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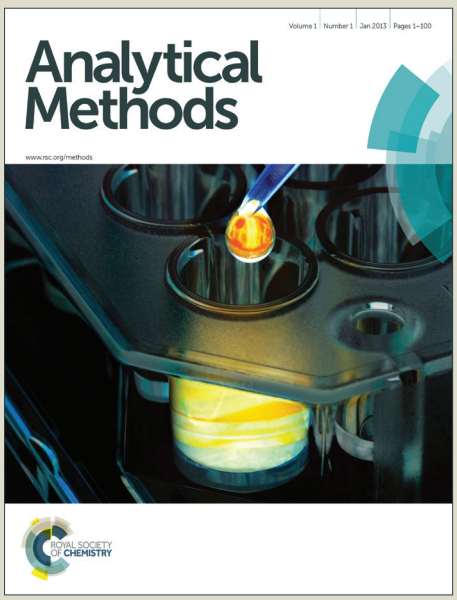

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Gas Chromatographic Analysis of Amino Acids in Jams, Fruits and Tablets Using Trifluoroacetylacetone and Ethylchloroformate as Derivatizing Reagents

S. A. Majidano[1], M. Y. Khuhawar[1], R. A. Zounr[1*], A. H. Channar[1], T. M. Jahangir[1], M. Mughal[2]

[1]Institute of Advanced Research Studies in Chemical Sciences, University of Sindh, Jamshoro, Pakistan

[2]Dr. M. A. Kazi Institute of Chemistry, University of Sindh, Jamshoro, Pakistan.

Abstract

GC-FID procedure was used for the analysis of amino acids from a pharmaceutical preparation (Aminess N^{TN} tablets), jams (Apple, Mango, Strawberry and Mixed fruits), juices (Lemon and Orange) and vegetable (Kundur). Free amino acids were extracted as aqueous or aqueous – methanol solution and acid hydrolyzed by acid treatment with 6N HCl. GC was carried out by pre-column derivatization with trifluoroacetylacetone (FAA) and ethylchloroformate (ECF) from the column HP-5 (30 m × 0.32 mm id). Complete separation between 19 amino acids was repeatable with relative standard deviation (RSD) in terms of retention time and peak height / peak area within 3.8%. The limits of detections (LODs) were obtained within 0.1-0.2 µg / mL each. A variation in the contents of the amino acids was observed in both free and acid hydrolyzed in different jams, fruit juices and vegetables and is discussed.

Keywords: GC-FID, Amino acids**,** Pharmaceutical preparation, Fruits, Trifluoroacetylacetone, Ethyl chloroformate

*Corresponding author Email: rizwan_zounr@yahoo.com

1. Introduction

Amino acids are building blocks of proteins and are present in most foods. The food stuffs may be characterized by their relative amounts of amino acids present.[1] They are also source of energy and serve as precursors for the biosynthesis of neurotransmitters, porpherins, polyamines and nitric oxide.[2] The needs for the amino acids analysis from foods and pharmaceutical preparations are unlimited, including detection of fraudulent, manipulations or economically profitable falsications.[3] The analysis could also work as characteristic finger print for the food under investigation. Further more the knowledge of concentration of amino acids for particular food may be necessary for manufacture and control processes for better food quality.[4] The amino acids are present in foods and fruits in free and combined state. They are present in combined state in proteins and polypeptides. Silva et al[3] evaluated free amino acids in quince fruit and jam. Zaini examined kundur as a potential source for variable nutrients and functional foods[5]**.** The analysis of citrus fruits, citrus juices and concentrates have been reported[4, 6, 7] and a number of amino acids have been identified. Widmer et al[8] have reviewed methods for determining adulteration of citrus juices and has indicated extensive use of amino acid profiles for detection of adulteration.[9] The determination of amino acid has wide range of applications in food industry. An alteration in amino acid contents from normal value may be an indication of an adulteration. The determination and identification of several amino acids simultaneously may be helpful in characterization of particular food product.[10] The analytical procedures used for the analysis of amino acids are mostly based on high performance liquid chromatography (HPLC),[11-17] gas chromatography (GC) [3, 6, 18-22] and capillary electrophoresis (CE).[23-25]

HPLC determinations of amino acids are generally based on ion exchange separation coupled with post column derivatization[26, 27] or pre column derivatization with reversed phase HPLC.[14, 28] Some of the commonly used pre column derivatizing reagents are o-phthaldehyde,[29] phenylisothiocyanate,[30] 6-dimethyl-amino-1-nephthalene sulphonyl chloride (dansyl chloride),[31] and 9-fluorenylmethyl chloroformate.[32] A number of difficulties have been discussed using HPLC procedures.[14] GC determinations of amino acids after pre column derivatization have been reported, because of high resolution power of GC with ease of operation, lower running cost and shorter analysis time. The GC of amino acids with different silyl derivatizing reagents require non aqueous medium for derivatization.[33-35] However, the derivatization with ethyl chloroformate and related compounds could be carried out in a aqueous phase.[36-40] The sensitivities of GC determinations have been further enhanced by the use of fluorosubstituted chloroformates and mass-spectrometric detection.[39, 41-43] Recently GC of amino acids has been carried out by using trifluoroacetylacetone (FAA) and ethyl chloroformate (ECF) as derivatization reagents and is reported to give better sensitivity and separation of amino acids as compared to the use of only ECF as derivatizing reagent.[44] The present work also reports the use of FAA and ECF as reagents for pre column derivatization for the GC determination of amino acids from pharmaceutical preparations, jams, fruit juices and vegetables.

