used to explore the variability of the juice samples. Data points with values below the detection limit (labelled as *Not Detected*, *ND*) and quantitation limit (labelled as *Trace amount*) of the instrument were treated as missing values.

Results and Discussion

Brix value, titratable acidity and Ratio of brix-acidity

The results of total soluble solids (°Brix), total titratable acidity (%) and the ratio of total soluble solids to titratable acidity of fresh squeezed orange juice and packed orange juice samples are summarized in Table 3. Brix value and acidity, as well as the brix-acidity ratio are the common quality indicators used to determine sweetness, tartness of a fruit juice, and degree of maturity of the fruits from which the juice was extracted, respectively. High brix value indicates high sugar content as well as high in amino acids, minerals, organic acids and other water soluble components whereas high titratable acidity indicates high organic acid contents.

According to Codex Stan 45-1981, Standards for orange juice preserved exclusively by physical means, when the brix-acidity ratio expressed as anhydrous citric acid is greater than 15, the word “sweetened” may be used in the label to indicate the product being sweetened artificially. From Table 3, result shows that brix-acidity ratio of fresh squeezed orange juice samples ranged from 10.9 to 15.3. This means the fresh squeezed samples comply to Codex Stan 45-1981 except Kangara fruit juice which ratio slightly higher than 15.

As evident from Table 3, brix-acidity ratio for some commercially packed orange juice samples that claimed 100% orange juice are high. For Brand B, D, E, F, and G their respective brix-acidity ratio are 17.6, 17.1, 23.3, 19.6 and 30.3. This provides evidence that some of these products could have been adjusted or contained additional substances such as sweetener. It is noteworthy that Brand B and F claimed ‘Unsweetened’.

What does ‘100% orange juice’ mean? It may simply mean all contents in the carton were expressed from orange fruit with or without addition of other substances, or it may also mean 100% from concentrate, to which water was added back for reconstitution. Apparently, consumers are confused and may even equate the term ‘100%’ with ‘Natural’, which means a totally different thing. On 1st April 2014, FDA required all juice products containing fruit and vegetables juice to declare percentage of juice it contains (21CFR101.30). The regulation also states that for 100% juice, it shall not contain non-juice ingredients that result in a diminution of the juice soluble solids, otherwise it must be accompanied by the phrase “with added”.

Referring to Codex Stan 247-2005, Codex General Standards for Fruit Juice and Nectars, the term Fruit Juice means fruit juice directly expressed by mechanical extraction processes, whenever additive is added to fruit juice it shall be called Fruit Nectars. Apart from that, Codex Stan 247-2005 does state that fruit juice from concentrate is prepared by reconstituting concentrated fruit juice with potable water to a brix value not lower than the minimum Brix level specified in the Standard, namely 10 °Brix for reconstituted orange juice. This helps to control excessive dilution made to juice products. These definitions and criterion are not found in Malaysia Food Regulation 1985.

Table 3. Ratio of total soluble solids (°Brix) to total titratable acidity (%) of fresh squeezed orange juice and packed commercial orange juice samples

Samples	Total soluble solids (°Brix)	Total titratable acidity (%)	Ratio of total soluble solids (°Brix) to total titratable acidity (%): B/A
Fresh orange juices			
Australian Citrus	10.6±0.0	0.97±0.01	10.9±0.1
Kangara	11.3±0.1	0.74±0.01	15.3±0.1
MFC	11.3±0.1	1.01±0.01	11.2±0.1
Unifrutti	9.2±0.0	0.81±0.01	11.4±0.1
Vitor	10.6±0.0	0.86±0.01	12.3±0.1
Commercial samples claiming 100% juice			
A	9.9±0.1	0.81±0.01	12.2±0.1
B	11.8±0.0	0.67±0.01	17.6±0.1
C	11.2±0.1	0.76±0.04	14.7±0.8
D	12.0±0.1	0.70±0.00	17.1±0.1
E	10.7±0.2	0.46±0.02	23.3±0.7
F	10.8±0.2	0.55±0.01	19.6±0.2
G	10.6±0.0	0.35±0.00	30.3±0.3
H	8.9±0.1	0.66±0.01	13.5±0.2
I	11.4±0.3	0.86±0.02	13.3±0.2
Not claiming 100% juice			
J	12.0±0.0	0.34±0.00	35.3±0.1
K	9.8±0.0	0.32±0.00	30.6±0.1
L	10.5±0.1	0.24±0.00	43.8±0.1
M	9.4±0.0	0.43±0.01	21.9±0.1
N	10.4±0.3	0.74±0.02	14.0±0.2

