Amino Acid Analysis using EZ:faast™ to Assess Juice Purity

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Introduction

Customer demand for quality fruit juices has continued to grow dramatically over the last decade, due much in part to a desire for healthy beverage choices as well as a concern over the composition of such juices. This high demand has also led to increased incidences of fraudulent beverage adulteration as suppliers have added sugar, corn syrups, and inexpensive juices as a means of extending their juice volumes (thus increasing their profits). Such fraudulence has lead to the need for better analytical methods to detect juice tampering. Two excellent methods for identity information of a juice source are monosaccharide analysis as well as amino acid analysis[1,2].

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Figure 1. EZ:faast GC Chromatogram of Several Different Juices; APP ID: 15727, 15728, 15729

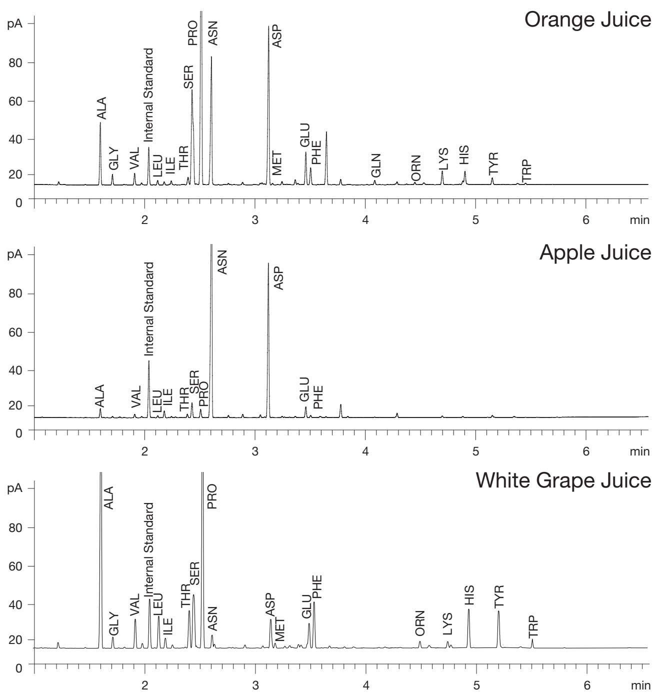

Figure 1: GC Chromatograms for EZ:faast amino acid analysis of pure: A) Orange Juice, B) Apple Juice, or C) Grape Juice. Note the different amino acid profiles between the three juices.

Materials and Methods

All samples were analyzed using the EZ:faast physiological kits for GC-FID and GC-MS (Phenomenex, Torrance, CA, USA); innovative sample preparation and analysis kits specifically designed for analyzing amino acids in complex sample matrices. Juice samples were analyzed directly; all steps including SPE sample cleanup, derivatization, and analysis were all performed as described in the kit; no additional sample preparation was performed before EZ:faast analysis. All analyses were performed on the Agilent 6890 GC equipped with either a FID detector or 5973 MS detector, and using Chemstation software (Agilent, Palo Alto, CA, USA). The GC column used was the ZB-AAA GC column provided in the EZ:faast kit and standard analysis conditions were used as described in the kit. Orange, apple, and white grape juice, as well as orange drink were all obtained from consumer sources. Adulteration of juices was performed by either mixing juices in a 3:1 ratio, or by adding orange drink to orange juice in a similar ratio before analysis.

Table 1. Levels for Key Amino Acids from Different Juice Sources analyzed by EZ:faast*

Amino Acid	Orange Juice	Apple Juice	Grape Juice
ALA	0.97	0.09	3.26
GLY	0.16	0.01	0.12
VAL	0.14	0.03	0.29
SER	1.40	0.17	0.70
PRO	4.69	0.07	2.52
ASN	2.82	3.52	0.18
ASP	2.78	1.78	0.33
GLU	0.65	0.17	0.45
HIS	0.05	0.03	0.48

*Units in µmole/mL

Results and Discussion

Efforts were undertaken to demonstrate the utility of using EZ:faast for fruit juice analysis. Other amino acid technologies give spurious results and require complicated sample preparation due to the presence of sugars, salts, and other matrix components in the sample. EZ:faast, with its integrated SPE sample preparation technology, is unaffected by sample matrix components.