2.Experimental

2.1 Chemicals and Solutions

The compounds glycine (Gly), L-alanine (Ala), L-valine (Val), L-phenylalanine (Phe), tryptophan (Trp) (Sigma, Loius, USA), tyrosine (Tyr), serine (Ser), leucine (Leu), isoleucine (Ile), methionine (Met), threonine (Thr), proline (Pro), (Sigma, Deisenhofen, Germany), glutamic acid (Glu), glutamine (Gln), aspartic acid (Asp), asparagine (Asn), cysteine (Cys), lysine (Lys), and histidine (His), (Sigma, GmbH, Germany), trifluoroacetylacetone (FAA) and ethyl chloroformate (ECF) (Fluka, Buchs, Switzerland), methanol (RDH, Chemical Co, Spring Valley, CA, USA) were used. Guaranteed reagent grade hydrochloric acid (37 %), potassium chloride, acetic acid, sodium acetate, ammonium acetate, sodium tetraborate, boric acid, sodium bicarbonate, sodium carbonate, ammonium chloride and ammonia solution were from E-Merck, Darmstadt, Germany. The three bottles of each jam samples of four different flavors (apple, mango, strawberry and mixed) of Mitchells Fruit Farms Ltd. Okara, Punjab, Pakistan, were purchased from local market (Hyderabad, Sindh, Pakistan). Amines N^{TM} tablets (Medisure Laboratories, Pak. (Pvt) Ltd., Karachi, Pakistan) were analyzed. The vegetable of bottle guord (Loki and Kadu), and grape fruits, lemons and oranges were purchased from local market of Hyderabad, Sindh, Pakistan. Stock solutions of amino acids containing 1000 μg/mL were prepared in methanol and water. Further solutions were prepared by appropriate dilution. Buffer solutions (0.1M) between pH 1-10 at unit interval were prepared from the following: potassium chloride adjusted with HCl for pH 1-2, acetic acid-sodium acetate (pH 3-6), ammonium acetate (pH 7), boric acid-sodium

tetraborate (pH 7.5-8.5), sodium bicarbonate-sodium carbonate (pH 9), ammonium chloride-ammonia (pH 10).

2.2. Equipment

The pH measurements were made with an Orion 420 A pH meter (Orion Research Inc., Boston, USA) with combined glass electrode and reference internal electrode. GC studies were carried out on an Agilent model 6890 network GC system connected with flame ionization detector (FID) and split injector (Agilent Technologies, Santa Clara, CA, USA), hydrogen generator (Parker Balston, Analytical Gas System, H_2-90, Parker Hnnifin Havorhill, MA, USA) and pure nitrogen (British Oxygen Company (BOC) Karachi); computer with Chemstation software controlled the gas chromatograph.

2.3. GC Analytical Procedure

GC analytical procedure was carried out as previously reported[44] as follows: The solution (0.2-3.0 mL) containing a mixture of amino acids (1-25 µg each) was added to 0.2 mL ammonia acetate buffer pH 7 and 0.2 mL FAA (2 % v/v in methanol). The contents were heated on water bath at 95^0 C for 20 min. The mixture was cooled at room temperature and 0.2 mL of solvent system (acetonitrile – water – methanol – pyridine 42 : 42 : 8 : 8 v/v/v/v) was added to it. The mixture was then added to ECF (0.2 mL) and carbonate buffer pH 9 (0.2 mL). The mixture was sonicated at room temperature (30^0C) for 15 min. Chloroform (1.0 mL) was then added and contents were mixed well. The layers were allowed to separate and an aliquot of organic layer was transferred to screw capped sample vial. The solution (1.0 µL) was injected in GC with split ratio 10:1 v/v on the column HP-5 (30 m × 0.32 mm id) with film thickness 0.25 µm (J & W Scientific GC

column USA) at column temperature 100 ^{0}C for 2 min., followed by ramping at a rate of 20 ^{0}C / min up to 250 ^{0}C. The nitrogen flow rate was 3 mL / min. The injector and detector temperatures were 270 and 280 ^{0}C respectively. The flow rates for FID were fixed for nitrogen as make up gas 45 mL / min, hydrogen 40 mL / min and air 250 mL / min.

2.4. Analysis of Amino acids in Jams after acid hydrolysis

Jam sample (5 g) from each of three bottles for each flavor was mixed thoroughly to form composite sample. Jam sample (1 g) was added 5 mL of 6N hydrochloric acid and contents were heated at 110 ^{0}C for 24 h in screw capped sample vial. The mixture was cooled and centrifuged for 20 min at 3000 g. The clear supernatant layer was separated and the residue was washed with deionized water (1 mL). The solvent from combined clear solution was evaporated gently under nitrogen atmosphere. The residue was dissolved in water and volume was brought to 10 mL. The solution (3 mL) was removed and GC Analytical Procedure was followed. The quantitation was made from external calibration curve prepared from linear regression equation $Y = ax + b$, within the range 0.5 – 25 µg / mL.

2.5. Analysis of free amino acids in Jams

Jam sample (5 g) was added methanol water (1:1 v/v) (10 mL) and was heated on water bath on 60 ^{0}C for 25 min. The solution was allowed to cool at room temperature for 15 min and filtered. The final volume was adjusted to 15 mL. Three mL of the solution was taken and GC Analytical Procedure was followed.

2.6. Recovery of amino acids from Spiked Jam sample

Apple jam (5 g) was treated as for analysis of free amino acids in jams. Solution (3 mL) in duplicate were transferred to two screw capped vials and one of the samples was added a mixture of amino acids solution (0.5 mL) containing 10 µg / mL each and both the solutions were processed as GC Analytical Procedure. The quantitation was made from the increase in the response with added standards and using external calibration curves.

2.7. Analysis of amino acids in Aminess N^{TM} tablets

Five tablets were weighed and ground to fine powder. The 0.5 g of powdered tablets was dissolved in water and final volume was adjusted to 50 mL. The solution (3 mL) was taken and GC Analytical Procedure was followed.

2.8. Recovery of amino acids from spiked solution of tablets

Five tablets were processed as analysis of amino acids in aminess N^{TM} tablets and two solutions (3 mL each) were transferred to sample vials. A solution was added standard solution (0.5 mL) containing mixture of amino acids (10 µg / mL each) and both the solutions were analyzed following GC Analytical Procedure.