Note: Values are mean±standard deviation (n=6)

Apart from that, when comparing those samples which does not come with the claim of 100% juice, results show that brix-acidity ratios of the orange fruit drink were extremely high, 35.3, 30.6 and 43.8 for brand J, K and L respectively. These values are higher than those found in sample Brand M (orange fruit juice drink; 21.9) and N (reconstituted orange juice; 14.0). Malaysia Food Regulation 1985 requires fruit juice drink and fruit drink to contain not less than 35%(w/v) and 5% (w/v) of fruit juice, respectively. These products may be added with permitted substances, such as sweeteners, preservative, colouring and others.

Major sugar profiles

Major sugars of the freshly squeezed and commercial juice samples are shown in Table 4. These sugars are sucrose, glucose and fructose. At a glance of those freshly squeezed orange juice samples, one can deduce that sucrose is the primary sugar in orange juice and the ratio of fructose, glucose and sucrose is 1:1:1 for MFC orange juice and approximately 1:1:2 for Australian Citrus, Kangara, Unifrutti and Vitor orange juice. Our result is in good agreement with Fry [11], who stated that, the ratio of fructose, glucose and sucrose is 1:1:1 for orange juice from Israel and Brazil, and 1:1:2 for orange juice from North America. This information indicates that determining the sugars composition could help identifying whether the juice has been adulterated.

For samples claiming 100% juice, if the samples are authentic with no adjustment, the ratio of fructose, glucose and sucrose should be either 1:1:1 or 1:1:2. Obviously, Brand B and G show substantial difference from the expected

ratio. As for Brand B, primary sugars were found to be fructose and glucose, and sucrose content is very minimal. This sugar profile is very similar to those of grape juice, a cheaper juice than orange juice. McLellan and Race (1990)[12] reported that the sugars in grape juice are mainly fructose and glucose, and the content of sucrose is less than 10% of total sugar. As for Brand G, sucrose content is found to be 15 times higher than the fructose or glucose content. This is a strong sign that sucrose could have been added to the product.

Table 4. Major sugar profiles for fresh squeezed orange juice and commercial orange juice samples

Samples	Fructose, F (mg/ml)	Glucose, G (mg/ml)	Sucrose, S (mg/ml)	Ratio of F:G:S
Fresh orange juices				
Australian Citrus	9.4±0.8	8.3±0.9	18±2	1:1:2
Kangara	19±2	17±2	37±3	1:1:2
MFC	23.85±0.07	24.3±0.2	28±1	1:1:1
Unifrutti	14±2	13±2	30±4	1:1:2
Vitor	14±1	13±2	23±2	1:1:2
Claiming 100% juice				
A	23±2	21±2	23±2	1:1:1
B	46±7	36±6	6.7±0.7	7:5:1
C	31±7	30±7	25±9	1:1:1
D	23±2	20±2	48±5	1:1:2
E	25±2	21±1	34±2	1:1:1
F	32±1	22±2	23.1±0.4	1:1:1
G	6.7±0.8	5.6±0.4	89.4±0.5	1:1:16
H	21±1	16.4±0.7	21.4±0.9	1:1:1
I	29.5±0.1	19.9±0.2	38.0±0.1	1:1:2
Not claiming 100% juice				
J	24.9±0.2	27±1	66.9±0.5	1:1:3
K	5.7±0.2	5.3±0.7	86±2	1:1:16
L	29±2	30±1	40.6±0.8	1:1:1
M	8.4±0.5	8.0±0.6	60±3	1:1:7
N	31±2	29±2	18±1	2:2:1

Values are means±standard deviation (n=6).

For samples not claiming 100%, sucrose content when compared to fructose or glucose, it is found to be high in Brand J, K and M samples. There is no issue because these samples claimed to be either fruit drink or fruit juice drink, in which sugars addition is permitted.

