Analytical Methods

Atmospheric pressure chemical ionisation mass spectrometry analysis linked with chemometrics for food classification – A case study: Geographical provenance and cultivar classification of monovarietal clarified apple juices ☆

Heng-Hui Gan, Christos Soukoulis, Ian Fisk *

Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, United Kingdom

ABSTRACT

In the present work, we have evaluated for first time the feasibility of APCI-MS volatile compound fingerprinting in conjunction with chemometrics (PLS-DA) as a new strategy for rapid and non-destructive food classification. For this purpose 202 clarified monovarietal juices extracted from apples differing in their botanical and geographical origin were used for evaluation of the performance of APCI-MS as a classification tool. For an independent test set PLS-DA analyses of pre-treated spectral data gave 100% and 94.2% correct classification rate for the classification by cultivar and geographical origin, respectively. Moreover, PLS-DA analysis of APCI-MS in conjunction with GC–MS data revealed that masses within the spectral ACPI-MS data set were related with parent ions or fragments of alkyesters, carbonyl compounds (hexanal, trans-2-hexenal) and alcohols (1-hexanol, 1-butanol, cis-3-hexenol) and had significant discriminating power both in terms of cultivar and geographical origin.

© 2013 The Authors. Published by Elsevier Ltd. All rights reserved.

1. Introduction

There is a growing consumer awareness of the need for traceable authenticity of foods; this is partially in response to authenticity scares and lack of Protected Designation of Origin (PDO) traceability, but also as a result of recent cases of food producers' malpractice. Food authenticity issues may be classified into four main groups: adulteration; mislabeling associated with geographical provenance, botanical or species origin; implementation of non-authorised practices and non-compliance to legislative standards (Carcea et al., 2009). One response to these maybe through legislation, the European Union Council Regulation (EC) 510/2006 exists to identify and protect geographical indications and designations of origin for agricultural products and foods across Europe, this ensures easier traceability of issues associated with food authenticity allowing more efficient quality and safety control of the food market. There is therefore clearly a need for rapid non-destructive analytical methods to support the consumers right for confidence in authenticity; these approaches

must allow rapid monitoring of food origins, quality and safety, with the minimum processing time and cost per sample; reducing sample pre-treatment and simple measurement protocols are also of paramount importance (Reid, O'Donnell, & Downey, 2006).

There are a number of emerging rapid non-destructive methods for chemical grouping of foods such as the direct injection mass spectrometric techniques (DIMS), atmospheric pressure chemical ionisation mass spectrometry (APCI-MS) (Davies, Linforth, Wilkinson, Smart, & Cook, 2011), proton transfer reaction mass spectrometry (PTR-MS) (Biasioli, Yeretzian, Gasperi, & Mark, 2011) and selected ion flow tube mass spectrometry (SIFT-MS) (Langford et al., 2012) have gained the attention of the researchers working in the field for classification and authenticity, due to their ability to perform real time non-invasive analysis with high sensitivity and limited sample pre-treatment. PTR in combination with a time-of-flight mass spectrometer (PTR-ToF-MS) have been extensively used for classification studies of a broad range of food products including PDO cheese, olive oil and dry cured hams, intact fruits and their derivatives (Aprea et al., 2006; Biasioli et al., 2003; Cappellin et al., 2012; Del Pulgar et al., 2011; Galle et al., 2011). In these cases, classification typically uses the data matrix resulting from the entire mass spectrum (spectral fingerprint) and statistical treatment to identify clusters, trends or correlations, appropriate data mining techniques may include partial least squares discriminant analysis (PLS-DA), K-nearest neighbours

---

* Corresponding author. Tel.: +44 (0) 115 951 16037; fax: +44 (0) 115 951 16142.
E-mail address: Ian.Fisk@nottingham.ac.uk (I. Fisk).

(KNN), soft independent modelling of class analogies (SIMCA) (Fisk, Virdie, Kenny, & Ullrich, 2010) support vector machine (SVM) and random forest (RF) (Cappellin et al., 2012).

Whist direct injection mass spectrometric techniques are rapid and information rich, gas phase chemometric classification approaches should always take into consideration the availability of volatile compounds in the gas-phase and the equilibrium concentration difference between the product and its gas phase. The chemical potential of a volatile component is dependent firstly on the physicochemical properties of the analyte, the physical structure of the matrix (Yang et al., 2012; Yu et al., 2012), the presence of multiple phases (Fernández-Vázquez et al., 2013; Fisk, Linforth, Taylor, & Gray, 2011) and chemical composition of the product being analysed (Fisk, Boyer, & Linforth, 2012). It is therefore important to consider that modifications to the product nonvolatile composition may have a significant impact on the aroma profile and therefore where appropriate, standardisations should be applied.

For fruit juice, the main authenticity issues are related with false labelling of products in terms of their cultivar or geographical origin, blending of expensive fruit juices with juices extracted from lower value fruits, adulteration of juice with pulp wash and peel derived by-products, addition of unauthorised sugars and the use of juice concentrates of undeclared origin (Singhal, Kulkarni, & Rege, 1997). To date several techniques have been used for the authentication and classification of apple juices and similar beverages, these include chemical profiling (Souza et al., 2011) stable isotopes analysis (Magdas & Puscas, 2011), infra-red spectroscopy e.g. NIR, MIR, FT-IR (Kelly & Downey, 2005; León, Daniel Kelly, & Downey, 2005; Sivakesava, Irudayaraj, & Korach, 2001), chromatographic techniques e.g. GC–MS (Fisk, Kettle, Hofmeister, Virdie, & Silanes Kenny, 2012; Guo, Yue, & Yuan, 2012; Lignou, Parker, Oruna-Concha, & Mottram, 2013; Montero-Prado, Bantayeb, & Nerín, 2013) and HPLC (Yamamoto et al., 2008) and direct injection spectrometric techniques such as PTR-MS (Biasioli et al., 2003, 2011).

Direct injection APCI-MS has been successfully applied in a number of areas, most of these relate to the real time tracking of volatile compound release (Taylor, Linforth, Harvey, & Blake, 2000) to understand the dynamic partitioning from complex systems such as food (Linforth, Baek, & Taylor, 1999) and beverages (Shojaei, Linforth, & Taylor, 2007) or as tool to evaluate different processing methodologies (Fisk et al., 2011, 2012; Yang et al., 2012; Yu et al., 2012) Notwithstanding its use as tool for real time aroma analysis, APCI-MS can also provide a rapid and informative mass spectral fingerprint of a foods volatile compliment; it can therefore be hypothesised that APCI-MS could be used for the monitoring of food authenticity. The aim of the present work was to evaluate APCI-MS as a novel tool for the classification (based on geographical and botanical origin) of a foods volatile compliment, using a real food (clarified apple juice) with broad commercial diversity as an exemplar.