2.9. Analysis of amino acids (free and acid hydrolyzed) in Juices

The skins of fresh grapefruits, lemons and oranges were removed and juice from each fruit was extracted separately by clean electrical juicer machine. The juice was filtered with Watman filter paper 42 and 1.0 mL of the juice sample was evaporated

under nitrogen at 50 – 60 ^{0}C. The residue was dissolved in water and volume was adjusted to 10 mL. The solution (3 mL) was processed as GC Analytical Procedure for free amino acids. The juice (0.5 mL from each) was hydrolyzed with hydrochloric acid (6N), (5 mL) at 110 ^{0}C in screw capped vials for 24 h and cooled at room temperature. The solution was centrifuged for 20 min at 3000 g. The clear supernatant layer was separated and the residue was washed with deionized water (1 mL). The solvent from the combined solution was evaporated gently under nitrogen atmosphere. The residue was dissolved in water and volume was brought to 10 mL. The solution (1 mL) was taken and GC analytical procedure was followed.

2.10. Recovery of amino acids from Spiked Juice

The juice (0.5 mL) was processed as analysis of free amino acids from juice and final volume was adjusted to 10 mL. Two solutions (3 mL each) were transferred to screw capped sample vial and one was added standard solution (0.5 mL) containing mixture of amino acids (10 µg / mL each) and both the solutions were processed as GC analytical procedure.

2.11. Analysis of amino acids in Bottle Guord (*Lagenaria Siceraria*)

The skin, pulp and seeds of the fruit were separated with sharp stainless steel knife. Each part of the fruit (5 g) was ground and water (10 mL) was added. The contents were shaken on mechanical shaker for 1 h. The contents were filtered and residue was washed with water (5 mL). The final volume was adjusted to 15 mL. The solution (0.5 mL) was analyzed for free amino acids contents following GC analytical procedure. Well

ground each part of the fruit (skin, pulp and seeds) (0.5 g) was added hydrochloric acid 6N (5 mL) and heated at 110 ^{0}C for 24 h in a sealed sample vial. The contents were cooled at room temperature and mixture was centrifuged for 20 min at 3000 g. The clear supernatant layer was separated and the residue was washed with deionized water (1 mL). The solvent from the combined clear solution was evaporated gently under nitrogen atmosphere. The residue was dissolved in water and volume was brought to 10 mL. The solution (0.5 mL) was taken and GC analytical procedure was used.

2.12. Recovery of free amino acids from Spiked Bottle Guord Seeds Solution

Well ground seed (5 g) was treated as analysis of free amino acids in bottle guord. The solution (3 mL) in duplicate was taken and a solution was added a mixture of amino acids (0.5 mL) containing 10 µg / mL each. Both the solutions were processed as GC analytical procedure.

3. Results and Discussion

FAA and ECF are used as labeling reagents for the GC determination of amino acids. The derivatization reaction takes place in aqueous or aqueous miscible organic solvents to form volatile derivatives by the reaction with both functional groups (-NH_2 and –COOH) of AAs and elute from GC column. The 19 amino acids Val, Trp, Leu, Ile, Pro, Glu, Asn, Cys, Phe, Tyr, Lys, Ser, Met, Thr, Gln, Asp, Gly, His and Ala were derivatized with FAA and ECF as reported[44]. The extraction of the derivatives was carried out in chloroform and were eluted and separated on column HP-5 (30 m × 0.32 mm id). The peak of each AA was identified by comparing the retention time of the

standard amino acid and by spiking with standard AA one by one in sequence. All 19 AAs separated completely and eluted from GC column within 10 min. The repeatability of the separation (n = 5) was checked by analyzing the standard AAs mixture at concentration of 6 µg / mL each. The relative standard deviation (RSD) in terms of peak height and retention time was obtained below 2.4% and 3.2%. Linear calibration ranges were obtained within 0.6 - 25 µg / mL and limit of detection as 0.1 – 0.2 µg / mL (**Table 1**) as reported[44]. The method was examined for the analysis of AAs in foods (jams, vegetables and juices).

3.1. Analysis of amino acids in Jams

Jams are commonly used as food along with bread and other food products. They not only provide the pleasant flavor and sweetness to the food, but contain a number of nutrients, including amino acids. They are the common components of the fast foods. Four different flavors of the jams (apple, mango, strawberry and mixed) were analyzed for their nutritional values in terms of AAs contents and their variation in these brands of the jams. The results indicated the presence of all the 19 AAs (**Table 2**) in the four different flavors of jam samples examined. The apple jam indicated 19 free AAs (**Fig. 1**), which were present within the concentration range from below the limit of detection (BLOD) to 0.7 mg / 100 g and total (free + hydrolyzed) AAs within the concentration 0.11 – 8.47 mg / 100 g. The contents of free 9 essential AAs (Met, Thr, Trp, Val, Ile, Leu, Phe, His, Lys) were found between BLOD and 0.49 mg / 100 g, with maximum concentration of Lys. The concentration of total essential AAs were found in the range 0.11 – 5.474 mg / 100 g with maximum of Phe. The apple jam also contained non

essential free AAs in the concentration range 0.1 – 0.7 mg / 100 g and after acid hydrolysis 0.32 – 8.47 mg / 100 g.

Mango jam contained free (essential and non essential) AAs ranging in between the concentration of BLOD – 0.976 mg / 100 g and total (free + hydrolyzed) AAs 0.12 – 7.044 mg / 100 g with maximum for Glu. The contents of essential free AAs were BLOD – 0.564 mg / 100 g, and total 0.14 – 5.872 mg / 100 g with Thr as highest (5.872 mg / 100 g). Non essential free AAs were found BLOD – 0.976 mg / 100g and after acid hydrolysis 0.32 – 7.046 mg / 100 g.

