Fingerprinting Fruits Using Coumarins & Psoralens, Authentication Tool for Meyer Lemons

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Abstract:

Screening, using RP-HPLC, for a range of Coumarins & Psoralens has been shown to be useful to differentiate between lemons and limes juices as well as their oils. Data presented in this poster will show the extension of this technique to the differentiation between lemons from Meyer lemons.

Introduction

Unfortunately there is a long history of economic adulteration of fruit juices. Screening, using RP-HPLC, for a range of Coumarins & Psoralens has been shown to be useful to differentiate between lemon and lime juices and oils. Data presented in this poster will show the extension of this technique to the differentiation of Meyer lemons (*Citrus × meyeri)*, from lemon (*Citrus limon* (L.) Osbeck) and lime (*Citrus aurantifolia* and *Citrus Latifolia)*. Meyer lemon is considered to be a cross between a citron (lemon) and a mandarin/pomelo hybrid[11].

Meyer lemons have become more popular and are changing from a purely ornamental use to one with a culinary function, which means it would be susceptible to fraud and adulteration. Although in 2009 the FDA officially recognized food fraud as an emerging risk to the consumer, in fringe industries that use juice based products, this risk had not been recognized and few companies have QA programs in place[10]. It should be remembered that product "verification" is a key element in the 2011 Food safety modernization act[1].

Juices are typically assessed for a range of components (acids, minerals, sugars, by ^{13}C-IRMS) and by the Kirksey[2] (fig 7) fingerprinting method to detect juice mixtures. However, the latter fails to discriminate between lemon and their hybrid blends. Reported here is an additional procedure, based on polymethoxylated phenols (PMF) which shows the phenolic pattern of this hybrid fruit that may be used to identify its juice or juice concentrate.

Methodologies:

Key tests are:- **Brix (AOAC 983.17), tit. acidity (IFU 3)[8], citric acid (AOAC 986.13), the enzymic method for isocitric acid (IFU 54), sugars (IFU67), K, Mg, Ca (IFU 33), oligosaccharides (IFU Rec 4), ^{13}C-IRMS (AOAC 981.09), and the internal isotopic method for C_4 and C_3 acid addition[6]. Other useful tests are malic and fumaric acids (AOAC 986.13) and $\delta^{18}O$ values for NFC juices (AOAC 992.09).**

PMFs

Juices are prepared at single strength and cloudy juices are centrifuged (2500 x g) for 10 minutes. Clarified single strength juices can be used "as is".
The clear or centrifuged sample (20 ml) is passed through the activated reverse phase SPE cartridge over a 2 minute period. The sugars and acids are then eluted with water (5 ml) over 15 seconds. The less polar materials are subsequently eluted with 28 % acetonitrile in water (5 ml) over 30 seconds. Traces of this solvent are "blown out" of the SPE cartridge with air, prior to the final elution of the PMFs using MeOH/CH_3Cl (1:1), 5 ml) over 1 minute. The solvent is collected in a small flask and removed by evaporation under vacuum at 35°C. The residue is taken up in methanol (1 ml) and filtered, if required, prior to LC analysis.

Polymethoxyflavones UPLC conditions

Column: 15 cm C_{18} column 1.7 µm (BEH or equivalent)
Solvent A: HPLC grade water
Solvent B: 100 % acetonitrile
Flow rate: 0.3 ml/min
Injection volume: 2 µl
UV detection: 330 nm, DAD can replace UV detector
Analysis time: 50 minutes

Gradient conditions

Time	%B
0	25
30	25
30.1	End

References:
1) Food safety modernization act (2011) http://www.fda.gov/Food/GuidanceRegulation/FSMA/default.htm
http://www.natlconsumersleague.org/food/85-food-labeling/637-watered-down-lemon-juice-making-advocatessour
2) Kirksey S.T. *et al* (1995). In: Methods to detect adulteration of fruit juice beverages Vol 1, page 145 – 166. Pub.by AG Science
3) McHale D. & Sheridan J.B. (1989). The Oxygen Heterocyclic Compounds of Citrus Peel Oils. Journal of Essential Oil Research 1, 4, 139-149
4) Jamin E. R. *et al*. Detection of exogenous citric acid in fruit juices by stable isotope ratio analysis. *J Agric. Food Chem*. 53 ,5130 – 5133, (2005)
5) Ooghe W.C., Ooghe S. J., Detavernier C.M & Huyghebaert A. (1994) Characterization of Orange Juice (*Citrus sinensis*) by Polymethoxylated Flavones . J. Agric. Food Chem., 42 (10), 2191–2195
6) Lehnert N. & Ara V. Authenticity analysis of lemon juices concerning the adulteration lime. (2014) Fruit Processing Nov/Dec 242 - 248
7) Pupin A.M., Dennis, M.J. & Toledo M.C.F. (1998). Polymethoxylated flavones in Brazilian orange juice. Food Chemistry 63,(4), 513–518
8) IFU methods www.ifu-fruitjuice.com (for a fee)
9) AIJN code of practice www.aijn.org (for a fee)
10) https://www.beveragedaily.com/Article/2012/03/26/National-Consumer-League-urges-FDA-crackdown-on-US-lemon-juice-swindle
11) Curk, Franck; Ollitrault, Frédérique; Garcia-Lor, Andres; Luro, François; Navarro, Luis; Ollitrault, Patrick (2016). Phylogentic origin of limes and and lemons revealed by cytoplasmic and nuclear markers. *Annals of Botany*. **11**: 565–583.