2. Materials and methods

2.1. Sampling and juice preparation

Five cultivars (Braeburn, Golden Delicious, Granny Smith, Jazz (Scifresh), and Pink Lady) harvested in three different countries of the South hemisphere (New Zealand, South Africa, Chile) were purchased from four local supermarkets. For each cultivar, 12 apples were randomly selected and used for the preparation of apple juice samples. Apples were peeled, cored, sliced and placed in an antioxidant solution to retard enzymatic browning, as previously illustrated by Ting et al. (2012). Apple flesh was squeezed using a household juicer (Philips, UK) and the freshly extracted apple juice was immediately heat treated at 60 °C for 30 s using a water bath to retard any further enzyme activity. Excessive pulp and foam were removed from the juice by filtering through a 100-mesh cloth filter. Clarification of the apple juice was conducted by pectinase (Sigma–Aldrich, UK) treatment at 37 °C for 60 min and subsequent centrifugation of the juices at 5000 rpm (Beckman Ltd., J2-21M, UK) for 10 min. A total of 210 apple juices were prepared.

2.2. GC–MS analysis

For GC–MS headspace analyses six individual apple juices samples per cultivar referring to different market suppliers and geographical origin were selected. Headspace solid phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC–MS) was applied to analyse the volatile compounds of apple juices. An automated SPME sampling unit (CombiPal. Zwingen, Switzerland) was used with a SPME StableFlex fibre with 50/30 µm divinylbenzene/carboxen on polydimethylsiloxane coating (DVB/CAR/PDMS) purchased from Supelco (Sigma Aldrich, UK). Five mL of juice sample was transferred to a 30 mL vial crimp-sealed with 23 mm diameter aluminium seal and a Teflon septum. In addition, pure aqueous systems of cis-3-hexenol (25 µL/L) were prepared and analysed together with apple juice samples in a fully randomised order. After 10 min equilibration at 20 °C, the SPME fibre was exposed to the sample headspace for 15 min. The fibre was then removed from the vial and immediately inserted into the injector port of the GC–MS system for thermal desorption at 220 °C for 10 min.

Analysis of the aroma components were performed on a Trace GC Ultra (Thermo Scientific, USA) that was attached to a DSQ series mass spectrometer (Thermo Scientific, USA). The gas chromatograph was equipped with a low bleed/fused-silica ZB-Wax capillary column (100% polyethylene glycol phase, 30 m $\times$ 0.25 mm $\times$ 1.0 µm) (Phenomenex, UK). Helium was the carrier gas with a constant flow rate of 1.5 ml/min into the GC–MS. The GC oven was held for 2 min at 40 °C and heated to 220 °C at a rate of 8 °C/min. The GC to MS transfer line was maintained at 250 °C. Analysis was carried out in the electron impact mode with a source temperature of 230 °C, ionising voltage of 70 eV, and a scanned mass range of m/z = 50–200. Pure apple juices were run in triplicate. Compounds were identified by comparison to NIST Library and the retention time of authentic standards.

2.3. APCI-MS analysis

A MS Nose interface (Micromass, Manchester, UK) fitted to a Quattro Ultima mass spectrometer (Milford, Waters) was used for the static headspace analysis of apple juice samples. Fifty mL aliquots of samples were placed in 100 mL flasks fitted with a one port lid. After a 30 min equilibration period at room temperature (20 °C), the headspace was drawn into the APCI-MS source at a rate of 5 mL/min. The samples were analysed in full scan mode, monitoring ions of mass to charge (m/z) ratios from 40 to 200. The intensity of these ions was measured at cone voltage of 20 V, source temperature of 75 °C and dwell time of 0.5 s. Moreover, headspace analysis was carried out in the splitless injection mode, at a flow of 20 mL/min, splitless valve time of 1.5 min and constant pressure of 124 kPa. All analyses were run in triplicate.

2.4. Statistical analyses

The chromatographic data was subject to one-way ANOVA followed by Duncan's post hoc means comparison test. Moreover, principal components analysis (PCA) was also performed on the chromatographic dataset (36 samples, 16 variables) after

Table 1

Volatile compounds identified in the headspace above monocultivar apple juices using SPME-GC-MS. Data refers to the normalised peak area of the identified compounds relative to the intensity of pure cis-3-hexenol (25 μL/L). Results are reported as means of 6 individual measurements for each apple cultivar and ($\times 10^{-03}$) indicates ($\times 10^{-03}$).

	Aroma descriptors[A]	Braeburn	Golden delicious	Granny Smith	Jazz	Pink Lady	p-value
Carbonyl compounds							
2-Methylbutanal	Chocolate, sweet	1.77×10^{-03a}	2.35×10^{-03a}	2.84×10^{-03a}	1.40×10^{-03a}	1.69×10^{-03a}	0.399
3-Methylbutanal	Caramel	7.77×10^{-05a}	6.21×10^{-05a}	9.12×10^{-05a}	2.28×10^{-05a}	4.10×10^{-05a}	0.116
Hexanal	Green, grassy	1.86×10^{-01a}	7.39×10^{-01c}	3.11×10^{-01b}	1.12×10^{-01a}	1.28×10^{-01a}	<0.001
Trans-2-hexenal	Green, grassy	3.45×10^{-01b}	3.11×10^{-01b}	3.87×10^{-01b}	1.57×10^{-01a}	1.60×10^{-01a}	<0.001
Alcohols							
1-Butanol	Light-fruity	2.91×10^{-01b}	4.02×10^{-02a}	2.05×10^{-02a}	5.02×10^{-01c}	3.11×10^{-01b}	<0.001
2-Methyl-1-butanol	Alcohol, solvent	6.43×10^{-02bc}	4.60×10^{-02a}	7.93×10^{-02b}	1.25×10^{-01c}	6.82×10^{-02bc}	<0.001
1-Hexanol	Light-apple	1.79×10^{-02b}	1.75×10^{-02b}	6.96×10^{-03a}	3.39×10^{-02d}	2.55×10^{-02c}	<0.001
cis-3-Hexenol	Fresh, green, grassy	4.42×10^{-05ab}	1.12×10^{-05a}	6.35×10^{-05b}	4.56×10^{-05b}	7.98×10^{-06a}	0.050
Esters							
Butyl acetate	Sweet, fruity	4.76×10^{-01b}	1.29×10^{-02a}	6.00×10^{-03a}	$1.66 \times 10^{+00c}$	4.65×10^{-01b}	<0.001
2-Methylpropyl acetate	Sweet, fresh	1.22×10^{-02c}	2.75×10^{-04a}	1.36×10^{-04a}	9.69×10^{-03bc}	6.73×10^{-03b}	<0.001
2-Methylbutyl acetate	Fresh, banana	4.80×10^{-02b}	3.38×10^{-03a}	9.29×10^{-03a}	1.30×10^{-01c}	3.50×10^{-02b}	<0.001
Hexyl acetate	Sweet, fruity	2.99×10^{-01a}	4.79×10^{-02a}	3.50×10^{-03a}	$1.93 \times 10^{+00c}$	6.59×10^{-01b}	<0.001
Methyl butanoate	Fruity, apple	3.53×10^{-03a}	7.16×10^{-05a}	1.76×10^{-03a}	2.10×10^{-04a}	3.17×10^{-02b}	<0.001
Ethyl butanoate	Sweet, fruity	1.38×10^{-03a}	4.35×10^{-05a}	3.27×10^{-04a}	2.75×10^{-04a}	1.54×10^{-02b}	<0.001
Ethyl hexanoate	Fruity	2.61×10^{-02b}	2.32×10^{-02b}	2.87×10^{-02b}	1.09×10^{-02a}	1.12×10^{-02a}	<0.001

