APPLE JUICES ADULTERATION'S ASSESSMENT USING A COMBINED APPROACH: IC-HPLC-CHEMOMETRY

Edward MUNTEAN

University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania
E-mail: edimuntean@yahoo.com / Phone: +40754074375

Introduction

Fruit juices' authenticity is an important issue in food safety and food quality control; hence, objective analytical methods and appropriate data analysis procedures are required for guaranteeing it for consumers. This work proves the usefulness of ion chromatography (IC) in conjunction with high performance liquid chromatography (HPLC) for obtaining conclusive data on apple juices' composition in one run, using a hybrid system equipped with conductivity detection and differential refractive index detection. By the simultaneous determination of three carbohydrates (fructose, glucose and saccharose) and four cations (Na^+, K^+, Mg^{+2} and Ca^{+2}), this approach saves analytical time, sample pre-treatment and reagents.

The major objective of this research was to establish a method involving a minimal sample workup, able to reveal the chromatographic fingerprints for the above-mentioned analytes, for assessing both the apple juices' quality and authenticity. Chromatographic analysis in conjunction with multivariate data analysis proved to be appropriate complimentary tools in discriminating between sweetened and non-sweetened apple juices, between genuine and adulterated products.

Materials and methods

Commercial apple juices were obtained from the local supermarkets; 36 juice brands were selected, from these twelve being Romanian (R1-R12), eight Polish (P1-P8), six Austrian (A1-A6), two French (F1, F2), two German (G1, G2), two Hungarian (H1, H2), two Italian (I1, I2) and two American (U1, U2). All the samples were analyzed in triplicates, using 10 mL apple juice from each brand, which were diluted 1/ 10 (v/v) with ultrapure water, then filtered through 0.45 mm membrane filters (Millipore). Reference apple juices were obtained from Starkinson and Red-Star apples using a centrifugal juice-extractor; after homogenization, the freshly-made juices were processed as mentioned before.

Mobile phases were prepared from high purity nitric acid (Merck, Darmstadt, Germany) and HPLC grade acetonitrile (Merck); ultrapure water (18.2 MΩ. cm^{-1}) was utilized for mobile phase's preparation as well as for samples' dilution, being obtained from a Direct Q 3UV Smart (Millipore). Mobile phases were filtered through 0.45 μm membranes (Millipore), and then degassed under vacuum using an Elmasonic S30 H ultrasonic bath. Standard working calibration solutions for cation analysis were prepared daily by dilution from a "six cation standard- II" (Dionex Corporation), while carbohydrate calibration solutions were prepared from reagent-grade glucose, fructose and saccharose (Merck).

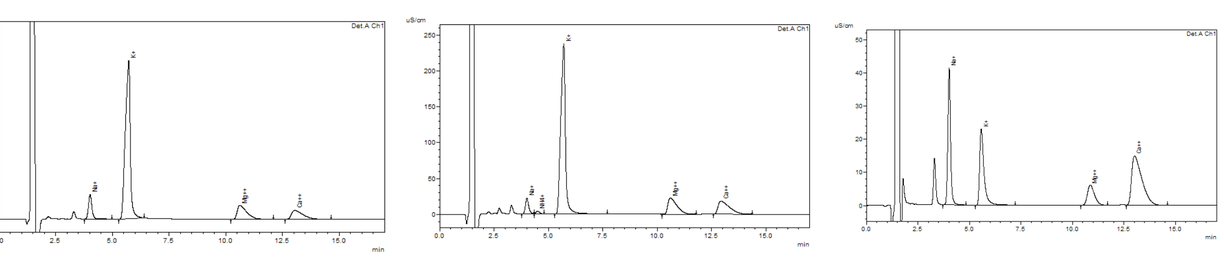
Chromatographic analyses were performed on a Shimadzu system, consisting of two Prominence LC-20AP solvent delivery modules, a Prominence DGU 20As online degasser, an automatic sample injector SIL-10AF, an RID-10A differential refractive index detector, a CDD-10Avp dual channel conductivity detector, a Prominence CTO-20A column oven, an FCV-10AH2 valve unit and a Prominence CBM-20A system controller. Isocratic separations were conducted at 40^0C, using a Universal Cation 7u column (100 x 4.6 mm–Alltech Associates) for cation analysis, the mobile phase being a 3 mM HNO_3 solution, at 0.5 mL min^{-1}, while carbohydrates were separated using an EC 250/4 Nucleodur 100–5 NH_2 RP column (250 x 4.6 mm, Macherey-Nagel) and 77:23 v/v % acetonitrile in water as mobile phase at 1 mL min^{-1}; injection sample volumes were of 200 μL. The external standard method was used for quantification; recoveries were established spiking samples with known concentrations of analytes, the obtained values being in the range 96.28-104.58%. Instrument control, data acquisition and data analysis were accomplished by „LCsolution" ver.1.2. software.