Strawberry jam indicated (both essential and non essential) free AAs within BLOD – .97 mg / 100 g and total AAs 0.83 – 8.23 mg / 100 g. The contents of essential free AAs were found BLOD – 0.97 mg / 100 g and total 1.421 – 6.21 mg / 100 g with Thr as maximum. The non essential free AAs were BLOD – 0.87 mg / 100 g and total 0.83 – 8.23 mg / 100 g with Asp as maximum (8.23 mg / 100 g). The mixed jam indicated the presence of all 19 AAs and essential and non essential free AAs varied between BLOD – 0.723 mg / 100 g and 0.121 – 0.695 mg / 100 g respectively and total essential and non essential AAs 1.724 – 6.111 mg / 100 g and 0.65 – 8.45 mg / 100 g respectively (**Table 2**). All the four flavors of jams contained a reasonable amount of AAs including essential free and protein AAs and can be used as a potential source of food for essential and non essential AAs. The RSD for the analysis of free and after hydrolysis AAs was found below 5.5 %. The % recovery of AAs calculated by standard addition was calculated 87.5 – 96.5% with RSDs within 3.5 – 5.0%.

3.2. Analysis of AAs in Pharmaceutical Preparations

The aminess N^{TM} tablets contained 9 essential and 1 non essential AA and are used as a source of these AAs. The analysis of the tablet indicated the concentration of AAs within 23.1 – 130.4 mg / tablet with RSD 2.8 – 5.2% and agreed with the labeled values (**Table 3**). The % recovery of AAs from the pharmaceutical preparation by standard addition method was calculated 95.9, 97.1, 96.4, 97.4, 98.7, 97.4, 95.1, 95.8, 95.5 and 90.9% with RSD within 1.2 – 5.1% for Val, Leu, Thr, Ile, Met, Lys, His, Phe, Tyr and Trp respectively.

3.3. Analysis of AAs in Grapefruit, Lemon and Orange juice

Orange (*Citrus aurantium*), lemon (*Citrus limonum*) and grapefruit (*Citrus paradise*) are citrus fruits and are the members of Rutaceae family. They are most commonly used as fruits world wide and are used as source of vitamins and flavoring agents. The fresh juice of orange, lemon and grapefruits was collected and analyzed. The analysis indicated mainly the presence of four non essential amino acids (Ala, Asp, Glu and Pro). The concentrations of AAs in lemon juice were within the ranges 3120 – 20100 µg / mL as free AAs and 3946 – 20900 µg / mL after hydrolysis with maximum concentration of Pro with RSDs within 2.1 – 4.4% and 2.9 – 4.1% respectively. The orange juice indicated presence of free AAs within 173 – 1449 µg / mL and after acid hydrolysis 1175 – 3592 µg / mL with RSDs within 2.6 – 4.4% and 2.1 – 3.9% respectively. Similarly grapefruit juice contained free AAs 2758 - 8547 µg / mL and after hydrolysis 3325 – 9840 µg / mL with maximum concentration of Pro with RSDs 2.4 – 4.3% and 2.4 – 5.0% respectively (**Table 4**). The recovery of the free AAs from lemon juice calculated by standard addition method was within 89.6 – 95.9% with RSD 1.7 – 5.0%.

3.4. Analysis of AAs in Bottle Guord

Bottle Guord or White Guord (*Lagenaria siceraria*) is a member of Cucurbitaceae family. It is used as vegetable and is simple and light food due to the greater percentage of water. Skin, pulp and seeds of two varieties of the Bottle Guord (elongated loki and spherical Kadu) were analyzed for AAs contents. All the parts of loki contained 17 AAs, including 9 essential (Met, Thr, Trp, Val, Ile, Leu, Phe, His and Lys) and 8 non essential (Asp, Ser, Glu, Pro, Gly, Ala, Cys and Tyr). The concentrations of free essential AAs in pulp, seed and skin of loki were found in the ranges 0.6 – 9.2, 2.0 – 11.0 and 0.7 – 17.2 mg / 100 g and non essential AAs BLOD – 22.8, BLOD – 140 and BLOD – 47.1 mg / 100 g respectively with RSD within 1.9 – 4.8%. Total essential AAs (after acid hydrolysis) in pulp, seeds and skin of loki were 2.3 – 44.0, 5.1 – 336.2 and 2.8 – 60.6 mg / 100g and non essential AAs 2.9 – 44.0, 38.6 – 870.5 and 1.9 – 109.2 mg / 100g respectively with RSD 1.0 – 4.9% (**Table 5**). The free AAs contents in pulp commonly used as food indicated Pro < Gly < Val < Tyr = Lys < Cys = Met < Leu = His < Trp < Ala < Ile < Phe < Ser < Thr < Asp < Glu, while total AAs indicated the following order: Cys < Pro < Met < Trp < Ala < Gly = His < Tyr < Thr < Ser < Phe < Ile < Val < Lys < Leu < Asp < Glu. The pulp, skin and seed of the second variety of Bottle Guord (Spherical, Kadu) also indicated the presence of 17 AAs (9 essential and 8 non essential) for both with and without acid hydrolysis. Pulp, skin and seed samples indicated free essential AAs contents of 0.6 – 8.3, 1.7 – 10.1 and 0.6 – 15.0 mg / 100 g and non essential BLOD – 20.4, BLOD – 137.1 and BLOD – 47.1 mg / 100 g, respectively with RSD 1.1 – 5.5%. A sample was spiked with standard AAs and % recovery was calculated

within 90.0 – 116.0% (**Table 6**). This variety (Kadu) also indicated a similar trend in AAs contents as loki and pulp indicated the following order of increasing concentration: Pro < Gly < Val < Lys = Trp < Cys =Met < His = Leu < Trp < Ala < Ile < Phe < Ser < Thr < Asp < Glu, while the total AAs contents indicated the following order: Cys < Pro < Met < Ala < Gly = His < Tyr < Thr < Ser < Phe < Ile < Val < Lys < Leu < Asp < Glu. The results indicated that both the varieties are a reasonable source of essential and non essential AAs, however the seeds which are not commonly consumed as a food is also a rich source of both essential and non essential AAs. Now comparing the results of analysis with reported values, Silva et al[21] have reported the presence of all the 19 AAs in the jams as free AAs at the concentration level similar to the reported in present work. Zaini et al[5] have reported the AAs contents of *Benincasa hispida* of the family Cucurbitaceae at the similar concentrations as reported for *Lageraria siceraria* of same family with the observations that the seeds contained higher concentration of AAs than pulp and skin as has been observed in the present work.

