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Research Article

Amino Acid Profile as a Feasible Tool for Determination of the Authenticity of Fruit Juices

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Abstract
Purpose: Fruit juice is a nutrient rich food product with a direct connection to public health. The purpose of this research was to determine the amino acid profile of juices and provide a quick and accurate indicator for determining their authenticity.
Methods: The method of analysis was HPLC with fluorescence detector and pre-column derivatization by orthophtaldialdehyde (OPA). Sixty-six samples of fruit juices were analyzed, and fourteen amino acids were identified and determined in the sampled fruit juices. The fruit samples used for this analysis were apples, oranges, cherry, pineapple, mango, apricot, pomegranate, peach and grapes.
Results: The results showed that 32% of samples tested in this study had a lower concentrate percentage as compared to that of their labels and/or other possible authenticity problems in the manufacturing process. The following samples showed probable adulteration: four cherry juice samples, two pomegranate juice samples, one mango, three grape, four peach, seven orange, two apple and one apricot juice samples.
Conclusion: In general, determining the amount of amino acids and comparing sample amino acids profiles with the standard values seems to be an indicator for quality control. This method can provide the regulatory agencies with a tool, to help produce a healthier juice. The aim of this study is the analytical control of the fruit juice composition is becoming an important issue, and HPLC can provide an important and essential tool for more accurate research as well as for routine analysis.

Introduction

The fruit juice industry is one of the most important agricultural businesses in the world. There are many advantages interrelated with the manufacturing of this food. Fruits are perishable items and their harvest is seasonal, but the consumption of these fruit has been made available throughout the year with the processing technologies we use today. The juices of these fruits and its concentrates have become a valuable semi-finished product. A large variety of fruits are used for commercial manufacturing of fruit juice, such as the orange, apple, peach, pomegranate, apricot, pineapple and grapes. The detection of adulteration requires a clear definition of what in fact constitutes a juice. The authentication of these juices is imperative in the food industry, where substitution with cheaper ingredients is common practice and has a potential for financial gain. Numerous chemical, physical and microbiological techniques have been proposed for testing the quality of these juices. These tests assess the presence of naturally occurring substances, although other compounds are also measured such as additives and decomposition products. Moreover, many specific chemical parameters can be used to detect adulterations, mainly those based on the profile analysis, e.g., sugars, amino acids, carotenoids, flavonoids, organic acids and others.

There are many known classes of juice adulteration in today's manufacturing process. The most frequent types of adulteration include a simple dilution with water, by the addition of natural constituents from juices. As well as from other sources such as the addition of sugar syrup, which decreases the total amino acid value; additionally the use of constituents not naturally present in the juice such as colorants and the addition of inexpensive juice from other types of a lesser expensive fruit. More sophisticated forms of adulteration consists in the use of in- expensive amino acids such as glycine or glutamic acid or protein hydrolysates with the objective of increasing the total amino acid content.[1] These adulterations can be detected by characterization of amino acids profile. The approach for detecting these types of fraudulent acts includes the measurement of specific individual amino acids or the characteristic amino acids ratio estimation.

Amino acids are one of the most important types of natural compounds because they take part in many essential and

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well-known physiological processes. One of these is the peptide and protein creation, where twenty amino acids take part in the building process. Therefore, free amino acids can be found in many biological tissues, body fluids, foods and drugs.[2,3]

Amino acids in their native form are generally weak chromophores (do not absorb UV light) and do not possess electrochemical activity. This means that for analytical purposes, they must first be chemically modified (derivatized). These products can be detected at much higher sensitivity by certain types of liquid chromatographic detectors.

The purpose of this study was to determine the amino acid profile of commercially available fruit juices for the purpose of evaluating their authenticity.

Materials and Methods

Reagents and apparatus

The reagents and solvents used in the experiments were of analytical grade from Merck (Darmstadt, Germany). Amino acids standards were purchased from Sigma Chemical Co. (Saint Louis, USA). Deionized water was prepared using a Mili- Q System (Tehran Absaz co, Iran). The amino acids standard solution was stored in a dark area of the refrigerator and refrigerated at 4°C, the samples were diluted with water to obtain working standard solutions. The concentration of the amino acids was determined using calibration curves of the standard solutions. These calibration curves were linear in the interval of concentration studied for each amino acid.

Sample preparation

All sample juices were purchased from the local grocery markets in Tabriz, Iran. An assortment of various flavored juices included non-carbonated fruit drinks, fruit juices, and all natural fruit juice and fruit nectars. Prior to analysis, all purchased samples were filtered through a 0.45 µm filter.