[A] The same letter within a row indicates no significant difference according to Duncan's mean post hoc comparison test ($p < 0.05$).

[A] Aprea et al. (2012, 2011), Dimick et al. (1983), Komthong et al. (2006), López et al. (2007), Burdock (2009), Lignou et al. (2013).

standardization in order to explore the clustering of the apple juices in terms of their flavour volatile compounds composition. All analysis were performed using MINITAB release 16 (Minitab Inc., Pennsylvania, US).

The APCI-MS dataset matrix consisted of 210 samples and 120 variables (*m/z* 40-160), these were log transformed, auto-scaled and consequently subject to principle component analysis (PCA). The unsupervised PCA was used to identify potential outliers (according to Hotelling's ellipse and Leverage plot) and natural clusters (Tres, Ruiz-Samblas, van der Veer, & van Ruth, 2013). PCA revealed the presence of natural clusters for both geographical origin and cultivar type. Eight samples (3 Jazz, 2 Golden Delicious,

and 3 Braeburn) were removed from further analyses after classification as outliers.

Partial least squares discriminant analysis (PLS-DA) was conducted using the APCI-MS fingerprint (matrix comprised of 202 samples and 120 variables) to construct the classification models for the verification of the cultivar and geographical provenance of the clarified apple juices. Log transformation, mean centring and auto-scaling of the spectral dataset was applied prior to conducting the PLS-DA analysis. Pretreatment of spectral dataset allowed the removal of the offset from the data, reduced the heteroscedasticity (skewness) of the data and enable comparison of the spectral data based on an equal basis. The entire dataset
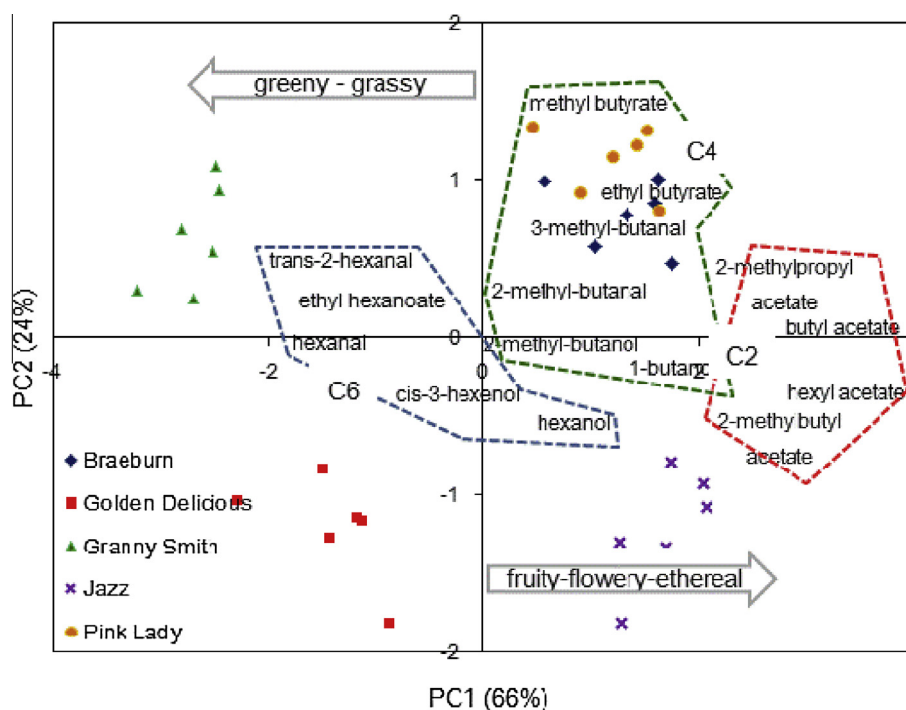

Fig. 1. Principal components analysis (PCA) biplot on the data (averaged, mean centred and auto-scaled) obtained by GC-MS headspace analysis of the clarified fresh apple juices. C2, C4 and C6 indicate volatiles compounds participating in the metabolic pathways of the development of acetates, butyrates and hexanoates respectively.

was divided randomly into subsets that were used for the development of the classification models (143 samples, 70.8% of the total samples, namely internal validation set) and their validation (59 samples, 29.2%, namely external validation set). A leave-one-out (LOO) full cross validation was also used to evaluate the performance of the models constructed using the training dataset and the optimal number of principle components (PCs) required to achieve the best classification from the constructed models was also calculated. All statistical treatments of the APCI-MS fingerprint were conducted using Unscrambler version 9.7 (Camo A/S, Norway).

3. Results and discussion

3.1. Headspace analysis by GC–MS

Sixteen volatile flavour compounds were detected and identified in the headspace of the fresh monocultivar apple juices (Table 1), the identified compounds were mainly aldehydes, alkyl-esters, alcohols and carboxylic acids. The compositional flavour profile of the apple juices was found to be in accordance with previously published data in apple juices and fresh cut apple samples (Aprea et al., 2011, 2012; Dimick, Hoskin, & Acree, 1983; Komthong, Igura, & Shimoda, 2007; López et al., 2007).