4. Conclusion

The use of two stages derivatization with FAA and ECF has been examined for the GC analysis of AAs in pharmaceutical preparation, jams, fresh fruit juices and vegetable. The method indicated adequate sensitivity and selectivity for the determination of 4 to 19 AAs. The variation in the AAs contents within different fruits and different parts of vegetable have been noted and reported.

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Figures Captions

Fig.1. Chromatogram of free amino acids in apple jam. Peak Numbers: 1. Ala, 2. Gly, 3. Val, 4. Leu, 5. Ile, 6. Pro, 7. Thr, 8. Ser, 9. Asn, 10. Asp, 11. Glu, 12. Gln, 13. Cys, 14. Met, 15. Phe, 16. Lys, 17. His, 18. Tyr, 19. Trp. GC conditions: Column HP-5 (30 m × 0.32 mm id) with film thickness of 0.25 μm at column temperature 100^0 C for 2 min with ramping of 20^0 C/min up to 250^0 C with nitrogen flow rate of 3 mL/min with split ratio 10:1. The injector and detector temperatures were 270^0 C and 280^0 C respectively.

Fig. 2. Chromatogram of free amino acids in lemon juice. Peak Numbers and GC Conditions as Fig. 1.

Table 1. Limits of quantitation, limits of detection, calibration range, coefficient of determination and regression equations for the amino acids analysis

Amino acid	LOQ (μg/ml)	LOD (μg/ml)	Callibration range (μg/ml)	Coefficient of determination R^2	Linear Regression equation
Gly	0.3	0.1	0.5-25	0.9966	y=3.4143x+0.9333
Ala	0.3	0.1	0.5-25	0.9977	y=3.3429x-1.4520
Val	0.3	0.1	0.5-25	0.9921	y=3.3857x+0.4667
Leu	0.3	0.1	0.5-25	0.9947	y=3.7643x+2.0667
Ile	0.3	0.1	0.5-25	0.9964	y=3.907x+1.4321
His	0.6	0.2	0.5-25	0.9976	y=3.2071x-0.3667
Ser	0.6	0.2	0.5-25	0.9986	y=2.0643x-0.7667
Thr	0.6	0.2	0.5-25	0.9968	y=2.7286x-2.2667
Cys	0.6	0.2	0.5-25	0.9924	y=3.7743x+2.3467
Met	0.6	0.2	0.5-25	0.9963	y=3.5286x+2.1333
Asp	0.9	0.3	0.5-25	0.9984	y=1.6532x-0.812
Asn	0.6	0.2	0.5-25	0.9963	y=1.7214x-0.533
Pro	0.6	0.2	0.5-25	0.9916	y=3.450x-1.0667
Glu	0.9	0.3	0.5-25	0.9993	y=3.0714x-1.6667
Gln	0.6	0.2	0.5-25	0.9949	y=3.6814x+1.7467
Lys	0.6	0.2	0.5-25	0.9928	y=3.5714x+1.3333
Tyr	0.6	0.2	0.5-25	0.9975	y=3.3857x-1.5333
Trp	0.6	0.2	0.5-25	0.9954	y=3.5533x+0.5667
Phe	0.6	0.2	0.5-25	0.9928	y=3.450x+0.2667

Table 2. Concentration (mg/100g) of free and acid hydrolyzed amino acids in jams with different flavors