Discussion:

Some expensive juices can be blended with cheaper materials, such as apple and grape. Meyer lemon is also sometimes blended with/or substituted for lemon or lime. As these juices have very similar compositions this type of blending is very difficult to detect from the regular juice parameters. Although the "Kirksey" method shows slightly different LC profiles for lemon and lime, which allows their discrimination, it does not provide a very good procedure for the detection of blends of these juices, due to their similarities. However, there are other, less polar phenolics, which can be very useful to identify these juice blends. The procedure described here was developed in the 90's as a method to detect mandarin in orange juice[5] but has been adapted by us for use on a UPLC system to detect the presence of lime in lemon.

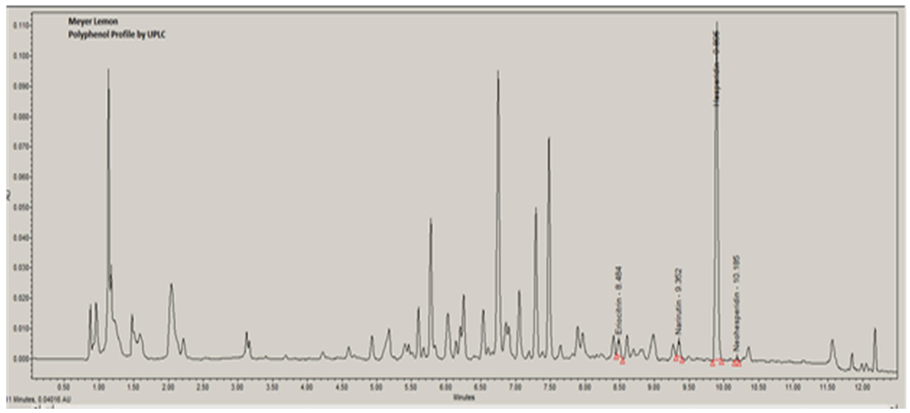

Fig 7 Meyer lemon polyphenol profile

As illustrated in Figures 1 & 5 it is clear that the pattern of polymethoxyphenols (PMF) seen in lemon and lime are very different and allows a clear differentiation of these juices and their blends. Meyer lemons contain sinensetin (Fig 4) which is not seen in lemon or lime but present in orange and mandarin (Figs 2 & 3), which shows its genetic heritage. This was achieved by the use of appropriate standards (Sigma) and use of LC-DAD. Typical DAD spectra for sinensetin is shown in figure 8 and lime markers are shown in Fig 5. It has been found that using the procedure developed by Ooghe *et al* and adapted here offers certain advantages over the method employed by Lehnert & Ara in their study[6]. Although this procedure requires some careful manipulation of solid phase extraction cartridges, it still offers a very efficient and rapid method to screen Meyer lemon juice for the presence of added lime and also its own profile fingerprint.

Conclusions

The adulteration of fruit juice still remains an issue and new methods of detection have gained more attention. This method can be used as a tool to look for markers in authentic Meyer lemon juice as well as absence of lemon and lime juice addition.

