Original Article

Rapid Test for Traceability Assessment in Lemon Juice by High-Performance Liquid Chromatography Fluorescence

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Abstract

Aims: Fruit juices, especially lemon juice, are the most targeted food goods for adulteration and fraud in Iran. The aim of this study was to investigate the profile and concentration of free primary amino acids as a marker for adulteration in lemon juice. **Materials and Methods:** Amino acids were determined with high-performance liquid chromatography fluorescence. The limit of detection and limit of quantification were in the range of 0.008–0.01 ng/ml and 0.03 ng/ml, respectively. **Results:** The method is suitable for distinguishing authentic juices from drinking products that may contain little or no fruit juice. In our study, total amino acid concentration in natural lemon was 13.15 mmol/l and in other brands was in the range of 1.81–14.84 mmol/l. Further, the concentration of aspartic acid, glutamic acid, asparagine, and serine was more than other amino acids. The findings showed that only brands 1 and 2 were considered similar to natural lemon juice. **Conclusion:** The present study demonstrated that the concentration of the first four amino acids can use as a marker to determine lemon juice adulteration.

Keywords: Adulteration, amino acid, high-performance liquid chromatography, lemon juice

Introduction

The originality of fruit juice is so important for consumers. Fruit juice is the liquid obtained from fresh fruits without the chemical process.[1] Among fruit juices, lemons have a special place in the Iranian household's food basket and contain high amounts of natural antioxidant compounds.[2] With the advancement of chemistry, serious and widespread food adulteration occurred. Lemon juice is one of the cases in which fraud has been carried out since past times.[3,4] Adulteration usually points out to blending matters with inferior or sometimes detrimental material in foodstuffs or drink intended to be sold.[5] Adulteration included the replacement of one material or substance instead of the other, for example, a manufactured product is labeled improperly, or dosage information does not meet the requirements.[6] For the detection of fruit juice authenticity, chemical, biomolecular, and isotopic procedures could be used depending on the structure of matter for obtaining the target information.[7]

There are different types of lemon juice adulteration including miss-labeling, the addition of water, addition of food additive, and substitution of very valuable beverages with cheaper ones coming from other fruits being be recognized.[8,9] Economic adulteration has been concerning for a long time. Besides, dilution problems, mislabeling, and insignificant or entire

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substitution of expensive ingredients are common, which can cause notable damage to the companies involved.[10] As nonauthentic products are economic concerns that also can lead to health risks, the necessity for authentic products is in favor of the consumers.[11] In addition to nutritional properties of lemon juice, many studies have shown that the free amino acid profile of vegetable foods can be useful in validity and quality control approaches.[12] Most complex forms of adulteration consist of using inexpensive amino acids such as glycine, glutamic acid, or protein hydrolysates to raise the total amino acid amount.[13] The amino acids that have been found in juices in low quantities may be related to sensory quality losses. Certain sensory defects in food products originate from side reactions during the manufacturing process that involves amino acids. Amino acids are noticeable because of their accessibility in many biochemistry samples.[14] They have vital functions in metabolism and can be seen as the protein constructor blocks.[15,16] Thus, in fruit, free amino acids are useful some indicators for recognizing the validity of fruit juices.[17] Amino acids are normally weak chromophores in their natural shape (do not absorb ultraviolet light) and do not have electrochemical activity.[18,19] By doing so, for analytical aims, they need to be modified in terms of chemically. The products are detectable by certain types of liquid chromatographic detectors at higher precision. O-phthalaldehyde (OPA) is a basic amine-reactive fluorescent tracing reagent that can be applied as a postcolumn detection reagent for amino acid analysis (high-performance liquid chromatography [HPLC]).[20] The reaction of OPA with primary amino acids yields linear outcomes over an extensive range of concentrations. This work uses primary amino acid determination as rapid and robust method, as well as specific and reliable markers for authentication of lemon juices.

Materials and Methods

Chemicals and reagents

Amino acid standards were purchased from Sigma Chemical Co. (Saint Louis, USA). Stock standard solutions of each amino acid were gravimetrically done in 0.1 M HCl solution at 1000 mg/l. Then, they were stored at 4°C in a dark place. The mixture of the amino acid standard was ready for the calibration run by diluting the stock solution with water. The fresh solution of 70 mg OPA, 1 ml methanol, 95 ml buffer with pH 10.5 (25 g/l of boric acid), and 0.2% of 2-mercaptoethanol was prepared and purged with N_2. There were two mobile phases. Mobile phase A included 10 mM Na_2HPO_4 and 10 mM $Na_2B_4O_7$ (pH 8.2), and mobile phase B contained acetonitrile, methanol, water (45:45:10, v: v: v). Then, they were filtered through Millipore 0.45-µm before use.