OPA/MPA in methanol was used as derivatisation reagent. Derivatisation for primary amino acids was performed by a Spark autosampler (Triathlon type 900). The autosampler was programmed to transfer successively sodium borate buffer (pH 9.5, 0.14 M, 100 µl) and OPA/MPA reagent (50µl) to a sample vial (100 µl). After 1 min 25 µl, HCl 0.7M was added to stop the reactions. Then 200 µl of mobile phase A and 50µl of sample were transferred to an autosampler vial and injected on to the HPLC column (injection volume = 20 µl).

Chromatographic separation

The chromatographic system was a KNAUER HPLC instrument (Knauer, Berlin, Germany) consisting of a K-1000 Knauer controller Quaternary pump, a Spark Triathlon autosampler and a fluorescence detector (Shimadzu, RF-551) operating with the Chromgate 3.7 software. Separations were achieved using a spherimage 250mm×4 mm, reversed-phase ODS column (Knauer; Berlin, Germany). For OPA/MPA derivatives the eluent system consisted of two components: eluent (A) was methanol–sodium phosphate (pH 6.5, 12.5 mM) (10:90, v/v), while eluent (B) was methanol– tetrahydrofuran (97:3, v/v). The separation (gradient) conditions were as follows: 15–20% B in 5 min, 20–32% B in 12 min, 32–60% B in 10 min, 60%–90% B in 3 min and 90–15% B in 2 min. The eluent flow rate was 1.0 ml/min. Detection was performed using the fluorescence (Fl) (Shimadzu; RF −551) detector. The optimum excitation (λex)/emission (λem) wavelengths were as λex/λem = 330/450 nm.[4]

Results and Discussion

Fruit juice adulterations create serious ethical and economic difficulties. Consumers suffer from losses, because they expect juices of standard value and an authentic product, and the honest manufacturers loose a good portion of their market to the dishonest competitor.[5]

There is an ongoing interest in developing a reliable and rapid method to assess the quality of foods for nutritional and regulatory purposes.[6]

Gomez-Ariza et al published the results of their study on the amino acid profile of orange juices. According to their study, the usual approach used for adulteration assessment in commercially produced orange juice were based on the study of the most abundant amino acids in the juices and this method was used for the evaluation of samples in this study. They used fresh hand-squeezed juice as a reference point for the amino acids typically profiled in this food. In this analysis, the amino acid content of the commercially produced juices was compared with that of the fresh juice. Thus, the total content of amino acids and proline concentration may indicate dilution with water and/or addition of sugar syrup to the commercial juices.[2]

Versari et al studied the apricot juice in order to characterization of Italian apricot juices. They showed that asparagine was the main amino acid found in apricot juice, representing up to 78% of the total amino acid content.[6]

Zhang et al reported that the presence of the amino acid proline at >25 mg/L is indicative of added grape products to pomegranate juice.[7]

In this study, sixty-six juice samples were studied. The detailed results of their amino acid analysis were as the follows:

1. Grape juice: A total of eight grape Juice samples were tested. Three samples had amino acid concentration (37.5%) lower than labeled.
2. Apple juice: Four apple samples were analyzed and two samples (50%) showed amino acids lower than concentration.
3. Orange juice: Thirteen samples were evaluated. Seven samples (53.8%) showed adulteration, the concentrations were lower than labeled.
4. Sour Cherry juice: Thirteen samples were tested. Four samples (30.7%) had chromatogram compatibility with the standard value but with a lower concentration levels.

5. Pineapple juice: Five Pineapple samples analyzed, two samples (40%) had adulteration as lower concentration.
6. Peach juice: Eleven peach samples were tested and three samples (27.2%) showed lower than concentration and one sample had no concentrate.
7. Pomegranate juice: Of the six pomegranate samples, two samples (33.3%) showed adulteration at lower concentration levels (in one sample glutamic acid was added as adulteration).
8. Apricot juice: Three apricot samples were analyzed and one sample (33.3%) had lower concentration levels.
9. Mango juice: Three mango samples were tested and one sample (33.3%) showed lower concentration levels.

In the present study, at least two types of adulteration in production of fruit juices were observed:
1) The available concentration of total amino acids was under the labeled concentrate.
2) Additionally, one or more amino acids were used to increase their protein content, thus creating a false positive in the formol index test, which is a common test in the food control laboratories in Iran.

Every fruit juice product needs to comply with the amino acid profile. On the other hand, the mean concentration of amino acids as compared to the amino acid concentrations in pure fruit indicates the percentage of the natural concentrate, consumed in the production process.
Table 1 shows the typical amino acid profile of the fruit juice, which were used as a base control for comparison of the results for this study.[8]
Table 2 illustrates the results of this study and the adulteration types in these samples.