	Apple			Mango		Strawberry		Mixed	
	Free	Spiked	Hyd	Free	Hyd[a]	Free	Hyd	Free	Hyd
Gly	0.58(3.3)	0.56(3.0)	2.0(3.6)	0.446(1.4)	0.996(4.2)	0.65(3.3)	2.652(3.0)	0.514 (3.7)	2.133(1.6)
Ala	0.62(2.3)	0.6(1.3)	1.8(3.4)	0.546(3.4)	1.9(4.6)	0.59(2.4)	2.124(1.9)	0.447 (3.0)	1.918(2.7)
Val	0.15(4.5)	0.44(3.7)	2.35(5.0)	BLOD	3.215(1.9)	0.52(2.9)	2.544(1.3)	0.65 (3.3)	2.442(3.4)
Leu	0.12(3.0)	0.12(4.4)	0.11(5.3)	0.21(3.8)	4.0(1.3)	BLOD	3.487(1.7)	BLOD	3.547(3.8)
Ser	0.14(4.4)	0.3(2.4)	0.32 (2.3)	0.534(4.3)	0.82(2.5)	0.33(4.3)	0.83(2.6)	0.473 (4.3)	0.65(3.0)
Thr	0.13(2.4)	0.13(2.9)	5.1(2.4)	0.151(5.0)	5.872(3.0)	0.13(2.3)	6.21(2.3)	0.15(4.1)	6.111(2.3)
Ile	0.2(2.9)	0.29(3.3)	5.42(2.0)	0.322(4.3)	5.121(3.9)	0.97(5.2)	4.894(2.7)	0.247(2.3)	4.972(4.3)
Cys	0.14(3.4)	0.12(4.0)	3.21(2.3)	0.141(2.7)	3.291(3.9)	BLOD	2.894(3.8)	0.148(2.0)	3.141(5.3)
Met	BLOD	BLOD	1.24(2.4)	BLOD	1.355(4.8)	BLOD	1.421(3.0)	BLOD	1.724(5.0)
Asp	0.4(2.8)	0.73(1.4)	8.47(2.9)	0.774(2.8)	0.99(4.4)	0.57(4.4)	8.23(4.0)	0.392(3.4)	8.45(5.1)
Asn	0.7(2.5)	0.86(3.0)	6.54(1.3)	0.976(3.3)	7.016(4.3)	0.75(1.4)	7.115(4.3)	0.695(4.4)	6.89(1.3)
Glu	0.17(4.6)	0.51(3.7)	7.21(3.6)	0.891(3.0)	7.044(4.0)	0.87(1.3)	8.02(2.3)	0.523(5.3)	7.54(4.3)
Gln	0.12(1.3)	0.11(1.7)	2.14(3.0)	0.194(4.3)	3.019(5.0)	BLOD	2.18(3.2)	0.134(5.0)	2.225(4.4)
Pro	0.13(4.2)	0.14(3.4)	2.14(4.4)	0.122(5.3)	0.32(1.3)	0.16(3.7)	2.0(2.2)	0.158(3.0)	2.385(3.0)
Lys	0.49(2.6)	0.15(4.7)	4.574(4.3)	0.564(2.3)	0.93(5.4)	0.19(3.4)	4.893(3.6)	0.52(2.3)	5.054(3.9)
His	0.39(1.9)	0.33(1.0)	2.544(2.3)	0.291(1.8)	0.98(1.5)	0.59(4.4)	2.877(3.8)	0.723(2.5)	2.63(3.6)
Phe	0.35(2.8)	0.18(3.5)	5.474(3.9)	0.114(2.9)	0.18(3.9)	0.13(5.3)	4.983(4.8)	0.112(3.5)	5.214(1.8)
Tyr	0.1(4.3)	BLOD	1.21(3.4)	BLOD	0.52(2.3)	0.12(1.7)	1.873(1.3)	0.121(2.4)	1.544(1.3)
Trp	0.13(4.0)	0.13(2.4)	2.543(3.0)	BLOD	0.14(4.3)*	0.14(3.0)	2.575(3.3)	BLOD	2.967(1.3)

*(%RSD) n = 3

[a]Hyd = Acid Hydrolyzed

BLOD = below limit of detection

Table 3. Concentration (mg/tablet) of free amino acids in Aminess N™ tablet

Amino acids	Concentration of amino acids (%RSD), n = 3		
	Found	Labelled values	Analyzed after spiking with standard amino acids and deduction of amount of standard added
Val	130.4 (4.2)	135	125.0 (1.2)
Leu	86.8 (4.0)	90	84.3 (4.3)
Thr	64.7 (3.2)	65	62.4 (2.1)
Ile	61.6 (2.8)	60	60.0 (1.9)
Met	93.3 (3.9)	90	92.1 (2.7)
Lys	42.2 (5.2)	46	41.1 (4.7)
His	43.1 (3.0)	45	41.0 (2.7)
Phe	68.3 (2.9)	70	65.4 (4.8)
Tyr	71.2 (3.7)	75	68.0 (5.1)
Trp	23.1 (4.5)	25	21.0 (3.8)

Table 4. Concentration of amino acids (µg/ml) in grapefruit, lemon and orange juice (%RSD), n = 3

	Lemon Juice			Orange Juice		Grapefruit juice	
	Free	Spiked	Hyd	Free	Hyd*	Free	Hyd
Ala	3120 (3.0)	2918 (2.4)	3946 (4.0)	173 (4.1)	3592 (2.1)	3521 (4.3)	3774 (2.7)
Asp	6124 (2.1)	5549 (3.4)	10081(2.9)	313 (2.6)	1175 (2.5)	3214 (2.4)	4574 (2.4)
Glu	3511 (4.4)	3147 (5.0)	4010 (4.1)	812 (3.8)	1004 (3.9)	2758 (5.1)	3325 (3.5)
Pro	20100(2.6)	19200(1.7)	20900(4.0)	1949 (4.4)	2401 (2.3)	8547 (3.3)	9840(5.0)

*Hyd = Acid Hydrolyzed sample

Table 5. AAs contents (mg/100g) in fruits of Bottle Guord (elongated; Loki)

(%RSD), n = 4

Amino acid	Free			Acid Hydrolyzed		
	Pulp	Seed	Skin	Pulp	Seed	Skin
Met	0.6 (3.8)	2.3 (3.6)	0.7 (2.1)	2.3 (2.3)	28.9 (5.1)	2.8 (4.1)
Val	0.9 (2.1)	4.3 (3.5)	2.8 (3.1)	8.4 (4.7)	187.8(3.5)	35.7 (4.0)
Phe	3.9 (4.4)	9.3 (3.9)	5.2 (3.0)	7.6 (3.9)	259.4(1.0)	49.0 (3.0)
Lys	0.9 (3.3)	2.0 (3.5)	2.0 (3.9)	9.2 (3.1)	256.6(1.1)	55.8 (3.3)
Tyr	0.6 (4.8)	2.1 (3.0)	0.8 (4.4)	5.0 (2.8)	66.4 (3.0)	27.1 (2.2)
Ile	2.9 (3.1)	11.0 (2.4)	7.1 (2.8)	7.8 (3.0)	185.7(4.4)	37.5 (3.6)
Leu	0.8 (4.1)	4.5 (2.1)	1.8 (4.1)	10.1 (2.8)	336.2(3.2)	60.6 (4.9)
His	1.0 (3.0)	2.9 (1.0)	3.1 (4.1)	5.9 (2.0)	129.8(2.1)	22.0 (3.1)
Ser	8.9 (3.8)	11.2 (5.0)	2.8 (2.6)	7.3 (3.0)	247.3(2.7)	47.2 (3.8)
Cys	0.9 (2.5)	4.1 (4.7)	1.3 (5.1)	0.9 (4.1)	38.6 (3.0)	1.9 (3.6)
Glu	22.8 (1.9)	10.1 (4.1)	47.1 (3.0)	44.0 (2.6)	870.5(5.0)	109.2(4.7)
Asp	10.0 (4.0)	140.0(1.8)	13.1 (3.3)	32.1 (2.8)	499.1(3.1)	95.1 (2.9)
Pro	BLOD	BLOD	BLOD	2.9 (3.6)	142.9(2.8)	32.4 (1.1)
Thr	9.2 (2.8)	4.1 (1.1)	17.2 (3.6)	8.1 (2.1)	165.1(2.7)	31.7 (3.0)
Gly	0.3 (2.8)	0.5 (2.1)	0.6 (3.1)	5.7 (4.8)	332.1(4.1)	44.0 (3.2)
Ala	1.8 (3.6)	2.8 (3.7)	11.7 (5.5)	7.3 (4.1)	227.6(4.6)	50.2 (3.6)
Trp	1.2 (3.1)	3.1 (1.1)	2.1 (5.1)	3.0 (1.7)	5.1 (2.0)	3.4 (3.0)