Apparatus

The analytical chromatographic system including an Agilent 1200 series vacuum degasser, an automatic sample injector, a quaternary pump, a scanning fluorescence detector (all from Agilent Technologies, Palo Alto, CA, USA), The samples were separated on a Symmetry® C18 column (3.5 µm particle diameter, 100 Å pore diameter, 4.6 mm id, 150 mm length, with matching guard column; Agilent Technologies, Palo Alto, USA) USA). The system was controlled controlled by a computer running Chem Station Software (Agilent Technologies). Analytical chromatography was carried out with a flow rate of 1.5 ml/min at 25°C ± 1°C. The gradient details are presented in Table 1. Excitation and emission wavelengths were 340 and 450 nm, respectively.

Juice preparation

One kilogram of lemon fruit was purchased from a local supermarket (Karaj, Iran) and kept at 4°C before treatment. Lemon fruits were washed and chopped. Then, the squeezed lemon juice was centrifuged at 5000 rpm for 5 min. Then, the supernatant was filtered with a steel sieve by a mesh of 2 mm. All lemon sample juices from different seven brands were obtained from local grocery markets in Karaj, Iran. Various fruit juices were filtered (0.45 µm) before analysis.

Extraction of samples

15 ml of acetonitrile containing 1% of acetic acid was added to 15 ml of sample and mixed well in a 50 ml centrifuge tube. Then, 4 g of $MgSO_4$ and 1 g of NaCl were added to the sample and shook for 30 s. The whole sample was centrifuged for 5 min at 4000 rpm, and the aqueous phase was taken and filtrated with a syringe filter (0.45 mm cellulose). Then, 1 ml of the obtaining solution mixed with the derivatizing agent solution with a ratio of 1:1, and after 2 min, 20 µl of the sample was injected into the HPLC instrument.

Results

Analyzing the kinds and amounts of amino acids in foodstuff can give us significant food nutrition information. The lemon juice sample presented an amino acid profile composed of 18 amino acids [Table 2]. The typical chromatogram of amino acids obtained by HPLC-fluorescence (FL) detection is presented in Figure 1.

The separation and fluorescence intensity were performed for 18 of the amino acids, except for glutamine, histidine, valine, and methionine. Further, the degradation peak from the old reagent (OPA) was observed near lysine (17.8 min). The curves, in the form of straight lines, were obtained by injecting amino acids with variable concentrations of amino acids (0.03–2500.0 ng/ml). All calibration curves were linear

Table 1: Instrument conditions for determination of amino acids

Time (min)	Flow rate (ml/min)	Percentage *B*
0	1.5	2
0.84	1.5	2
33.4	1.5	57
33.5	1.5	100
39.3	1.5	100
39.4	1.5	2
40	1.5	2

with correlation coefficients higher than 0.998. According to the IUPAC definition, limit of detection and limit of quantification were calculated based on the standard deviation of the blank signal divided by the slope of the regression equation and were in the range of 0.008–0.01 ng/ml and 0.03 ng/ml, respectively. The figure of the method for the extraction and determination of amino acids from samples is given in Table 2.

In the current study, the concentration of total amino acids in natural lemon juice was 13.15 mmol/l and for other brands was in the range of 1.81–14.82 mmol/l. Aspartic acid, glutamic acid, asparagine, serine profile, and concentration (first four amino acids) were the highest amino acids. The concentration for the first four amino acids in natural lemon juice was 1322 mg/l. Then, seven brands of lemon juices were surveyed. The findings indicated that amino acid concentration for brands 1–7 was 1320, 663, 195, 132, 42, 15, and 12 mg/l, respectively.