As mentioned earlier, the abovementioned parameters (brix, acidity, sugar profiles) are weak indicators for detection of adulterations in juice products because they can easily be manipulated. Therefore, to support the investigation, free amino acid composition were determined.

Amino acid profiles

Orange juice sample presented an amino acids profile composed of 20 amino acids (Table 5): alanine, glycine, valine, leucine, isoleucine, threonine, serine, proline, asparagine, aspartic acid, methionine, 4-hydroxyproline, glutamic acid, phenylalanine, glutamine, ornithine, lysine, histidine, tyrosine and tryptophan.

The most abundant amino acid was proline, followed by asparagines, aspartic acid or serine. However, from the five fresh orange juice samples studied (Australian Citrus, Kangara, MFC, Unifruitti and Vitor), it is found that these major amino acids constituents content are inconsistent. On the other hand, glycine, valine, leucine and isoleucine contents of these freshly prepared orange juices are quite consistent, they are around 230, 236, 76, and 68 nmoles/mL, respectively. The amino acids ratio estimation of both glycine: valine and leucine: isoleucine is consistently to be 1:1, which provide a good indicator that the content measurement of these four amino acids could be useful for detecting frauds. Apart from that, inexpensive amino acids such as glycine and glutamic acid have been found added to orange juice samples to increase the total amino acid content [5]. Therefore, it is believed that a multivariate approach using various amino acids as variables could be useful for classifying samples to detect odd or suspected samples, than a single specific amino acid.

Hierarchical Cluster analysis (HCA) was carried out to classify all samples studied using all amino acids composition as variables. Classification was done by Ward's method, which joins cases together in order to minimize variance within a cluster. From the dendrogram obtained (Fig. 1), samples studied can be classified into two main clusters at scale distance of 25, namely Cluster 1 (Brand J, L, K, G, and N) and Cluster 2 (contains all the other samples analyzed). It is interesting to find out that sample Brand G (claims as 100% juice in the label) has been classified together with those fruit drink products which may have only 5% juice according to Malaysia Food Regulation 1985. There are many factors account for the difference in these two main clusters; obviously dilution is one of it.