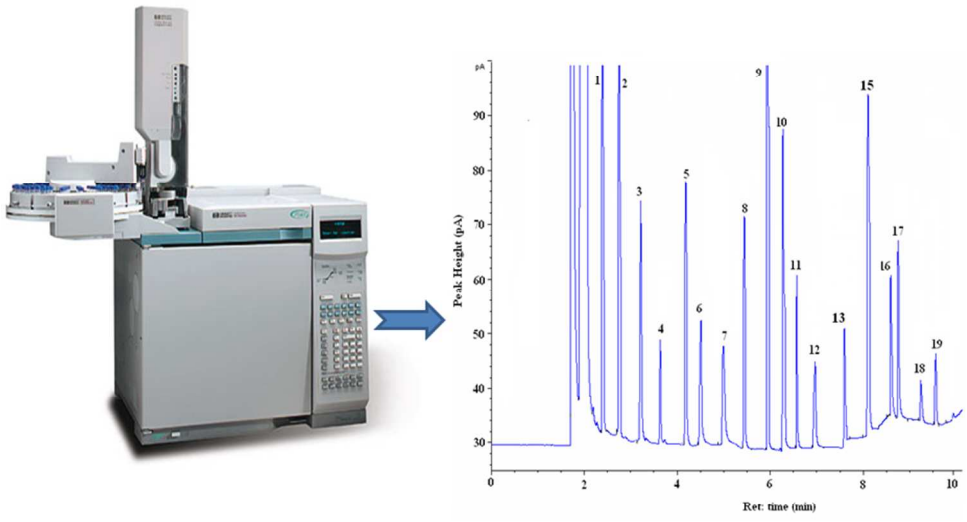
BLOD = below limit of detection

Table 6. AAs contents (mg/100g) in fruits of Bottle Guord (spherical; Kadu) (%RSD), n = 4

Amino acid	Free				Acid hydrolyzed		
	Pulp	Seed	Spiked	Skin	Pulp	Seed	Skin
Met	0.8 (2.1)	2.0 (4.0)	1.9 (4.0)	0.6 (2.2)	1.9 (2.3)	22.7 (3.2)	1.9 (3.7)
Val	0.6 (1.1)	3.9 (5.1)	3.7 (5.1)	2.0 (3.3)	7.3 (3.5)	175.4(5.3)	32.3 (5.1)
Phe	3.1 (5.1)	8.1 (5.0)	7.7 (5.0)	4.5 (5.1)	6.3 (4.4)	241.0(4.5)	44.2 (4.6)
Lys	0.7 (3.5)	1.7 (3.9)	1.6 (3.9)	1.6 (5.1)	7.9 (3.0)	247.3(5.0)	47.7 (3.5)
Tyr	0.7 (5.4)	1.8 (4.1)	1.7 (4.1)	0.7 (4.0)	4.4 (5.1)	58.5 (4.1)	26.6 (1.9)
Ile	2.2 (4.0)	10.1 (4.7)	9.7 (4.7)	6.3 (5.0)	6.4 (3.0)	174.4(3.9)	28.8 (3.0)
Leu	0.9 (4.1)	4.0 (3.8)	3.7 (3.8)	1.9 (3.5)	9.0 (2.9)	312.7(4.8)	56.4 (2.9)
His	0.9 (3.0)	2.0 (3.3)	1.8 (3.3)	2.7 (3.8)	4.3 (2.8)	122.4(4.1)	20.1 (3.1)
Ser	7.7 (4.1)	10.0 (4.1)	9.4 (4.1)	2.3 (5.5)	6.0 (4.1)	241.2(4.5)	40.8 (3.0)
Cys	0.8 (4.6)	3.7 (3.1)	3.4 (3.1)	1.6 (3.1)	1.0 (3.9)	37.4 (3.3)	0.8 (5.6)
Glu	20.4 (4.0)	9.6 (2.4)	9.1 (2.4)	47.1 (4.1)	38.8 (5.0)	813.0(4.6)	98.7 (2.0)
Asp	9.1 (3.4)	137.1(2.2)	130.5(2.2)	11.8 (3.7)	29.5 (4.8)	442.0(2.7)	89.3 (5.1)
Pro	BLOD	BLOD	------	BLOD	1.4 (2.0)	135.5(3.5)	27.7 (2.7)
Thr	8.3 (5.4)	3.5 (3.3)	3.3 (3.3)	15.0 (3.0)	5.3 (3.2)	157.5(3.7)	29.6 (4.4)
Gly	0.4 (3.5)	0.6 (3.0)	0.7 (3.0)	0.7 (4.8)	4.3 (1.1)	289.9(4.0)	38.3 (2.8)
Ala	1.9 (3.6)	2.1 (4.1)	1.9 (4.1)	10.9 (3.9)	4.2 (3.7)	221.4(4.4)	47.8 (4.1)
Trp	1.0 (3.8)	2.8 (2.1)	2.6 (2.1)	1.6 (3.0)	2.5 (3.0)	6.8 (1.1)	4.9 (2.4)

