Identification of Adulteration in High Quality Styrian Pumpkin Seed Oil Using Untargeted Analysis via LC-QTOF Followed by Analysis of Specific Entities via LC-QQQ

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Overview

- Scope: Detection of adulteration of Styrian pumpkin seed oil with vegetable oils
- Nontargeted analysis using UHPLC-QTOF and Agilent Profinder and Mass Profiler Professional software
- Selection of specific entities for the tested vegetable oils
- Targeted analysis of specific entities using UHPLC-QQQ
- Results: Canola-, and sunflower oil addition <5 % detected

Introduction

Pumpkin seed oil is a genuine specialty of the province of Styria in southern Austria, and a product of protected geographical indication (PGI). As a premium product with prices around 20 €/L it is a likely target for economically motivated adulteration, such as blending with cheaper plant oils.

Currently, nontargeted chemometric approaches are gaining importance in fighting food fraud. Mass spectrometers capable of measuring accurate mass and powerful software tools are essential for such tasks.

The aim of this work is to examine the adulteration of pumpkin seed oil with canola-, and sunflower oil. This task was addressed by starting with nontargeted analysis to identify specific entities for the tested oils, which is then followed by targeted analysis using a LC-QQQ system to acquire MRM transitions.

Methods

Sample preparation:

- Pumpkin seed oil mixed in different ratios (0, 1, 5, 10, 30, 50 %) with canola-, or sunflower oil
- Extraction with acetonitrile, dispersive SPE with C_{18} material
- Internal standards (deuterated pesticides) added before extraction

Measurement:

Instruments: Agilent 1290 Infinity II UHPLC coupled to a 6545 QTOF system for nontargeted analysis and fragmentation experiments
& Agilent 1290 Infinity UHPLC coupled to a 6460 QQQMS for targeted analysis

UHPLC-separation:

Reversed phase separation using a ZORBAX SB-Aq column and a gradient of 5 mM ammonium formate, 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile.

Mass spectrometry: electrospray ionization; scan between m/z 60 and m/z 1200 for nontargeted analysis (QTOF), fragmentation experiments at collision energy of 10, 20, 40 V (QTOF) & MRM (QQQMS) of certain m/z for targeted analysis

Data evaluation (using several Agilent software tools):

1) Recursive molecular feature extraction (Profinder); **2)** multivariate data analysis and identification of entities specific to each oil (Mass Profiler Professional); **3)** Assessment of test samples with a prediction model (Classifier); **4)** Assessment of specific entities (Mass Hunter Quantitative Analysis); **5)** Selection of m/z for MRM transitions (MassHunter Qualitative Analysis); **6)** Quantitation according to vegetable oil content (MassHunter Quantitative Analysis)

Results

Specific/unique entities for each oil could be identified (figure 1). Using the combined list of specific entities for the oils, principal component analysis allowed the grouping of samples according to the oil content (figure 2). Additionally, prediction models could be generated for the classification of samples (figure 4).