Granny Smith apple juices were characterised as having the lowest alkyl-esters concentration (with the exception of ethyl hexanoate) and the highest concentration of cis-3-hexen-1-ol and trans-2-hexenal and intermediate concentrations of hexanal, and 2-methylbutanol. Cis-3-hexen-1-ol and trans-2-hexenal are both related with strong green-grassy flavour notes which together with hexanal are considered the main contributor of green flavour in apples and their derivatives (Komthong, Hayakawa, Katoh, Igura, & Shimoda, 2006). Aprea et al. (2011) showed that apple cultivars such as Granny Smith, Topaz, Pilot or Renetta, that are generally known to emit lower amounts of esters, are characterised as having higher concentrations of alcohols such as cis-3-hexen-1-ol. As is detailed in the PCA bi-plot (Fig. 1) the apple juice extracted from Golden Delicious exhibited a similar flavour profile to that of Granny Smith with high concentrations of volatile compounds related with green-grassy notes (trans-2-hexenal and 1-hexanal and cis-3-hexenol) and low concentrations of acetates. The latter has been also confirmed by Ting et al. (2012) who reported lower concentrations of acetates in the headspace of fresh cut Golden Delicious samples compared to other apple cultivars i.e. Red Delicious, Jonagold or Fuji. Moreover, the aldehyde to alcohol ratio is indicative of ripeness, as aldehydes can be metabolised to alcohols and subsequently esterified with the present carboxylic acids (Defilippi, Dandekar, & Kader, 2005). Based on GC–MS data, the aldehydes and their corresponding alcohols ratios were higher for Golden Delicious and Granny Smith juices implying a lower level of ripeness for the specific fruit samples. Pink Lady and Braeburn were characterised as having moderate concentrations of most of the identified flavour compounds, apart from a marked ele-
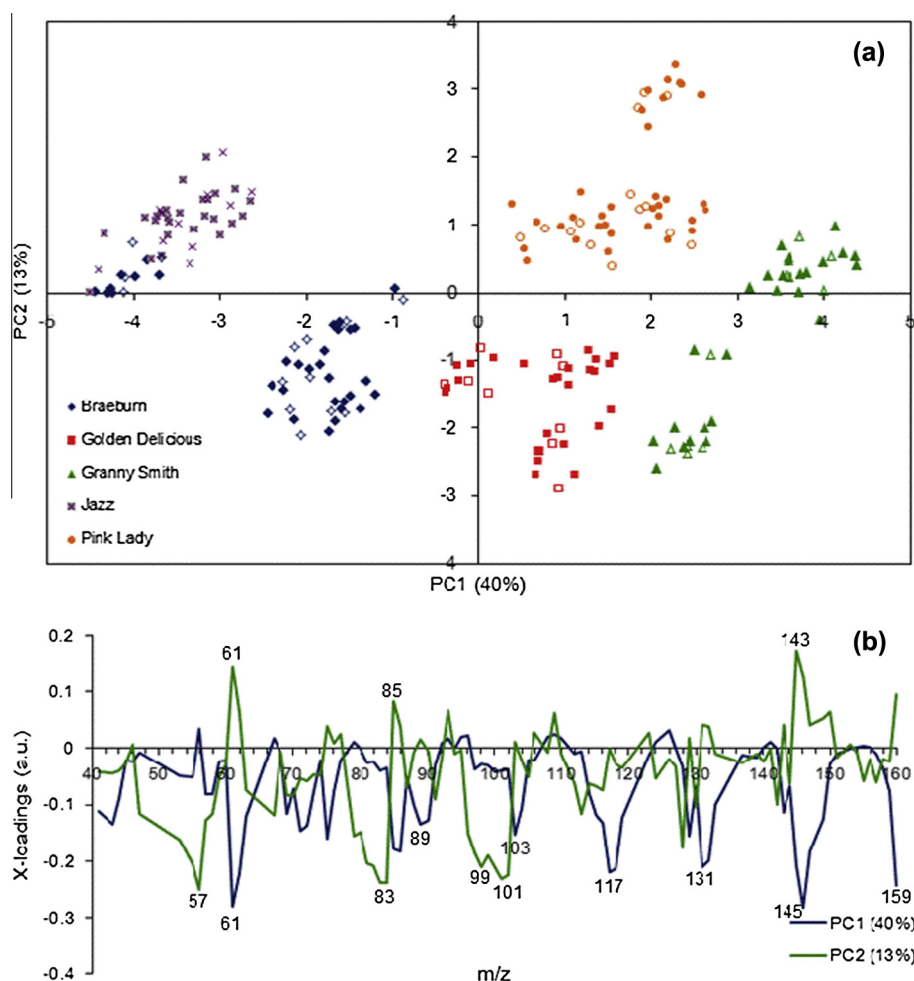

Fig. 2. PLS-DA scores (a) and loadings (b) for the first two factors of the classification models based on the APCI-MS data obtained by the headspace analysis of the clarified apples juices made of different apple cultivars. (Symbols in bold = classification samples and empty symbols = test samples).

Table 2
Results of PLS-DA analysis (based on 7 Principal Components accounting for the 81% of the total variance) applied for the classification of clarified apple juices by means of cultivar.

	Correctly classified	Misclassified	% correct classification
Internal validation			
Braeburn	32	1	96.7
Golden Delicious	25	0	100
Granny Smith	26	0	100
Jazz	26	0	100
Pink Lady	34	0	100
Total	143	1	99.3
External validation			
Braeburn	15	0	100
Golden Delicious	10	0	100
Granny Smith	10	0	100
Jazz	10	0	100
Pink Lady	14	0	100
Total	59	0	100

vation in concentration for trans-2-hexenal in Braeburn. Jazz had the greatest fruity-ethereal-flowery flavour type compounds as indicated by the higher concentration of acetates (2-methylpropyl, butyl, 2-methylbutyl, and hexyl acetates) and the low green-grassy odour related compounds (cis-3-hexen-1-ol and trans-2-hexenal).

Regardless the cultivar type, acetates and more specifically butyl and hexyl acetate were the dominant esters in the headspace of the apple juices, this has previously been reported in other studies (Aprea et al., 2012; Kato et al., 2003; Komthong et al., 2007; Ting et al., 2012). 1-Butanol was the most abundant alcohol in the headspace of the juices followed by 1-hexanol. In contrast to esters and aldehydes, alcohols are generally characterised as having higher odour threshold and thus they are considered as secondary contributors to apple flavour perception (Echeverría, Graell, López, & Lara, 2004). It is also interesting that 1-butanol was highly correlated (according to Pearson's test) with butyl acetate ($r = 0.926$, $p < 0.001$), hexyl acetate ($r = 0.898$, $p < 0.001$), trans-2-hexenal ($r = -0.777$, $p < 0.001$) and hexanal ($r = -0.748$, $p < 0.01$) and it could be surmised that these compounds are generated by a similar metabolic pathway during apple ripening. Finally, it should be noted that in the present work the major sesquiterpene found in the headspace of apples e.g. alpha-farnesene was not detected. This could be attributed either to the adopted protocol for the identification and quantification of the volatiles by GC or to the post juice extraction treatments e.g. enzymatic clarification and pectinase inactivation by heating. Su and Wiley (2006) investigated the impact of enzymatic clarification and pasteurisation on the major flavour volatile compounds of clarified apple juices and reported significant changes in concentration with processing.