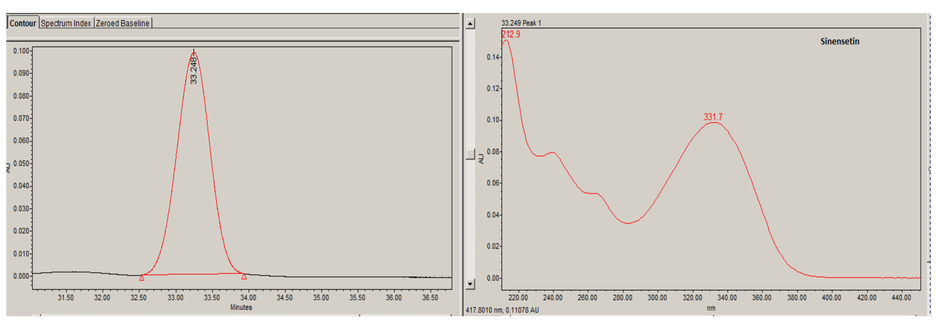

Fig 8 Sinensetin Diode Array

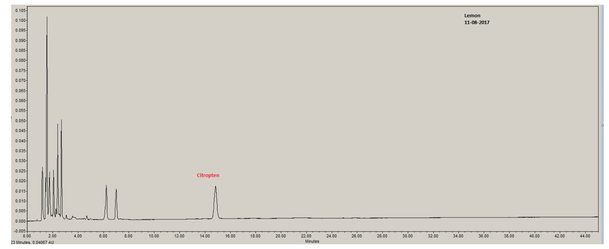

Fig 1 lemon Juice

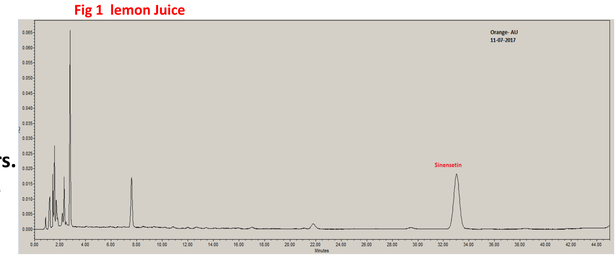

Fig. 2 Orange Juice

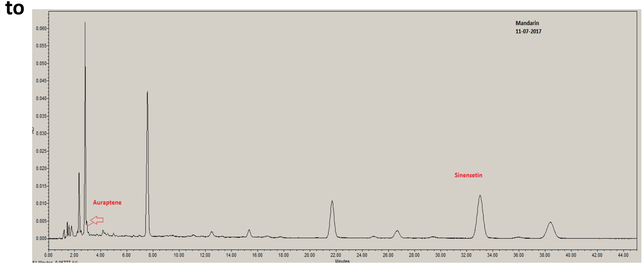

Fig 3 Mandarin

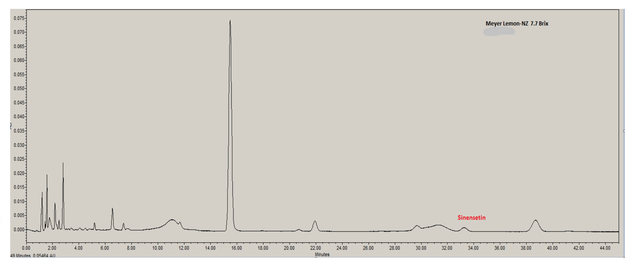

Fig 4 Meyer Lemon

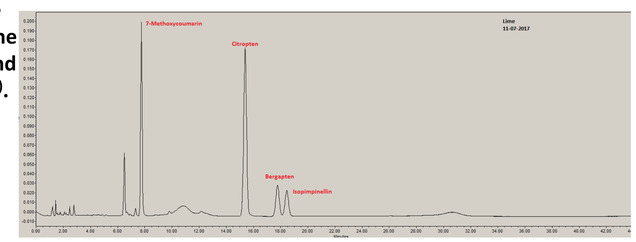

Fig 5 Lime Juice

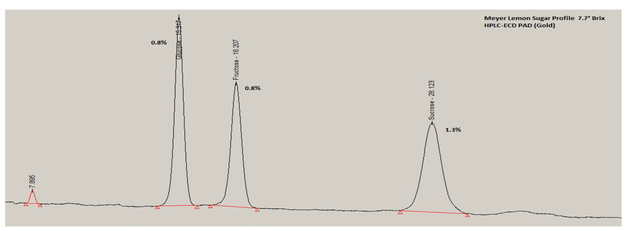

Fig 6 Meyer lemon sugar profile

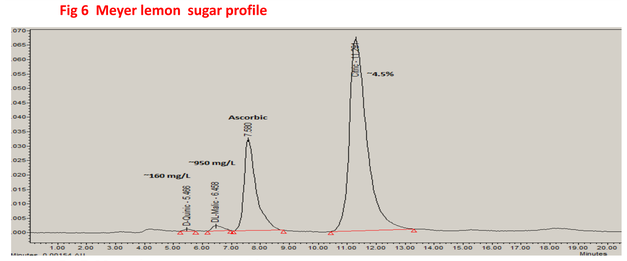

Fig 7 Meyer lemon organic acids