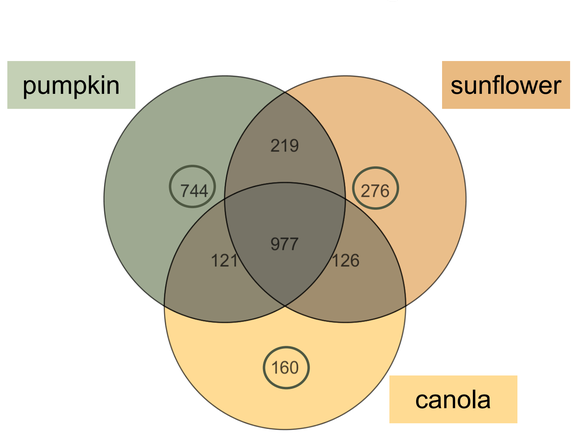

Figure 1: VENN diagram of 100 % oil samples

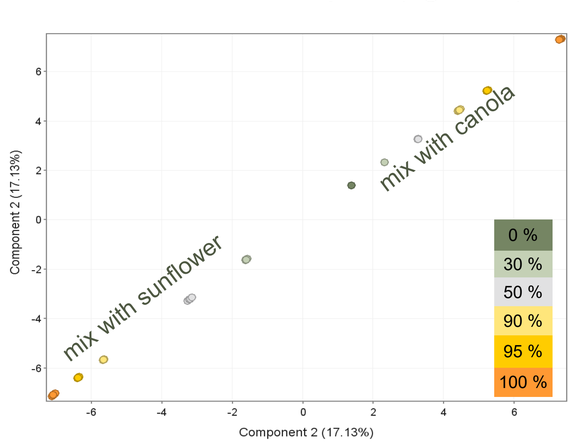

Figure 2: Principal component analysis allowed grouping in accordance with oil content

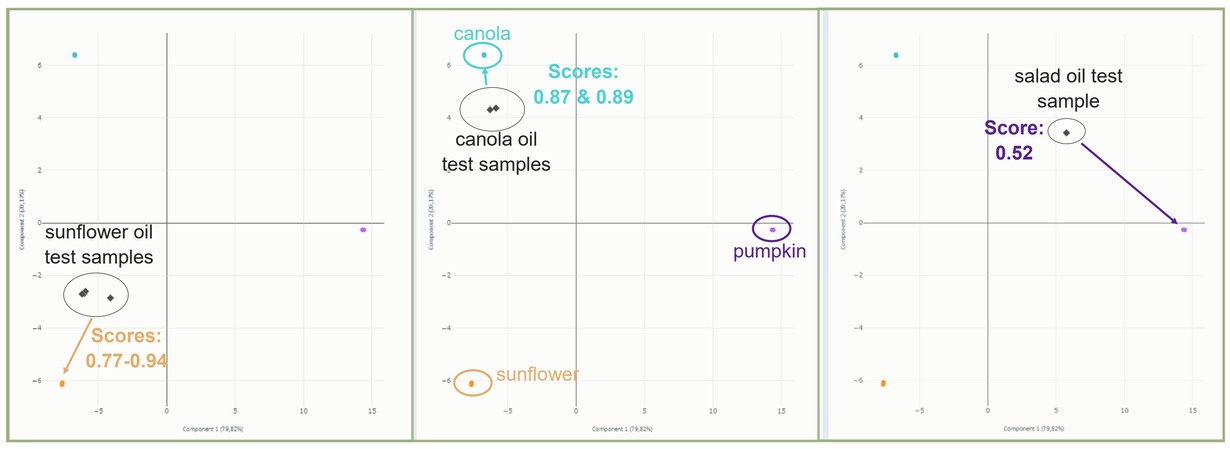

Figure 3: PLSD prediction model applied to test oil samples (Score 0 to 1; 1 is a perfect match)

Entities suitable for quantitation (sufficient response, correlation signal and oil content, absence in other oils) were chosen using Mass Hunter Quantitative Analysis as shown in figure 4. After fragmentation experiments, selected MRM transitions were used to establish calibration curves (figure 5).