Principal component analysis (PCA) and cluster analysis were completed on autoscaled data using Matlab (The Mathworks Inc., USA).



Results

- The four major cations in apple juices are potassium, magnesium and calcium; of these, magnesium and calcium levels may be significantly distorted by the addition of water to apple juice concentrate, while sodium and potassium may be introduced as counter ions by the added ingredients. Authentic apple juices have a distinct chromatographic fingerprint (IC displayed on fig.1.b), easily distinguishable by adulterated ones (Fig.1.c).
- Genuine apple juices are characterized by a minimum fructose/glucose ratio of 1.6 and a maximum sucrose content of 3.5% and a maximum sucrose content of 3.5 %; their chromatographic fingerprints exhibits major peaks for fructose and potassium (fig.1); the samples within the biggest cluster (figures 2-3) agree with these criteria, the determined values being in most cases in good agreement with the label information if comparing the total carbohydrate concentrations.
- The HPLC chromatographic fingerprints of apple juices with added sugar exhibit a distinct second major peak of saccharose besides that of fructose (not listed on this poster), while the potassium peak is usually smaller and that of calcium bigger comparing with genuine juices; these juices generate a distinct cluster, which can be easily differentiate by PCA (samples P1, P2, A3, A4 – fig.2 and fig.3).
- Apple juices sweetened using glucose-fructose syrup can be identified based on their chromatographic carbohydrate fingerprint (HPLC chromatogram not listed on this poster), having fructose and glucose as major peaks; in the corresponding ion chromatograms, the potassium peak is smaller and the calcium one bigger comparing with genuine juices, while these juices also generate a definite cluster, distinguishable by PCA (samples H1, H2, R3, R4, A5, A6, P7, P8 – fig.2 and fig.3).
- A fourth category of apple juices in this context are those with unusual carbohydrate and cation chromatographic fingerprints, dominated by glucose (higher than fructose - not listed on this poster) and sodium besides potassium; they form a different cluster (P3, P3, R11, R12 - fig.2 and fig.3) which is characterized by a high loading for glucose. The corresponding products' labels are not consistent with these findings, hence they can be considered adulterations.



a. freshly-pressed apple juice (St1) b. genuine apple juice (R2) c. adulterated apple juice (R12)

Figure 1. Ion chromatograms obtained for apple juices

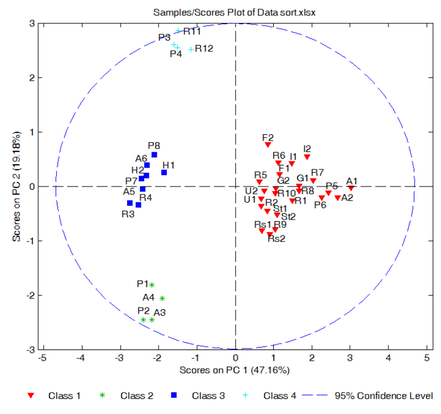


Figure 2. Scores plot obtained for the seven variables' variables model (95% confidence level, marked by the discontinuous line)

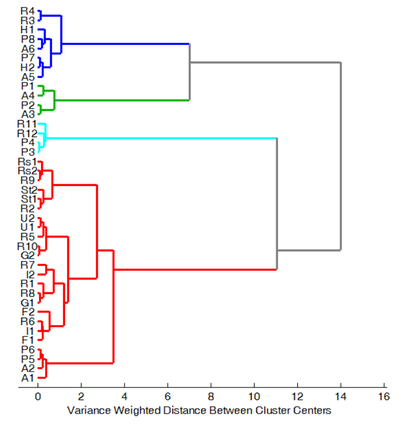


Figure 3. Dendrogram obtained with Ward's method using Mahalanobis distance and PCA

Conclusions

- Chromatographic analysis in conjunction with multivariate data analysis proved to be appropriate complimentary tools in discriminating between sweetened and non-sweetened apple juices, between genuine and adulterated products.
- Using a minimal sample workup, this approach can reveal the chromatographic fingerprints for three carbohydrates (fructose, glucose, saccharose), and four cations (sodium, potassium, magnesium and calcium), being thus a fast tool for assessing both the apple juices' quality and authenticity.