No significant differences were observed for 2-methylbutanal and 3-methylbutanal. Although, the latter is well known as a
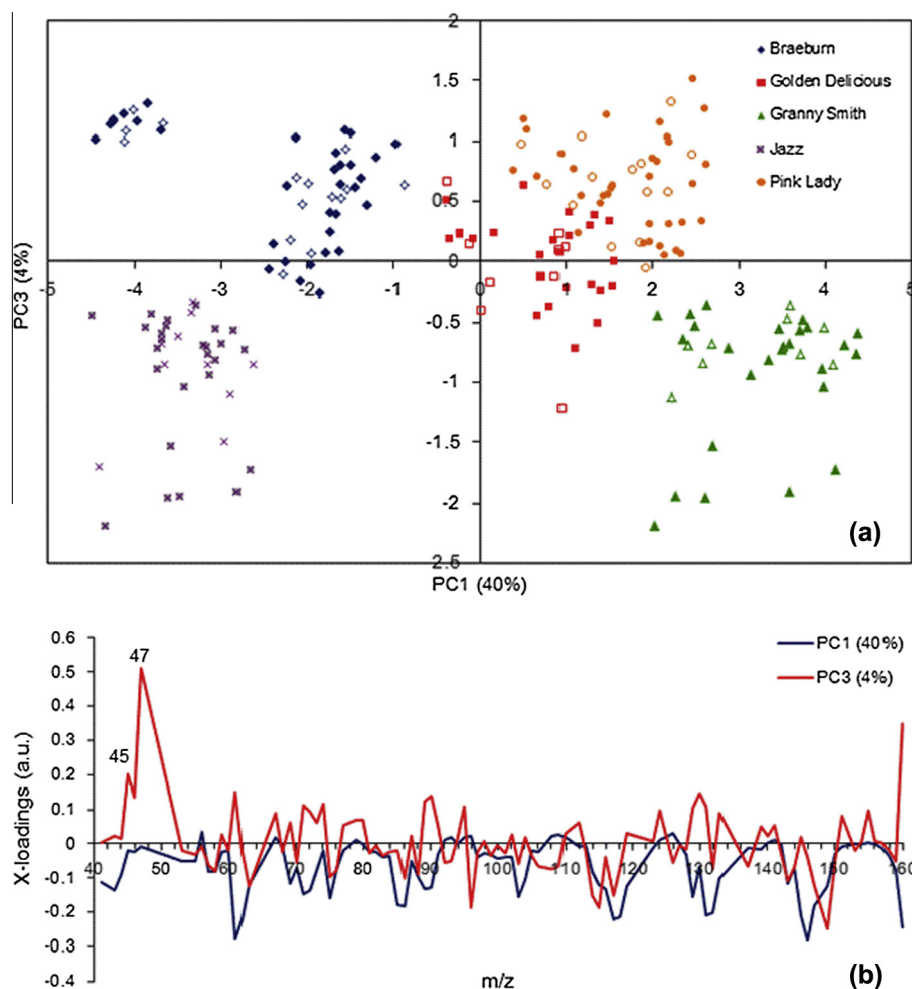

Fig. 3. PLS-DA scores (a) and loadings (b) for the first and third factors of the classification models based on the APCI-MS data obtained by the headspace analysis of the clarified apples juices made from different apple cultivars. Symbols in bold = classification samples and empty symbols = test samples).

precursor of the esters formed via the alcohol esterification pathway, the first two have been rarely identified in fresh cut apple samples. However, both aldehydes have been previously identified in processed fruit juices, including apple juice (Burdock, 2009; Sapers, Abbott, Massie, Watada, & Finney, 1977). Due to the presence of 2-methylbutanol at relatively high levels the presence of the former aldehydes is possibly related to the activity of enzymatic induced oxidation of alcohols.

3.2. Cultivars classification of apple juices by APCI-MS

For the classification of the apple juices according to their varietal origin the log transformed, mean centred and auto-scaled data were initially subjected to principal components analysis (PCA) to facilitate the formation of clusters and subsequently the dataset was subjected to the supervised classification technique PLS-DA. No specific pre-treatment of the data e.g. dimensionality reduction using PCA, was carried out apart from the log transformation of data in order to avoid the over fitting problems that have previously been reported by Granitto et al. (2007).

The scores and the X-loadings plots are represented In Fig. 2 for principle component one (PC1) and principle component two (PC2), PC1 and PC2 account for the 53% of total variance of the spectral data. For the PLS-DA models, seven principle components were used which accounted for 81% of the total variability

According to the PLS-DA scores plots, very good clustering was observed for the monocultivar apple juices used in the present study, with juices extracted from Jazz apples showing the largest distance from Granny Smith, Golden Delicious and Pink Lady. As is illustrated in the classification matrix for the calibration and validation (testing set) datasets (Table 2), juices produced from Golden Delicious, Jazz, Granny Smith, and Pink Lady apples were 100% correctly classified whilst in the case of the Braeburn extracted juices only one sample was misclassified. In both cases the total classification percentage was excellent (99.3% and 100% for internal and external validation) which indicates the robustness of the PLS-DA predictive models. Moreover, with an RMSE value ranging from 0.10 to 0.23 representing a total error of less than 5%, the predictive power of the herein constructed models is very good. A similar level of performance has previously been seen for geographical characterisation models using a PLS-DA approach constructed with the spectral fingerprint of other DIMS techniques (PTR-MS), applications include agro-industrial products with protected designation of origin such as olive oil, dry cured hams and truffle (Aprea, Biasioli, Carlin, Endrizzi, & Gasperi, 2009; Araghi-pour et al., 2008; Del Pulgar et al., 2011).