Chromatograms for different fruit juice samples are shown in figure 1. Note the excellent peak shape as well as the short analysis time of less than seven minutes for these highly complex samples. Quantitated results for 9 of the 50+ amino acids analyzed are show in table 1. Levels and ratios of these amino acids are useful for identifying individual juices as well as detecting any changes to a juice composition.

Common methods of adulteration of high priced fruit juices like orange or apple juice include either A: mixing a low cost juice (like white grape juice) in with the high priced juice to increase the total juice volume, or B: adding sugar, water and organic acids to a juice to increase the total liquid volume. One example is shown in figure 2.Chromatogram 2A is of a 75:25 mixture of apple and white grape juice. Note the change in the amino acid profile versus what is observed in pure apple juice (Figure 2B). Quantitative differences between the samples are listed in table 2. Also listed in table 2 are the differences between pure orange juice and an orange juice/ orange drink mixture; another example of juice adulteration.

In both adulteration cases, amino acid analysis is a highly accurate method for identifying juice adulteration (table 2). In the apple juice example, changes in alanine, serine, and proline levels between samples are sensitive indicators of juice alteration.

Figure 2. Amino Acid Profiles for Pure Apple Juice vs. Adulterated Juice Mixture; APP ID: 15728, 15731

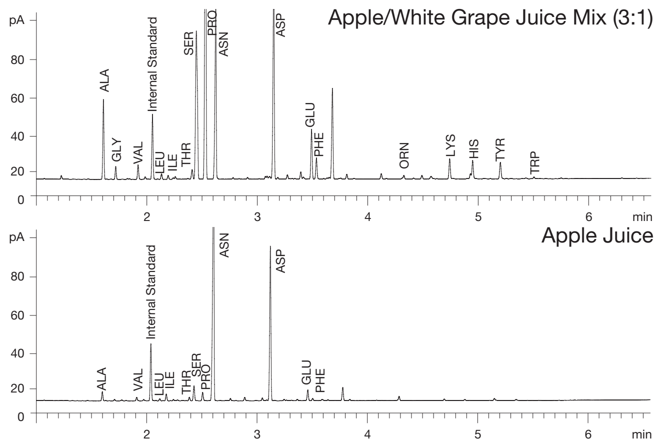

Figure 2: Comparison of the amino acid profiles as observed using EZ:faast A) apple and grape juice mixture (3:1) vs. B) pure apple juice. Note the major differences in ALA and PRO levels between the two samples.

In the orange juice example, an overall decrease in amino acid levels are suggestive of a dilution of the total orange juice content of the sample.

These results demonstrate the utility of amino acid analysis as a purity and concentration test for juice samples. The high through-put and resistance to variability caused by matrix effects make EZ:faast an excellent choice for QC/QA testing of juice samples.

Table 2. Levels for Pure vs. Adulterated Juice Samples*

Amino Acid	Pure Apple Juice	Apple + White Grape Juice Mix	Pure Orange Juice	Orange Juice + Drink Mixture
ALA	0.09	0.89	0.97	0.74
GLY	0.01	0.04	0.16	0.12
VAL	0.03	0.09	0.14	0.11
SER	0.17	0.32	1.40	1.19
PRO	0.07	0.78	4.69	3.99
ASN	3.52	2.70	2.82	2.25
ASP	1.78	1.46	2.78	2.20
GLU	0.17	0.27	0.65	0.51
HIS	0.03	0.14	0.05	0.04

*Units in µmole/mL

References

1. Harris, L.; Larson, S.; Hasel, K.; McPherson, A. Biochemistry 36: 1581 (1997)
2. Clark, M. Chemical Immunology 65, p. 88-110 (1997)

Ordering Information

Order No.	Description
KG0-7165	GC/FID Free (Physiological) Amino Acid Analysis Kit
KG0-7166	GC/MS Free (Physiological) Amino Acid Analysis Kit

3702_L