BLOD = below limit of detection

Graphical Abstract of GC-FID



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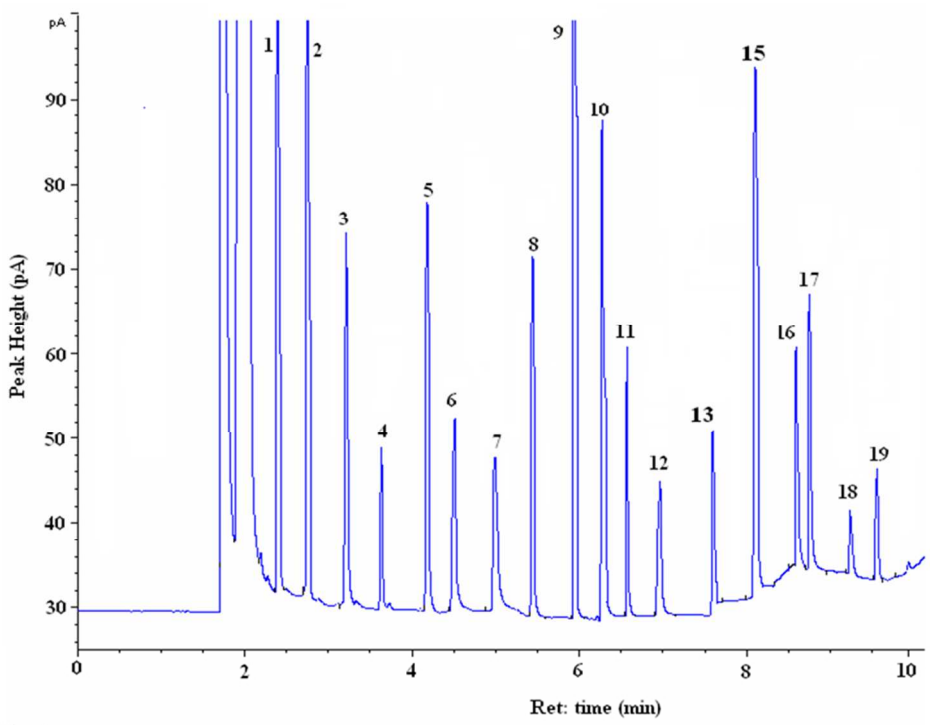

Fig. 1

Concentration of free amino acids in apple jam. Peak Numbers: 1. Ala, 2. Gly, 3. Val, 4. Leu, 5. Ile, 6. Pro, 7. Thr, 8. Ser, 9. Asn, 10. Asp, 11. Glu, 12. Gln, 13. Cys, 14. Met, 15. Phe, 16. Lys, 17. His, 18. Tyr, 19. Trp. Concentration 6 µg/ml each.
GC conditions: Ccolumn HP-5 (30 m × 0.32 mm id) with film thickness of 0.25 µm at column temperature 1000 C for 2 min with ramping of 200 C/min up to 2500 C with nitrogen flow rate of 3 ml/min with split ratio 10:1. The injector and detector temperatures were 2700 C and 2800 C respectively.

254x190mm (96 x 96 DPI)

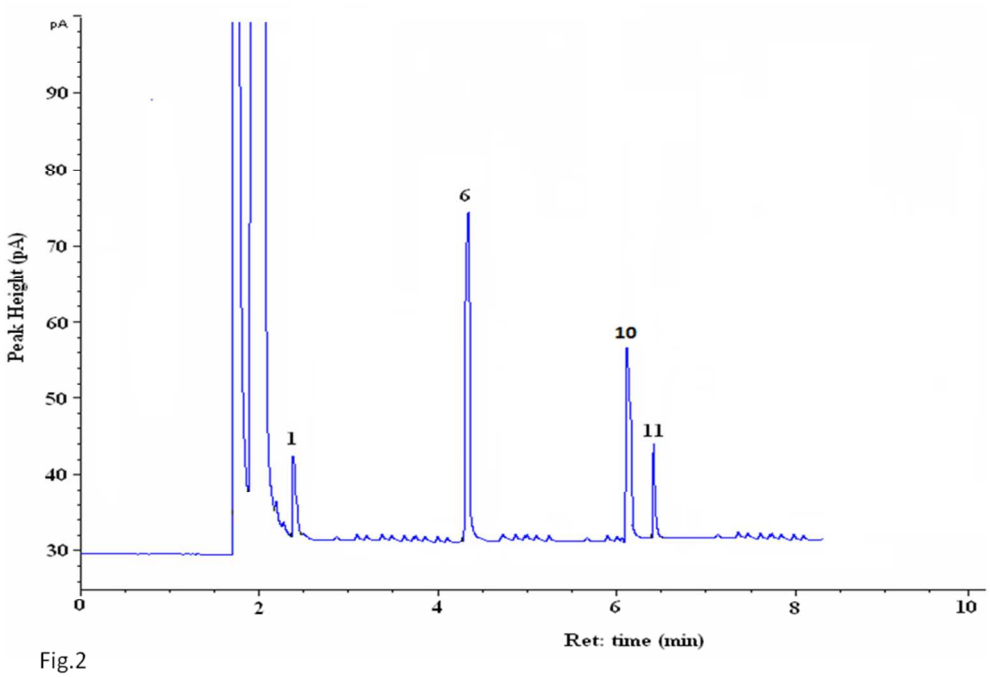

Fig.2

Concentration of free amino acids in lemon juice. Peak Numbers and GC Conditions as Fig. 1.
254x190mm (96 x 96 DPI)