The individual masses were also evaluated to gain an insight into the chemistry that is driving the multivariate discrimination of the apple juices (cultivar). The X-loading plot for the first two axes was constructed and is shown in Fig. 2b. PC-1 was mainly
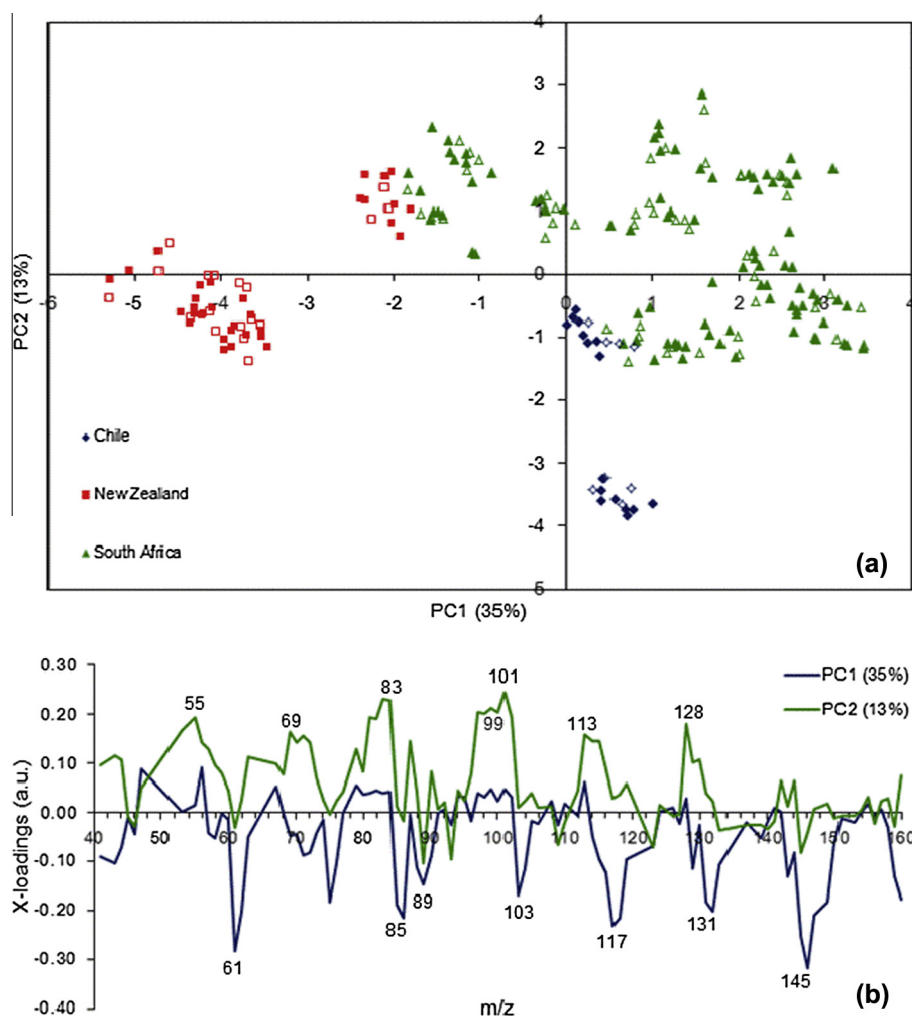

Fig. 4. PLS-DA scores (a) and loadings (b) for the first two factors of the classification models based on the APCI-MS data obtained by the headspace analysis of the clarified apples juices made from apples differing in their geographical origin. Symbols in bold = classification samples and empty symbols = test samples).

correlated with *m/z* 61, 75, 85, 89, 103, 117, 131, 145 and 159, these are well known parent and fragment ions of common alkyl-lesters (Aprea, Biasioli, Märk, & Gasperi, 2007). Similar fragments have also been reported in other DIMS studies. PC1 can therefore be tentatively identified as being related to the relative abundance of esters, and therefore the axis would be correlated to flavour notes such as fruity, ethereal, and fresh. Indeed, Jazz and Braeburn samples were clustered in the left side of the PCA map whilst the Granny Smith and Golden Delicious in the right. The second PC axis was also correlated with fragments of esters and alcohols, of which *m/z* 61 or 85 are tentatively attributed as fragments of acetates and 1-hexanol (Soukoulis et al., 2013), and 101 and 99 are proposed to be the parent ions of carbonyl compounds e.g. 1-hexanal (*m/z* 101) or trans-2-hexenal (*m/z* 99). Furthermore, *m/z* 83 was strongly discriminating and could be attributed to a dehydration product of 1-hexanol.

According to the X-loading plot for PC-1 and PC-3 (Fig. 3b) the peaks at *m/z* 47 and 45, which correspond to ethanol and acetaldehyde respectively (Davies et al., 2011), allowed the discrimination between Jazz and Braeburn apples supporting the classification data displayed in Table 2. Acetaldehyde is one of the most abundant volatile compounds present in the headspace of fresh cut apples (Ting et al., 2012). Apples juices extracted from Braeburn, Golden Delicious and Pink Lady were characterised by higher levels of acetaldehyde and ethanol which is in accordance with previously published data (Ting et al., 2012). Ethanol is considered as an indicator of post harvesting conditions e.g. exposure to hypoxia, stage of climacteric ripening (Dixon, 1999). According to Fig. 3a, juices extracted from Braeburn and Pink Lady had higher amounts of ethanol compared to Jazz and Granny Smith. The former observation implies that the APCI-MS fingerprinting may also provide important information associated not only with the genetic diversity of the samples but also with the adopted post-harvest practices, although further studies are recommended in this area.

3.3. Geographical provenance determination by APCI-MS

For the further evaluation of APCI-MS as a viable method for food authenticity testing and classification, the geographical provenance of the apples tested previously was also modelled. As it can be seen in Fig. 4a, effective clustering for the three apple juices was obtained, with New Zealand and South Africa being most clearly discriminated. The first two principle component axes accounted for 48% of total variability. For the PLS-DA models, five principle components were used which accounted for 79.7% of the total variability. The most robust classification performance was obtained in the case of internally validated PLS-DA models (97.1%) although the externally validated models were also successful (94.2%); this is further shown in Table 3. However, it should be further noted that in both cases the performance of the APCI-MS as a tool for geographical provenance determination was very good considering the high intrinsic variability due to the use of commercial samples. Whilst the use of commercial samples does allow the inclusion of true sample variability, it does not permit strict control of process parameters that support a mechanistic explanation of the model (e.g. cultivation and irrigation practices, environmental factors, edaphological parameters, post-harvesting practices). In both internal and external validation datasets the samples originating from New Zealand were all successfully classified, and of the total 135 samples only 4 were misclassified, resulting in an error rate of <3%.

There was a similar correlation of *m/z* to principle components, to that observed previously (Fig. 4b). More specifically, the first axis is proposed to be related to alkyl-esters (*m/z* 61, 75, 85, 89, 103, 117, 131, 145) and dehydrated alcohols (i.e. *m/z* 85 for 1-hexanol, m/z 57 for 1-butanol) in the form of fragments or parent ions.

Table 3
Results of PLS-DA analysis (based on 5 PCs accounting for 79.7% of the total variance) applied for the classification of clarified apple juices by means of geographical origin.

	Correctly classified	Misclassified	% correct classification
Internal validation			
Chile	16	0	100
New Zealand	32	0	100
South Africa	87	4	95.6
Total	135	4	97.1
External validation			
Chile	6	2	75
New Zealand	17	0	100
South Africa	44	2	95.6
Total	67	4	94.2

The second most powerful discriminating factor is shown on PC 2 and was found to associated with the green-grassy odour like volatiles such as 1-hexanal and trans-2-hexenal (*m/z* 101 and 99, respectively), or 1-hexanal and cis-hex-3-en-ol (*m/z* 83). Thus, complete discrimination between New Zealand and South Africa juices appear to be dependent on the ester-related flavour notes (fruity–flowery), whilst the Chilean samples appear to be discriminated by moderate ester concentration and low amounts of green-grassy flavour type volatiles. Finally, it should be noted that the variability of the New Zealand and South Africa labelled juices based on the green-grassy flavour criterion was quite high which would indicate differences in the ripening level of the sampled apples.