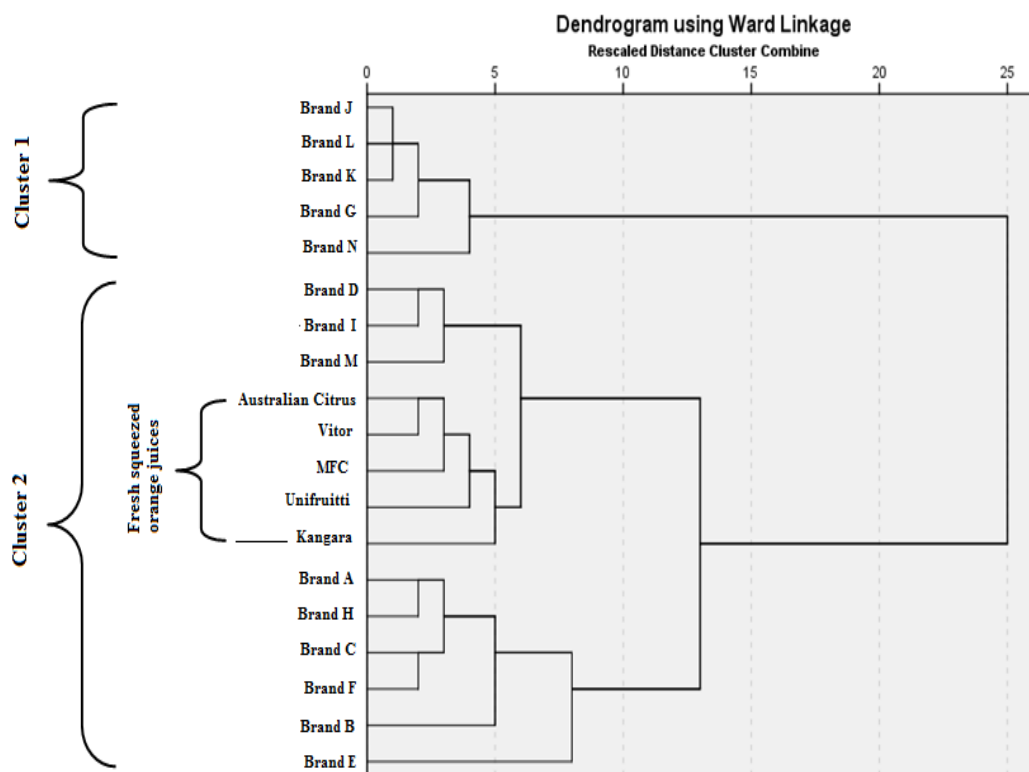


Figure 1. Dendrogram of cluster analysis of orange juice samples studied

At scale distance between 10 and 15, Cluster 2 can be further sub-classified into two more sub-cluster, namely sub-cluster 1 comprised of all freshly squeezed orange juices and commercial juices of Brand D, I and M, and sub-cluster 2 consists of juice Brand A, H, C, F, B and E. A closer look at sub-cluster 2, sample Brand E could be the

next case of suspect. This sample Brand E was found to contain high amount of glycine and glutamic acid (Table 5), which provides valid evidence that total amino acids content of this sample could have been adjusted.

Table 5. Amino acids analysis for commercial samples (nmoles/mL)