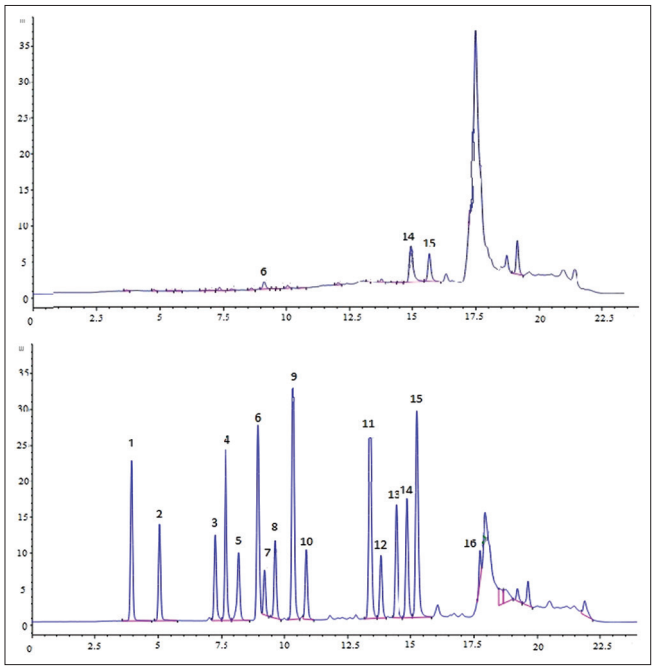

Figure 1: Chromatogram obtained for mixed amino acid at 1250 ng/mL (lower) and blank (upper)

Discussion

The test results of a juice need to be compared with authentic standards to detect fruit juice adulteration. This requires data information for the juices from diverse varieties and geographical origin. The most numerous amino acids in natural lemon juice were aspartic acid, glutamic acid, asparagine, and serine. Therefore, they can be used for traceability assessment. If the concentration of these amino acids were near to Association of the Industry of Juices and Nectars of the European Union[21] range, the lemon juice has a natural source and vice versa. There are diverse kinds of studies and techniques for lemon juice authenticity, for example, Bononi *et al*. assessed the authentication of lemon juice by High-performance liquid chromatography linked to isotope ratio mass spectrometry They found that 3 out of 20 lemon juice samples were adulterated.[22] Another study that was done by Guyon *et al*. reported authentication of lemon juice by HPLC-co-IRMS.[23] Both previous studies investigated the acid, glucose, and fructose for lemon juice fraud. Khodadadi *et al*. applied a colorimetric method in combination with the developed solid phase extraction-thin layer chromatography technique that could be used for the preliminary screening of adulterated lime juice products.[24] Although there are different methods for the determination of lemon juice adulteration, the assessment of amino acid profile in lemon juice is rapid and has studied rarely. In a study conducted by Gómez-Ariza

Table 2: Figure of merits for primary amino acids

Order	Amino acid	Rt (min)	Calibration range (ng/ml)	Calibration equation	R^2	LOD (ng/ml)	LOQ (ng/ml)
1	Aspartic acid	3.95	0.03-2500	Area=0.1089C−1.47	0.9997	0.008	0.03
2	Glutamic acid	5.05	0.03-2500	Area=0.066C+0.82	0.9998	0.01	0.03
3	Asparagine	7.24	0.03-2500	Area=0.063C+0.88	0.9998	0.01	0.03
4	Serine	7.67	0.03-2500	Area=0.128C−0.77	0.9997	0.008	0.03
5	Glutamine and histidine	8.2	0.03-2500	Area=0.058C+0.2	0.9998	0.01	0.03
6	Glycine	8.92	0.03-2500	Area=0.15C+3.7	0.9994	0.008	0.03
7	Threonine	9.22	0.03-2500	Area=0.04C−0.84	0.9991	0.01	0.03
8	Arginine	9.64	0.03-2500	Area=0.058C+0.83	0.9995	0.01	0.03
9	Alanine	10.34	0.03-2500	Area=0.20C+1.6	0.9997	0.008	0.03
10	Tyrosine	10.8	0.03-2500	Area=0.054C+1.4	0.9991	0.01	0.03
11	Valine and methionine	13.37	0.03-2500	Area=0.17C+0.9	0.9998	0.008	0.03
12	Tryptophane	13.8	0.03-2500	Area=0.05C+0.53	0.9985	0.01	0.03
13	Phenylalanine	14.42	0.03-2500	Area=0.086C+0.11	0.9997	0.01	0.03
14	Isoleucine	14.86	0.03-2500	Area=0.092C+0.48	0.9997	0.008	0.03
15	Leucine	15.25	0.03-2500	Area=0.176C+4.8	0.9938	0.008	0.03
16	Lysine	17.74	0.03-2500	Area=0.015C+0.34	0.9977	0.01	0.03

LOD: Limit of detection, LOQ: Limit of quantification

Table 3: Concentration of amino acid in various samples and parameters must be determined for the authenticity of lemon fruits

