

Amino Acids Changes during Citrus Storage

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Abstract

This study investigates the effect on of storage on the citrus fruits . The Brix scale is used to measure the amounts of the amino acids in the citrus juice . Seventy- four samples and six major amino acids have been tested and analyzed The study showed while the amounts of proline, butiric, arginine, aspartic acids increased, the amounts of serine and glumatic acids decreased. Some of the changes are attributed to errors in the freezing dehydration. The analysis results have confirmed fraud in the commercial grapefruit juice . Therefore the study recommends that the citrus chemo-graphic analysis of the free amino acids to discover the fraud . Pasteurization of the sample at a high temperature degree we can detect the existence of ammoniac. The study showed an evident storage effect on the concentration of amino acids in the lemon between December and May. There was a decrease in the concentrations of Serine, Arginine, and Aspartic amino acids in Jaffa oranges whereas there was an increase in the Alinine amino acid. In the storage period between May and October the Valencia type witnessed an increase in the amounts of the Asparagine acid and the Aspartic acid.

1. Introduction

This study aims to identify the amino acids changes that take place in the citrus syrups during storage. There are differences in the composition, taste, and flavor in citrus types and some of these differences are within the same type. These differences can be attributed the weather, climate, soil , irrigation , and types of fertilizers used. Generally, water constitutes 80% of the structure of the fresh vegetables and fruits contain whereas the other 20% is amino acids(that dissolve in water), carbohydrates and different kinds of ,sugar, proteins , and fats (that do not dissolve in water) .

Citrus juice is not a major source of amino acids , because citrus fruits contain little quantity of amino acids . However, the changes and concentration of amino acids in the juice may lead to: 1) creating new important compounds needed in the food industry,2) preserving the citrus juice for longer periods that vary according to the different types of citrus with different amino acids concentrations.

1.1.1 General information about Amino Acids :

Amino acids contain NH_2 , therefore, it has alkaline and acidic properties .

The general composition of the amino acid is:

The compound changing element of the compound: The general formula for an amino acid is: $NH_3 - CRH - COOH$.

In nature, there are about (80) amino acids some are proteins and others have protein in their structure.

2. Classification:

Amino acids are classified according to a number of different features. One of these features is whether or not people can acquire amino acids through the diet. According to this feature , scientists have identifies three types: the nonessential, essential, and conditionally essential amino acids. However, this classification as essential or nonessential doesn't really indicate their importance, as all twenty of them are indispensable for human health. The essential (or indispensable) can't be produced by the body and therefore should be supplied by food: Leucine, Isoleucine, Lysine, Threonine, Methionine, Phenylalanine, Valine, and Tryptophan. One more amino acid, Histidine, can be considered semi-essential, as the human body does not always need dietary sources of it. Meanwhile, conditionally essential amino acids are not usually required in the human diet, but are able to become essential under some circumstances. Finally, nonessential ones are produced by the human body either out of the essential ones or from normal proteins breakdown. These include Asparagine, Alanine, Arginine, Aspartic acid, Cysteine, Glutamic acid, Glutamine, Praline, Glycine, Tyrosine, and Serine. One more classification depends on the side chain structure, and experts recognize five types in this classification:

1. containing sulfur (Cysteine and Methionine)
2. neutral (Asparagine, Serine, Threonine, and Glutamine)
3. acidic (Glutamic acid and Aspartic acid) and basic (Arginine and Lysine)
4. alphatic (these include Leucine, Isoleucine, Glycine, Valine, and Alanine)
5. aromatic (these include Phenylalanine, Tryptophan, and Tyrosine)

There is another classification based on structure of the side chain which classifies the list of twenty into 4 groups, two are main groups and the other two are subgroups: non-polar, polar, acidic and polar, basic and polar. For example, side chains having pure hydrocarbon alkyl or aromatic groups are non-polar, and their list includes Phenylalanine, Glycine, Valine, Leucine, Alanine, Isoleucine, Proline, Methionine, and Tryptophan. In the meantime, if the side chain contains different polar groups like amides, acids, and alcohols, they are considered polar. Their list includes Tyrosine, Serine, Asparagine, Threonine, Glutamine, and Cysteine. Additional classification goes for acidic-polar (which includes Aspartic Acid and Glutamic Acid), if the side chain has a carboxylic acid, and basic-polar (includes Lysine, Arginine, and Histidine), if the side chain includes an amino group.