Amino Acid	Australian Citrus	Kangara	MFC	Unifruitti	Vitor	Brand A	Brand B	Brand C	Brand D	Brand E
ALA	662 $\pm$ 6	716 $\pm$ 8	680 $\pm$ 20	1070 $\pm$ 50	495 $\pm$ 3	710 $\pm$ 20	380 $\pm$ 20	1400 $\pm$ 200	1300 $\pm$ 40	320 $\pm$ 10
GLY	272 $\pm$ 4	244 $\pm$ 1	208 $\pm$ 1	220 $\pm$ 6	215 $\pm$ 4	228 $\pm$ 5	82 $\pm$ 3	280 $\pm$ 30	226 $\pm$ 7	1380 $\pm$ 50
VAL	229 $\pm$ 2	223.5 $\pm$ 0.6	265 $\pm$ 4	247 $\pm$ 6	217 $\pm$ 1	136 $\pm$ 4	158 $\pm$ 4	150 $\pm$ 20	199 $\pm$ 7	40 $\pm$ 2
IS	200.0 $\pm$ 0.1	200.0 $\pm$ 0.1	200.0 $\pm$ 0.1	200.0 $\pm$ 0.1	200.0 $\pm$ 0.1	200.0 $\pm$ 0.1	200.0 $\pm$ 0.1	200.0 $\pm$ 0.1	200.0 $\pm$ 0.1	200.0 $\pm$ 0.1
LEU	80.5 $\pm$ 0.7	76.5 $\pm$ 0.7	75.0 $\pm$ 0.2	80 $\pm$ 4	67 $\pm$ 2	34.0 $\pm$ 0.8	39 $\pm$ 2	48 $\pm$ 5	52 $\pm$ 1	12.8 $\pm$ 0.5
ILE	82.6 $\pm$ 0.6	79.6 $\pm$ 0.6	94 $\pm$ 1	86 $\pm$ 5	80 $\pm$ 1	42 $\pm$ 1	78 $\pm$ 4	41 $\pm$ 1	56 $\pm$ 2	17 $\pm$ 1
THR	213 $\pm$ 4	175.5 $\pm$ 0.5	94 $\pm$ 1	230 $\pm$ 30	193 $\pm$ 5	174 $\pm$ 5	125 $\pm$ 2	190 $\pm$ 20	206 $\pm$ 9	51 $\pm$ 2
SER	1951 $\pm$ 5	1540 $\pm$ 20	1320 $\pm$ 30	1990 $\pm$ 60	1480 $\pm$ 30	1710 $\pm$ 50	812 $\pm$ 5	2000 $\pm$ 200	1720 $\pm$ 70	500 $\pm$ 20
PRO	5450 $\pm$ 20	4670 $\pm$ 90	4349 $\pm$ 1	4820 $\pm$ 20	4300 $\pm$ 100	4150 $\pm$ 70	1690 $\pm$ 80	5800 $\pm$ 100	5400 $\pm$ 200	3200 $\pm$ 100
ASN	2662 $\pm$ 6	2190 $\pm$ 30	940 $\pm$ 20	3430 $\pm$ 30	1770 $\pm$ 30	2920 $\pm$ 70	4150 $\pm$ 80	3000 $\pm$ 600	2900 $\pm$ 100	1600 $\pm$ 200
ASP	1890 $\pm$ 10	1525 $\pm$ 1	1026 $\pm$ 5	750 $\pm$ 30	1290 $\pm$ 30	3240 $\pm$ 80	1590 $\pm$ 60	2500 $\pm$ 100	2600 $\pm$ 90	5500 $\pm$ 200
MET	26 $\pm$ 1	26.05 $\pm$ 0.08	33 $\pm$ 2	30.6 $\pm$ 0.7	24 $\pm$ 2	Trace amount	Trace amount	Trace amount	22.0 $\pm$ 0.8	Trace amount
HYP	16.6 $\pm$ 0.7	13.5 $\pm$ 0.7	9.1 $\pm$ 0.9	16 $\pm$ 2	11.0 $\pm$ 0.1	22 $\pm$ 1	Trace amount	13 $\pm$ 2	17 $\pm$ 1	Trace amount
GLU	530 $\pm$ 6	826 $\pm$ 5	680 $\pm$ 50	640 $\pm$ 20	510 $\pm$ 10	1110 $\pm$ 30	420 $\pm$ 30	800 $\pm$ 40	750 $\pm$ 50	2200 $\pm$ 100
PHE	343 $\pm$ 3	219.5 $\pm$ 0.7	229.6 $\pm$ 0.7	215 $\pm$ 5	271 $\pm$ 6	123 $\pm$ 3	61 $\pm$ 2	130 $\pm$ 10	170 $\pm$ 5	30 $\pm$ 1
GLN	89 $\pm$ 8	91.0 $\pm$ 0.9	122 $\pm$ 2	370 $\pm$ 60	129 $\pm$ 4	800 $\pm$ 200	554 $\pm$ 280	1400 $\pm$ 200	180 $\pm$ 10	1100 $\pm$ 200
ORN	71.5 $\pm$ 0.6	55 $\pm$ 2	38 $\pm$ 2	190 $\pm$ 50	55 $\pm$ 1	89 $\pm$ 5	19 $\pm$ 2	48 $\pm$ 5	59 $\pm$ 6	11 $\pm$ 1
LYS	411.5 $\pm$ 10	246 $\pm$ 4	260 $\pm$ 10	370 $\pm$ 10	412 $\pm$ 2	350 $\pm$ 20	59 $\pm$ 4	210 $\pm$ 30	290 $\pm$ 30	35 $\pm$ 4
HIS	133.5 $\pm$ 0.6	80 $\pm$ 20	70 $\pm$ 10	90 $\pm$ 10	140 $\pm$ 10	96 $\pm$ 8	13.00 $\pm$ 0.01	38 $\pm$ 3	66 $\pm$ 3	Trace amount
TYR	121 $\pm$ 3	74 $\pm$ 2	81 $\pm$ 2	123 $\pm$ 8	82 $\pm$ 2	36 $\pm$ 1	22 $\pm$ 2	60 $\pm$ 20	51 $\pm$ 1	Trace amount
TRP	31.5 $\pm$ 0.6	26 $\pm$ 1	25 $\pm$ 2	43 $\pm$ 7	38.6 $\pm$ 0.6	Trace amount	ND	12.0 $\pm$ 0.8	14.0 $\pm$ 0.8	Trace amount

Values are mean $\pm$ standard deviation (n=6). Abbreviation: ALA, alanine; GLY, glycine; VAL, valine; LEU, leucine; ILE, isoleucine; THR, threonine; SER, serine; PRO, proline; ASN, asparagine; ASP, aspartic acid; MET, methionine; HYP, 4-hydroxyproline; GLU, glutamic acid; PHE, phenylalanine; GLN, glutamine; ORN, ornithine; LYS, lysine; HIS, histidine; TYR, tyrosine; and TRP, tryptophan. IS, Internal standard; ND, Not detected (below limit of detection of the instrument; <LOD); Trace amount (below limit of quantification of the instrument; <LOQ).