Table 1. Typical amino acid profile of some fruits by mg/L

AA\fruit	Pineapple	Orange	Mango	Peach	Sour cherry	Apple	Grape	Pomegranate	Apricot
Asp	51	35	370	353	195	155	77	171	387
Glu	106	172	96	223	168	15	98	19	7
Ser	83	28	147	182	27	17	57	198	49
His	22	11	8	28	40	12	50	40	49
Gly	13	1	12	16	3	1	4	59	15
Arg	17	366	358	1	2	1	592	121	57
Ala	111	171	86	132	7	14	172	42	83
Tyr	26	5	50	9	1	1	26	30	66
Met	11	2	6	0.6	0.1	0.3	12	27	24
Val	24	15	16	42	4	5	47	33	25
Phe	20	6	19	27	1	1	21	5	9
Ile	12	6	10	30	ND	16	33	12	10
Luc	13	10	9.84	14	21	1	51	8	21
Lys	14	20	73	3	1	1	4	77	60

Table 2. The results of adulteration in fruit samples and types of adulteration.

Fruit juice	Sample count	Froude samples N(%)	Adulteration type		
			concentrate lower than label	Added amino acid	Lack of concentrate
Orange	13	7 (53.8%)	4	1	2
Sure Cherry	13	4 (30.7%)	2	2	-
Grape	8	3 (37.5%)	3	-	-
Apple	4	2 (50%)	2	-	-
Mango	3	1 (33.3%)	1	-	-
Pomegranate	6	2 (33.3%)	1	1	1
pineapple	5	2 (40%)	2	-	-
Peach	11	4 (36.3%)	3	-	1
Apricot	3	1 (33.3%)	-	-	1
Total	66	26 (39.4%)	18	4	5

Non-carbonated fruit drinks are one type of juice, products, which are manufactured by many factories in Iran, according to the Iranian National Standard Organization Regulations, it must contain at least 20% fruit juice. This product is sample of adulteration because of its lower price and higher benefit rate. In this study, twenty-six samples from various flavored fruit juices showed adulteration levels. A majority of these samples had (73%) lower concentrate than claimed on the label, and five samples were produced without the use of fruit concentrate. Additionally amino acids, such as glutamic acid and lysine were most likely added as well as aroma flavorings.
It seems that for economical and financial gain, some manufacturers take part in adulteration activities of their products, thus producing a low quality product rather than an authentic juice. The regulatory agencies need to increase their supervision and use regulations

that are more powerful to ensure the authenticity of the fruit juices quality and to protect the public health.

Conclusion

In general, determining the amount of amino acids and comparing the sample amino acids profile with the standard values seems to be an indicator for quality control. This accurate method almost can provide the regulatory agencies with a tool, assisting in producing a healthier juice. The aim of this study is the analytical control of the fruit juice composition has become an important issue, and High Performance Liquid Chromatography (HPLC) can provide an important and essential tool for research that is more accurate as well as for routine analysis.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Robards K, Antolovich M. Methods for assessing the authenticity of orange juice. *Analyst* 1995;120:1-28.
2. Gomez-Ariza JL, Villegas-Portero MJ, Bernal-Daza V. Characterization and analysis of amino acids in orange juice by HPLC–MS/MS for authenticity assessment. *Analytica Chimica Acta* 2005;540:221-30.
3. Sun DW. Modern techniques for food authentication. 1st ed. USA: Academic press; 2008.
4. Nemati M, Oveisi MR, Abdollahi H, Sabzevari O. Differentiation of bovine and porcine gelatins using principal component analysis. *J Pharm Biomed Anal* 2004;34(3):485-92.
5. Stój A, Targonski Z. Use of amino acid analysis for estimation of berry juice authenticity. *Acta Sci Pol Technol Aliment* 2006;5(1):61-72.
6. Versari A, Parpinello GP, Mattioli AU, Sergio Galassi S. Characterization of Italian commercial apricot juices by high-performance liquid chromatography analysis and multivariate analysis. *Food Chem* 2008;108:334-40.
7. Zhang Y, Krueger D, Durst R, Lee R, Wang D, Seeram N, et al. International multidimensional authenticity specification (IMAS) algorithm for detection of commercial pomegranate juice adulteration. *J Agric Food Chem* 2009;57(6):2550-7.
8. Bruckner H, Westhauser T. Chromatographic determination of L-and D-amino acids in plants. *Amino Acids* 2003;24(1-2):43-55.