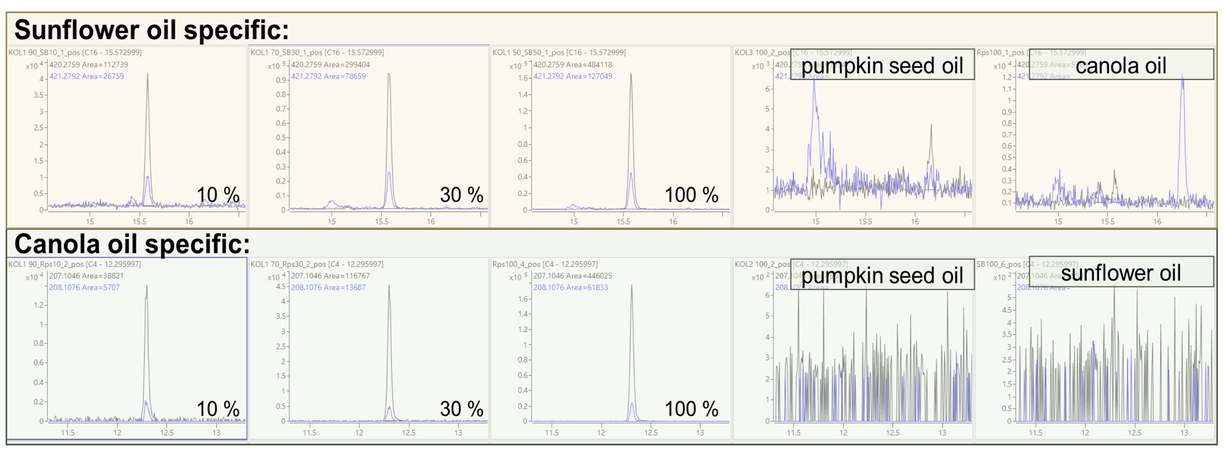

Figure 4: Selection of entities for further use in targeted measurements via compounds at a glance view

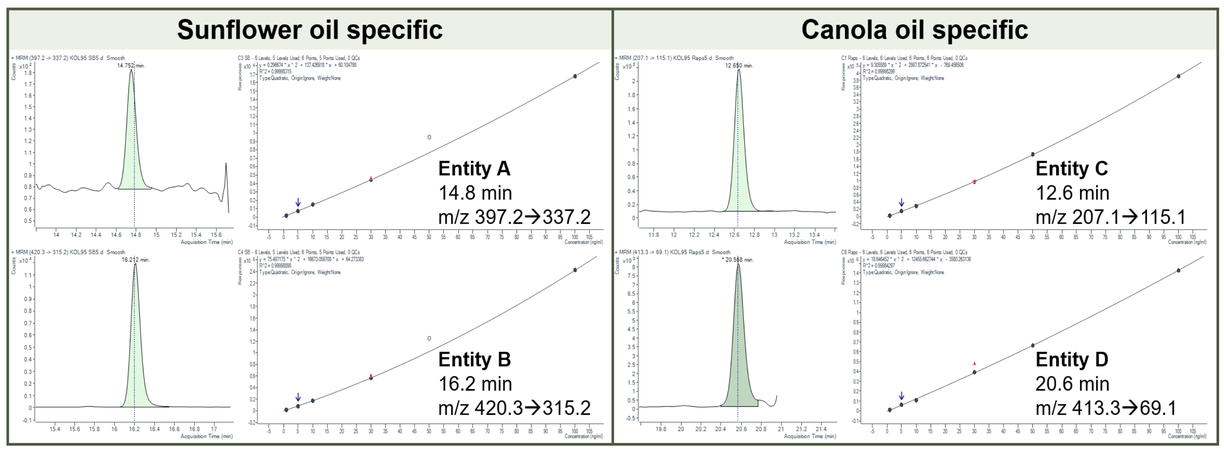

Figure 5: Signals and calibration curves (signal area vs. vegetable oil content) for target entities

Quantification experiments using test oils showed that the specific entities could be found in the respective oils (canola & sunflower), and mostly in insignificant quantities in the other oils (table 1). Deviations from the theoretical content could be due to biological-, or production variations, and can be checked with more samples.

Table 1: Tested vegetable oils and "quantified" oil content in wt% using entities shown in figure 5

	salad*	sunflower				canola		soy	olive	corn		peanut
used entity												
Sunflower (A)	< 1	80	72	81	66	< 1	< 1	< 1	< 1	< 1	4	7
Sunflower (B)	< 1	55	54	57	66	< 1	< 1	2	< 1	< 1	3	4
Canola (C)	60	< 1	< 1	< 1	< 1	142	150	< 1	< 1	2	2	< 1
Canola (D)	71	< 1	< 1	< 1	< 1	107	103	9	< 1	1	1	< 1

*theoretical content of salad oil: 80 % canola oil & 20 % pumpkin seed oil

Conclusion & Outlook

Untargeted analysis using UHPLC Q-TOF turned out to be a valuable tool to identify specific entities in the tested oils and allowed the identification of adulteration down to 5%. However, initial experiments with targeted measurements of specific entities enabled the detection of even lower percentages of blended vegetable oils. Also, it allows a faster and simpler analysis of real samples.

The scope of this project could be extended to other vegetable oils. To examine specificity and biological variability of the chosen entities, more samples should be analyzed. Certainly, an identification of the specific substances would be of interest.

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