LOD (nmoles/mL) and LOQ (nmoles/mL) limit of each amino acid is listed in respective parenthesis as follows: ALA (2.29,6.94); GLY(3.34;10.13); VAL(4.40,13.34); LEU(3.40,10.30); ILE(3.01,9.11); THR(3.97,12.02); SER(4.90,14.86);PRO(4.85,14.71); ASN(6.21,18.81); ASP(5.66,17.15); MET(6.87,20.82); HYP(3.23,9.79); GLU(9.33;28.29); PHE(4.70,14.24); GLN(8.99,27.24); ORN(2.66,8.07); LYS(4.81,14.58); HIS(3.17,9.60); TYR(5.40,16.36); TRP(3.66,11.10).

Table 5 (Cont'd). Amino acids analysis for commercial samples (nmoles/mL)

Amino Acid	Brand F	Brand G	Brand H	Brand I	Brand J	Brand K	Brand L	Brand M	Brand N
ALA	870±20	279±5	520±10	1370±70	224±3	Trace amount	119±5	1300±100	500±10
GLY	470±30	60±3	153±2	264±3	41.5±0.6	13±2	35±5	300±20	390±10
VAL	124±9	53±1	87.5±0.6	240±20	32.5±0.6	ND	24±1	220±20	49±1
IS	200.0±0.1	200.0±0.1	200.0±0.1	200.0±0.1	200.0±0.1	200.0±0.1	200.0±0.1	200.0±0.1	200.0±0.1
LEU	45±3	14.3±0.5	24.3±0.5	70±4	10.8±0.5	ND	Trace amount	62±4	18±1
ILE	38±4	15.0±0.1	28.3±0.5	69±5	9.8±0.5	ND	Trace amount	62±4	15.0±0.8
THR	144±7	52±1	133±3	218±9	34.0±0.8	ND	21±1	240±20	55±4
SER	1310±40	530±10	1230±20	1990±60	307±7	19±5	200±10	2200±100	510±30
PRO	5700±400	2240±40	3310±40	7200±400	1350±10	Trace amount	960±20	7300±300	2100±50
ASN	2080±50	856±9	2920±60	3090±80	520±10	ND	292±7	3300±200	800±40
ASP	3700±100	660±20	2670±40	2500±200	380±10	Trace amount	263±7	2700±200	1380±50
MET	Trace amount	ND	Trace amount	30±2	ND	ND	ND	30±2	ND
HYP	13.3±0.5	Trace amount	12.0±0.8	20±1	ND	ND	ND	24±2	Trace amount
GLU	1300±100	220±10	880±30	800±100	85±5	ND	67±1	810±80	353±6
PHE	124±6	52.5±0.6	97±2	260±20	30.5±0.6	ND	20±1	230±10	51±2
GLN	800±400	43±6	1000±300	730±70	600±100	700±200	600±200	500±200	56±6
ORN	42±6	13.5±0.6	80±10	55±8	11.3±0.5	Trace amount	9±1	63±7	21±3
LYS	170±20	52±3	290±10	270±20	30.0±0.8	ND	20.0±0.8	300±30	410±10
HIS	51±4	14.5±0.6	80±7	90±10	Trace amount	ND	Trace amount	88±7	16±1
TYR	43±7	18.0±0.1	23±1	100±10	Trace amount	ND	Trace amount	83±5	Trace amount
TRP	14.0±0.1	Trace amount	12.3±0.5	12.5±0.6	ND	ND	ND	22±2	Trace amount

Values are mean±standard deviation (n=6). Abbreviation: ALA, alanine; GLY, glycine; VAL, valine; LEU, leucine; ILE, isoleucine; THR, threonine; SER, serine; PRO, proline; ASN, asparagine; ASP, aspartic acid; MET, methionine; HYP, 4-hydroxyproline; GLU, glutamic acid; PHE, phenylalanine; GLN, glutamine; ORN, ornithine; LYS, lysine; HIS, histidine; TYR, tyrosine and TRP, tryptophan. IS, Internal standard; ND, Not detected (below limit of detection of the instrument); Trace amount (below limit of quantification of the instrument).