4. Conclusion

In conclusion, a PLS-DA chemometric approach was demonstrated to be a viable tool for the interpretation of raw APCI-MS data. The models generated were robust enough to reliably discriminate (100% correct classification with external validation set) apple juices prepared from Braeburn, Golden Delicious, Granny Smith, Jazz and Pink Lady varieties, furthermore developments on the model allowed the reliable (94.2% correct classification with external validation set) discrimination of the geographical provenance of monovarietal clarified apples from Chile, New Zealand and South Africa.

References

Aprea, E., Biasioli, F., Carlin, S., Endrizzi, I., & Gasperi, F. (2009). Investigation of volatile compounds in two raspberry cultivars by two headspace techniques: Solid-phase microextraction/gas chromatography-mass spectrometry (SPME/GC-MS) and proton-transfer reaction-mass spectrometry (PTR-MS). *Journal of Agricultural and Food Chemistry, 57*(10), 4011–4018.

Aprea, E., Biasioli, F., Märk, T. D., & Gasperi, F. (2007). PTR-MS study of esters in water and water/ethanol solutions: Fragmentation patterns and partition coefficients. *International Journal of Mass Spectrometry, 262*(1–2), 114–121.

Aprea, E., Biasioli, F., Sani, G., Cantini, C., Mark, T. D., & Gasperi, F. (2006). Proton transfer reaction-mass spectrometry (PTR-MS) headspace analysis for rapid detection of oxidative alteration of olive oil. *Journal of Agricultural and Food Chemistry, 54*(20), 7635–7640.

Aprea, E., Corollaro, M. L., Betta, E., Endrizzi, I., Demattè, M. L., Biasioli, F., et al. (2012). Sensory and instrumental profiling of 18 apple cultivars to investigate the relation between perceived quality and odour and flavour. *Food Research International, 49*(2), 677–686.

Aprea, E., Gika, H., Carlin, S., Theodoridis, G., Vrhovsek, U., & Mattivi, F. (2011). Metabolite profiling on apple volatile content based on solid phase microextraction and gas-chromatography time of flight mass spectrometry. *Journal of Chromatography A, 1218*(28), 4517–4524.

Araghipour, N., Colineau, J., Koot, A., Akkermans, W., Rojas, J. M. M., Beauchamp, J., et al. (2008). Geographical origin classification of olive oils by PTR-MS. *Food Chemistry, 108*(1), 374–383.

Biasioli, F., Gasperi, F., Aprea, E., Colato, L., Boscaini, E., & Märk, T. D. (2003). Fingerprinting mass spectrometry by PTR-MS: Heat treatment vs. pressure treatment of red orange juice – A case study. *International Journal of Mass Spectrometry, 223*, 343–353.

Biasioli, F., Yeretzian, C., Gasperi, F., & Mark, T. D. (2011). PTR-MS monitoring of VOCs and BVOCs in food science and technology. *Trends in Analytical Chemistry, 30*(7), 968–977.

Burdock, G. A. (2009). *Fenaroli's handbook of flavor ingredients/George A. Burdock*. CRC Press.

Cappellin, L., Soukoulis, C., Aprea, E., Granitto, P., Dallabetta, N., Costa, F., et al. (2012). PTR-ToF-MS and data mining methods: A new tool for fruit metabolomics. *Metabolomics, 8*(5), 761–770.

Carcea, M., Brereton, P., Hsu, R., Kelly, S., Marmiroli, N., Melini, F., et al. (2009). Food authenticity assessment: Ensuring compliance with food legislation and traceability requirements. *Quality Assurance and Safety of Crops & Foods, 1*(2), 93–100.

Davies, S. M., Linforth, R. S., Wilkinson, S. J., Smart, K. A., & Cook, D. J. (2011). Rapid analysis of formic acid, acetic acid, and furfural in pretreated wheat straw hydrolysates and ethanol in a bioethanol fermentation using atmospheric pressure chemical ionisation mass spectrometry. *Biotechnology for Biofuels, 4*(1), 1–8.

Defilippi, B. G., Dandekar, A. M., & Kader, A. A. (2005). Relationship of ethylene biosynthesis to volatile production, related enzymes, and precursor availability in apple peel and flesh tissues. *Journal of Agricultural and Food Chemistry, 53*(8), 3133–3141.

Del Pulgar, J. S., Soukoulis, C., Biasioli, F., Cappellin, L., García, C., Gasperi, F., et al. (2011). Rapid characterization of dry cured ham produced following different PDOs by proton transfer reaction time of flight mass spectrometry (PTR-ToF-MS). *Talanta, 85*(1), 386–393.

Dimick, P. S., Hoskin, J. C., & Acree, T. E. (1983). Review of apple flavor – State of the art. *Critical Reviews in Food Science & Nutrition, 18*(4), 387–409.

Dixon, J. (1999). *Enhancement of aroma and flavour volatiles in apple juice.* PhD thesis, Massey University, New Zealand.

Echeverría, G., Graell, J., López, M. L., & Lara, I. (2004). Volatile production, quality and aroma-related enzyme activities during maturation of 'Fuji' apples. *Postharvest Biology and Technology, 31*(3), 217–227.

Fernández-Vázquez, R., Linforth, R., Hort, J., Hewson, L., Vila, D. H., & Heredia Mira, F. J. (2013). Headspace delivery of limonene from the serum and non-serum fractions of orange juice in-vitro and in-vivo. *LWT - Food Science and Technology, 51*(1), 65–72.

Fisk, I. D., Boyer, M., & Linforth, R. S. T. (2012). Impact of protein, lipid and carbohydrate on the headspace delivery of volatile compounds from hydrating powders. *European Food Research and Technology, 235*(3), 517–525.

Fisk, I. D., Virdie, A., Kenny, J., & Ullrich, F. (2010). Soluble coffee classification through rapid scanning methodologies. In *Association for Science and Information on Coffee*. Bali.

Fisk, I. D., Kettle, A., Hofmeister, S., Virdie, A., & Silanes Kenny, J. (2012). Discrimination of roast and ground coffee aroma. *Flavour, 1*(1), 14.

Fisk, I. D., Linforth, R. S. T., Taylor, A. J., & Gray, D. A. (2011). Aroma encapsulation and aroma delivery by oil body suspensions derived from sunflower seeds (*Helianthus annus*). *European Food Research and Technology, 232*(5), 905–910.

Galle, S. A., Koot, A., Soukoulis, C., Cappellin, L., Biasioli, F., Alewijn, M., et al. (2011). Typicality and geographical origin markers of protected origin cheese from the Netherlands revealed by PTR-MS. *Journal of Agricultural and Food Chemistry, 59*(6), 2554–2563.