The above classifications can be summed into six main groups according to their structure and the general chemical properties of their R groups:

Table-1

Class	Name of the amino acids
1- Aliphatic	Glycine, Alanine, Valine, Leucine, Isoleucine
2- Hydroxyl or Sulfur/Selenium-containing	Serine, Cysteine, Selenocysteine, Threonine, Methionine
3- Cyclic	Proline
4- Aromatic	Phenylalanine, Tyrosine, Tryptophan
5- Basic	Histidine, Lysine, Arginine
6- Acidic and their Amide	Aspartate, Glutamate, Asparagine, Glutamine

3. Properties:

At temperature degree above 200°C all amino acids become solids and crystals that can dissolve in water but with different quantities. There amino acids crystals do not dissolve in organic solutions.

4. Procedure Material and method:

Citrus fruit were bought November from the local markets in Jordan. A pilot experiment was conducted to find out the quantity or the percentage of juice that could be extracted from a known quantity of citrus fruits.

Only Good fruits were selected ; diseased or bruised fruits were discarded. The fruits were washed to clean them from any dirt.

The Juice Preparation:

For the experiment, the fruits were weighed and divided into six lots for six observations:

- 1- Preservation by adding $K_2S_2O_5$ at the rate of 0.8 oz /100lbs and 1.0 oz/100 lbs respectively.
- 2- Preservation by adding sodium benzoate at the rate of 1.0 oz/100 lbs and 1.50 oz/100 lbs respectively.
- 3- Preservation by pasteurization at 165°F and 185°F for two minutes.

Extraction of the Juice :

An extraction machine was used to extract the juice which was filtered to obtain clear juice.

Bottling:

The juice was filled in sterilized glass bottles of 7 ounces capacity each. This enabled us to get six lots preserved by six different treatments:

- T1: preserved by potassium Meta bisulphite at the rate of 0.8oz/100 lbs of juice.
- T2: preserved by potassium Meta bisulphite at the rate of 1.0 oz/100 lbs of juice.
- T3: preserved by sodium benzoate at the rate of 1.0 oz/100 lbs of juice .
- T4 : preserved by sodium benzoate at the rate of 1.5 oz/100 lbs of juice .
- T5: preserved by pasteurization at 165°F for two minutes.
- T6: preserved by pasteurization at 185°F for two minutes.

Storage: all the six lots were stored at room temperature to observe the effect of storage on the amino acids of the citrus juice. Then , they were stored at zero storage and then at interval of 10 days up to 10 weeks.

Orange Juice:

Fruit juice are mixed in some cases with water or other ingredients making them impure juices. To know whether the juiced is natural or mixed , the Brix scale is used to test quantity of the Anthocyanin, vitamins B₁, C, asparagin , polyphenol, Isocitric ,...etc. This method measures the purity of the juice. Checking and testing seventy- four samples , six major amino acids have been identified.

Table-2

Amino acids	m mol /l
l-prolin	5.00
Arginine	2.00
Asparagin	1.75
Aminotutiric Acid	1.50
Aspartic acid	1.50
L-serin	0.70

Table-3: Orange Juice Amino Acids Distribution in Orange Juice

Amino acid	Mimum M mol/l	Maximum M mol/l
Aspartik acid	1.33	3.55
Serin	0.66	2.56
Asparagin	1.75	4.11
Glumatic acid	0.44	1.10
Glutamin	0.23	1.26
Prolin	2.61	14.67
Glisin	0.01	0.40
Alanin	0.49	1.66
Aminobutric acid	1.22	4.49
Arginin	2.16	5.61

The differences in the distribution of amino acids in orange juice should be determined to find out whether the juice is pure or ammonium salt , protein, or a dissolved mixture of proteins . The type of the free amino acid in the orange juice enables us to determine the type of the orange juice source whether it yellow orange or blood orange .