LOD (nmoles/mL) and LOQ (nmoles/mL) limit of each amino acid is listed in respective parenthesis as follows: ALA (2.29,6.94); GLY(3.34;10.13); VAL(4.40,13.34); LEU(3.40,10.30); ILE(3.01,9.11); THR(3.97,12.02); SER(4.90,14.86); PRO(4.85,14.71); ASN(6.21,18.81); ASP(5.66,17.15); MET(6.87,20.82); HYP(3.23,9.79); GLU(9.33;28.29); PHE(4.70,14.24); GLN(8.99,27.24); ORN(2.66,8.07); LYS(4.81,14.58); HIS(3.17,9.60); TYR(5.40,16.36); TRP(3.66,11.10).

From PCA, the Eigen values for PC 1 and PC 2 are 11.357 and 3.446, respectively. These two PC account for 74% total variability of the data (PC 1 = 56.79% and PC 2 = 17.23%). As shown in Fig. 2, sample Brand A, C, D, F, H, I, and M (all claim as 100% juice except Brand M that claimed as Fruit Juice Drink) are located closed to those freshly squeezed orange juice samples (Australian Citrus, Kangara, MFC, Unifrutti, and Vitor), and differ from other samples in a distinguishable pattern. Again, sample Brand G is found to appear among those samples declared as orange fruit drink or reconstituted juice (Brand J, K, L and N). This double confirmed the case that sample Brand G could be suspected of false claim as 100% juice. Similarly, sample Brand E (claims as 100% juice) is found far from the other clusters. This reinforces the case that Brand E could be a suspicious sample of inauthentic orange juice.

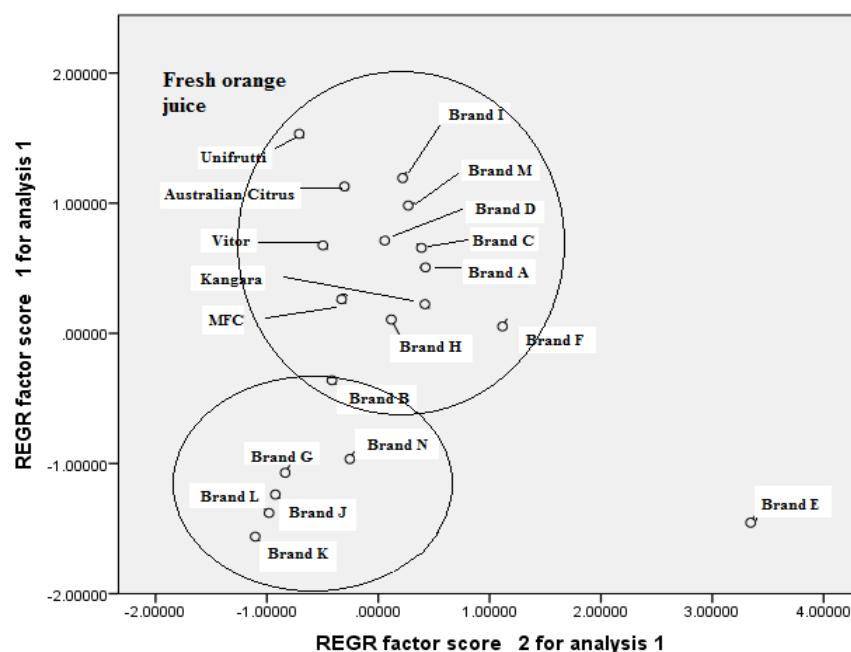


Figure 2. Graphical representation of orange juice samples on PCA. Fresh orange juice: Australian citrus, Kangara, MFC, Unifrutti, Vitor; Claiming 100% juice: Brand A, B, C, D, E, F, G, H, I; Not Claiming 100%: Brand J, K, L (Fruit drink, 5% juice), Brand M (Fruit juice drink, 35% juice) and Brand N (Reconstituted juice).

Conclusion

The data reported show that it is possible to distinguish and detect fraud products from other freshly squeezed orange juice and commercial orange juice products. Any sample can be analyzed and introduced to the data matrix and possible adulteration could be detected. Apart from that, results of the current study showed high variability especially among those products that declared and claimed as 100% juice. This scenario suggests an urgent need for establishing quality assurance standards and monitoring procedures in the country to control the quality of orange juice products in the market. With this, consumers will be protected from purchasing unethically traded products.

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