Granitto, P. M., Biasioli, F., Aprea, E., Mott, D., Furlanello, C., Märk, T. D., et al. (2007). Rapid and non-destructive identification of strawberry cultivars by direct PTR-MS headspace analysis and data mining techniques. *Sensors and Actuators B: Chemical, 121*(2), 379–385.

Guo, J., Yue, T., & Yuan, Y. (2012). Feature selection and recognition from nonspecific volatile profiles for discrimination of apple juices according to variety and geographical origin. *Journal of Food Science, 77*(10), C1090–C1096.

Kato, T., Shimoda, M., Suzuki, J., Kawaraya, A., Igura, N., & Hayakawa, I. (2003). Changes in the odors of squeezed apple juice during thermal processing. *Food Research International, 36*(8), 777–785.

Kelly, J. F. D., & Downey, G. (2005). Detection of sugar adulterants in apple juice using Fourier transform infrared spectroscopy and chemometrics. *Journal of Agricultural and Food Chemistry, 53*(9), 3281–3286.

Komthong, P., Hayakawa, S., Katoh, T., Igura, N., & Shimoda, M. (2006). Determination of potent odorants in apple by headspace gas dilution analysis. *LWT - Food Science and Technology, 39*(5), 472–478.

Komthong, P., Igura, N., & Shimoda, M. (2007). Effect of ascorbic acid on the odours of cloudy apple juice. *Food Chemistry, 100*(4), 1342–1349.

Langford, V. S., Reed, C. J., Milligan, D. B., McEwan, M. J., Barringer, S. A., & Harper, W. J. (2012). Headspace analysis of italian and new zealand parmesan cheeses. *Journal of Food Science, 77*(6), 1750–3841.

León, L., Daniel Kelly, J., & Downey, G. (2005). Detection of apple juice adulteration using near-infrared transflectance spectroscopy. *Applied Spectroscopy, 59*(5), 593–599.

Lignou, S., Parker, J. K., Oruna-Concha, M. J., & Mottram, D. S. (2013). Flavour profiles of three novel acidic varieties of muskmelon (*Cucumis melo* L.). *Food Chemistry, 139*(1–4), 1152–1160.

Linforth, R. S. T., Baek, I., & Taylor, A. J. (1999). Simultaneous instrumental and sensory analysis of volatile release from gelatine and pectin/gelatine gels. *Food Chemistry, 65*(1), 77–83.

López, M. L., Villatoro, C., Fuentes, T., Graell, J., Lara, I., & Echeverría, G. (2007). Volatile compounds, quality parameters and consumer acceptance of 'Pink Lady®' apples stored in different conditions. *Postharvest Biology and Technology, 43*(1), 55–66.

Magdas, D. A., & Puscas, R. (2011). Stable isotopes determination in some Romanian fruit juices. *Isotopes in Environmental and Health Studies, 47*(3), 372–378.

Montero-Prado, P., Bantayeb, K., & Nerín, C. (2013). Pattern recognition of peach cultivars (*Prunus persica* L.) from their volatile components. *Food Chemistry, 138*(1), 724–731.

Reid, L. M., O'Donnell, C. P., & Downey, G. (2006). Recent technological advances for the determination of food authenticity. *Trends in Food Science & Technology, 17*(7), 344–353.

Sapers, G. M., Abbott, J., Massie, D., Watada, A., & Finney, E. E. (1977). Volatile composition of Mcintosh apple juice as a function of maturity and ripeness indexes. *Journal of Food Science, 42*(1), 44–47.

Shojaei, Z. A., Linforth, R. S. T., & Taylor, A. J. (2007). Estimation of the oil water partition coefficient, experimental and theoretical approaches related to volatile behaviour in milk. *Food Chemistry, 103*(3), 689–694.

Singhal, R. S., Kulkarni, P. R., & Rege, D. V. (1997). *Handbook of indices of food quality and authenticity*. Woodhead Publishing.

Sivakesava, S., Irudayaraj, J. M. K., & Korach, R. L. (2001). Detection of adulteration in apple juice using mid infrared spectroscopy. *Applied Engineering in Agriculture, 17*(6), 815–820.

Soukoulis, C., Cappellin, L., Aprea, E., Costa, F., Viola, R., Märk, T. D., Gasperi, F., & Biasioli, F. (2013). PTR-ToF-MS, a novel, rapid, high sensitivity and non-invasive tool to monitor volatile compound release during fruit post-harvest storage: The case study of apple ripening. *Food Bioprocess Technology, 6*(10), 2831–2843. http://dx.doi.org/10.1007/s11947-012-0930-6.

Souza, S. S., Cruz, A. G., Walter, E. H. M., Faria, J. A. F., Celeghini, R. M. S., Ferreira, M. M. C., et al. (2011). Monitoring the authenticity of Brazilian UHT milk: A chemometric approach. *Food Chemistry, 124*(2), 692–695.

Su, S. K., & Wiley, R. C. (2006). Changes in apple juice flavor compounds during processing. *Journal of Food Science, 63*(4), 688–691.

Taylor, A. J., Linforth, R. S. T., Harvey, B. A., & Blake, A. (2000). Atmospheric pressure chemical ionisation mass spectrometry for in vivo analysis of volatile flavour release. *Food Chemistry, 71*(3), 327–338.

Ting, V. J., Soukoulis, C., Silcock, P., Cappellin, L., Romano, A., Aprea, E., et al. (2012). In vitro and in vivo flavor release from intact and fresh-cut apple in relation with genetic, textural, and physicochemical parameters. *Journal of Food Science, 77*(11), C1226–C1233.

Tres, A., Ruiz-Samblas, C., van der Veer, G., & van Ruth, S. M. (2013). Geographical provenance of palm oil by fatty acid and volatile compound fingerprinting techniques. *Food Chemistry, 137*(1–4), 142–150.

Yamamoto, A., Kawai, M. I. O., Miwa, T., Tsukamoto, T., Kodama, S., & Hayakawa, K. (2008). Determination of adulteration in apple juice by HPLC with novel optical rotation detector. *Journal of Agricultural and Food Chemistry, 56*(16), 7302–7304.

Yang, N., Fisk, I. D., Linforth, R., Brown, K., Walsh, S., Mooney, S., et al. (2012). Impact of flavour solvent on biscuit micro-structure as measured by X-ray micro-computed tomography and the distribution of vanillin and HMF (HPLC). *European Food Research and Technology, 235*(6), 1083–1091.

Yu, T. T., Macnaughtan, B., Boyer, M., Linfoith, R., Dinsdale, K., & Fisk, I. D. (2012). Aroma delivery from spray dried coffee containing pressurised internalised gas. *Food Research International, 49*(2), 702–709.