The following types of orange tested amino acids Amino acids are:

- 1.1 Blood oranges /Tarroco (newly extracted)
- 1.2 Blood-oranges / Robinson(newly extracted)
- 1.3 Blood oranges/ Moro(newly extracted)
- 1.4 Jaffa yellow Oranges
- 1.5 Spanish yellow Navel oranges
- 1.6 Jaffa oranges/ after storage of 140 days(stored in a tin envelope at 5°C)
- 1.7 Blood oranges/ Moro / after storage of 140 days(stored in a tin envelope at 5°C)

Table-4:

Amino acid	1.1	1.2	1.3	1.4	1.5	1.6	1.7
Aspartic	0.24	0.32	0.27	0.27	0.27	0.14	0.14
Serine	0.29	0.56	0.58	0.48	0.86	0.37	0.41
Glumatic	0.23	0.22	0.29	0.15	0.15	0.04	0.13
Proline	0.66	0.17	0.59	0.77	0.88	0.81	1.04
Glycine	0.01	0.01	0.01	0.01	0.02	0.02	0.02
Alanine	0.07	0.10	0.09	0.12	0.11	0.09	0.08
Cysteine	0.01	0.01	0.01	0.01	0.01	-	-
valine	0.02	0.01	0.02	0.02	0.02	0.03	0.02
Methionine	0.01	0.01	0.1	0.1	0.1	0.1	0.1
Isoleucine	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Leucine	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Tyrosine	0.01	0.01	0.01	0.01	0.01	0.3	0.01
Phenylalanine	0.01	0.01	0.01	0.02	0.02	0.03	0.01
L- Alanine	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Amino-butiric Acid	0.20	0.22	0.22	0.30	0.35	0.67	0.77
Lysine	0.04	0.04	0.04	0.04	0.06	0.06	0.05
Histidine	0.04	01	01	0.01	0.02	0.03	0.02
Arginine	0.39	0.05	0.49	0.65	1.02	0.75	0.52
Toatal	2.37	3.15	2.72	2.01	3.83	3.11	3.26

The table shows that the content of the free amino acids amino acids in the orange juice stored at low temperature ranges between 2.5 and 3.5 . While the amounts of proline, butiric, arginine, aspartic acids increase, the orange juice stored at a low temperature increases , the amounts of serine and glumatic acids decrease.

The following table shows the amounts of the amino acids according to the orange manufacturing:
 The material used:

- 2.1 Fresh orange juice without any added material
- 2.2 Frozen orange juice
- 2.3 Pasteurized orange juice at 78°C for 10 minutes
- 2.4 Pasteurized orange juice at 85°C for 10 minutes

Table -5:

Amino acid	1.1	1.2	1.3	1.4	1.5
Aspartic	0.29	0.23	0.26	0.29	0.27
Serine	0.65	0.49	0.60	0.65	0.64
Glumatic	0.12	013	0.11	0.12	0.12
Proline	0.83	0.75	079	0.85	0.80
Glycine	0.01	0.01	0.01	0.01	0.01
Alanine	0.07	0.07	0.06	07	07
Cysteine	0.01	0.01	0.01	0.01	0.01
Valine	0.01	0.01	0.01	0.01	0.01
Methionine	0.01	0.01	0.1	0.1	0.1
Isoleucine	0.01	0.01	0.01	0.01	0.01
Leucine	0.01	0.01	0.01	0.01	0.01
Tyrosine	0.01	0.01	0.01	0.01	0.01
Phenylalanine	0.01	0.01	0.01	0.01	0.01
B- Alanine	0.01	0.01	0.01	0.01	0.01
Amino-butiric Acid	0.18	0.18	0.18	0.18	0.18
Lysine	0.03	0.03	0.03	0.03	0.03
Histidine	0.01	01	01	0.01	0.01
Arginine	0.58	0.51	0.55	0.59	0.51
Total	0.62	2.43	2.53	2.83	2.72

There are changes in the table that are attributed to errors in the manufacturing processes. The greatest mistake was in the dehydration process that is the freezing dehydration. These changes, however, are not calculated or taken into account.

The amount of amino acids can be calculated according to the type of fruit and the treatment process (dehydrated, pressed as juice, fresh, ...etc.). The amount of the amino acids in natural juice ranges between 2.5 and 3.5. To know the amount of oranges needed or the juice produced, the following formula is used:

$$\text{total of amino acids} = 1\%$$

3

In this case, there is a relative error of 20% in the results obtained. This is attributed to the ripening degree of the fruits and their types. Moreover, the quantity of fruits can be calculated by finding out the amount of nitrogen:

$$\text{Fruit percentage} = N * 100$$

Thus, the content of nitrogen in the natural orange juice is 1%.

The Grapefruit Juice:

This study does give much concern about the types of fruit used in the samples. The amino acids studied, as shown in table-6, the aspartic, the Glutamic, the butyric, the proline, the Alanine as essential amino acids.