Amino acid	Mmol/L								
	Natural	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5	Brand 6	Brand 7	AIJN proposal
Aspartic acid	2.80	0.40	1.03	0.35	0.25	0.06	0.03	0.01	2.26-6.02
Glutamic acid	1.65	0.83	0.56	0.04	0.24	0.04	0.01	0.02	1.09-2.72
Asparagine	3.90	8.20	3.06	0.99	0.32	0.19	0.07	0.04	0.99-4.55
Serine	1.84	0.59	0.38	0.11	0.20	0.02	0.01	0.02	1.29-3.52
Glutamine and histidine	0.45	0.07	0.05	0.01	<0.01	<0.01	<0.01	<0.01	Maximum 0.38
Glycine	0.20	0.25	0.11	0.66	0.04	3.69	10.50	1.75	0.09-0.33
Threonine	0.12	0.28	0.20	0.06	0.03	0.02	0.01	2.41	0.08-0.25
Arginine	0.64	1.17	0.38	0.16	0.07	0.03	0.02	0.01	Maximum 0.58
Alanine	0.44	1.24	0.92	0.22	0.36	0.07	0.06	0.02	0.9-2.92
Tyrosine	0.02	0.03	0.02	0.01	0.01	0.01	0.01	0.01	Maximum 0.04
Valine and methionine	0.48	0.26	0.16	0.05	0.17	0.01	0.01	0.01	0.07-0.33
Tryptophane	0.04	0.03	0.06	0.01	0.02	0.05	0.02	0.89	-
Phenylalanine	0.17	0.12	0.05	0.05	0.03	0.01	<0.01	0.15	0.05-0.24
Isoleucine	0.10	0.07	0.04	0.01	<0.01	<0.01	<0.01	0.13	0.02-0.08
Leucine	0.10	0.05	0.06	0.06	0.04	0.03	0.01	0.02	0.02-0.08
Lysine	0.20	0.23	0.19	0.04	0.03	0.04	<0.01	9.32	0.03-0.14
Total amino acid	13.15	13.82	7.25	2.82	1.81	4.29	10.76	14.82	-
First four amino acid profile (%)	71	71	69	57	53	12	2	1	-
First four amino acid concentration (mg/l)	1322	1320	663	195	132	42	15	12	-
Total amino acid concentration (mg/l)	1861	1857	964	341	250	360	824	2026	-

AIJN: Association of the Industry of Juices and Nectars of the European Union

et al., the characterization and analysis of amino acids in orange juice were investigated using HPLC for authenticity assessment.[1]

The current study by HPLC-FL was applied for traceability assessment in different brands of lemon juice successfully. Our findings showed that only brands 1 and 2 are similar to natural lemon juice. Further, brands 3–7 are known as fraud. The concentration of amino acids is given in Table 3. The results demonstrated that the quality of lemon juice according to the sum of aspartic acid, glutamic acid, asparagine, and serine (first four amino acids) is as the following order: brand 1 > brand 2 > brand 3 > brand 4 > brand 5 > brand 6 > brand 7. For example, when the concentration of glycine is at a high range, the lemon juice is determined as a fraud. Inexpensive amino acids, such as glycine and glutamic acid, have been found added in lemon juice samples to rise the total amino acid amount. To determine the use of cheaper juices, we can calculate the amino acid profile (percentage of amino acids relative to total amino acids). The concentration of amino acids in a sample may be less than that of a natural juice. On the other hand, the amino acid profile is close to that of a normal sample, so it is calculated that the sample has been diluted with water. In our findings, in brands 3, 5, 6, and 7, glycine concentrations are higher than natural lemon juice. Therefore, they were considered adulterated in comparison with natural juice.

The amino acid profiles were calculated for samples. The results are shown in Table 3. The results display that brands 6 and 7 have not contained lemon juice, but other brands contained natural lemon juice. Aspartic acid, glutamic acid, asparagine and serine profile, concentration (first four amino acids), and total amino acid concentration must be determined to distinguish and detect fraud products from other freshly squeezed lemon juice.

Conclusions

The data show that it is feasible to recognize fraud products from other squeezed lemon juice and commercial lemon juice products. In addition, the results of our study demonstrated numerous variability, among lemon juices in terms of quality factors. This matter proposes an immediate need for establishing quality assurance standards and monitoring manner in Iran to control the quality of lemon juice products in the market. By doing so, consumers will be preserved from buying adulteration products. Aspartic acid, glutamic acid, asparagine, and serine were the most abundant free amino acids of lemon juice. Besides the interesting features of their compositions in the human diet, we suggest that these free amino acid profiles should be used as quality control parameters for manufactured products.

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Conflicts of interest

There are no conflicts of interest.

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