Table -6 shows the free amino acids in the grapefruit juice:

Mg/litre

Amino acid	1	2	3	4	5	6	7	The average	
								Mg/l	%
Alanine	131	164	79	180	200	171	106	147	7.3
Proline	600	521	662	734	763	834	558	662	27.9
α -amino-butyric Acid	227	251	216	174	355	247	328	271	11.4
Aspartic	139	682	808	1282	521	1011	992	919	38.8
Glumatic	438	606	439	322	329	-	-	426	18.0

Table -7 shows and proves the fraud in the commercial grapefruit juice as the analysis results has confirmed:

	1	2	3
Formalin amount(100ml)	2.5	17.2	13.6
Ash(9/1)	4.29	3.65	3.24
Potassium (mg/1)	1645	1460	1275
Sodium(mg/1)	127	18	228
Phosphate(mg/1)	246	604	174
Chlorine(mg/1)	72	-	-
Nitrate(mg/1)	1	2	16
Sulfate (k ₂ so ₄)(mg/1)	65	213	63
Calcium (mg/1)	134	-	107
Magnesium (mg/1)	26	-	46
Proline amino acid (mg/1)	-	50	-
Citric acid (mg/1)	-	-	-
Glycine amino acid	-	-	14
Isocitric acid (mg/1)	-	-	43.0
Vitamin B1(mg/1)	-	-	53
Free nitrogen	-	-	10.1

The table shows the following:

- 1- There are amounts of ash, potassium, and phosphate naturally distributed but evident. This indicates that salts are added to the juice. But, the non-organic materials change.

- 2- Outwardly, we cannot prove that there is anything which is not natural in the sample. But through the chemo-graphic analysis of the free amino acids we can discover the fraud. the results can show whether there is an increase or decrease in the concentration of the amino acids existing in the sample. we can also detect any added salts to the juice.
- 3- Pasteurization of the sample at a high temperature degree we can detect the existence of ammoniac, because during this process a rise in the amount of ammoniac occurs.

Lemon Juice:

This study involved a randomly chosen sample of 58 syrup types of Jaffa lemon. Fourteen of them containing free amino acids with values shown in table-8:

Table-8: Lemon Juice Free Amino Acids

Amino Acids	Average	STDV	Variable number(%)
Aspartic	4.91	0.954	19.4
Tyrosine	4.23	1.307	30.9
Serine	4.47	1.296	29.1
Glutamic	2.09	0.295	14.1
Proline	6.03	1.637	27.2
Glycine	0.23	0.036	15.8
Alanine	2.55	0.377	14.8
Cysteine	0.14	0.042	30.0
Valine	0.23	0.047	20.1
Methionine	0.02	0.010	47.8
Isoleucine	0.07	0.012	17.4
Leucine	0.07	0.013	17.3
Tyrosine	0.11	0.019	17.7
Phenylalanine	0.89	0.168	18.9
Lysine	0.09	0.019	17.9
Histidine	0.08	0.017	20.7
Arginine	0.40	0.100	25.0

As there is a chronic trust problem between the governmental institutions and the producer of fruit juices, a number of studies have been carried out to determine the orange and the lemon juice content of the free amino acids. Table -9 shows the average and standard deviations of the of the lemon juice:

Table-9: Lemon and orange Juice Free Amino Acids

Amino Acids	Orange juice(27 samples)		Lemon Juice(35 samples)	
	average	STDV	average	STDV
Aspartic	6.5	2.0	16.6	4.6
Asparagine	6.9	1.7	9.8	2.5
Serine	4.9	0.7	9.8	1.4
Glutamic	2.1	0.3	5.3	0.7
Glutamine	0.5	0.1	0.3	0.7
proline	44.0	6.0	30.0	5.0
Alanine	3.2	0.6	6.5	1.2
Cysteine	-	-	0.3	0.2
Valine	0.5	0.12	0.7	0.2
Methionine	0.12	0.08	0.2	0.1
Isoleucine	0.18	0.07	0.3	0.1
Leucine	0.24	0.08	0.4	0.1
Tyrosine	0.33	0.08	0.2	0.1
Phenylalanine	0.71	0.22	0.8	0.2
α -amino-butiric Acid	17.0	4.0	7.2	1.6
Lysine	1.2	0.4	7.2	1.6
Histidine	0.22	0.09	0.2	0.1
Arginine	10.4	3.1	1.0	0.8

In 1969, different analysis results of lemon juice were obtained in Lisbon, California and India. Mir's study showed that there were no significant differences in the amino acid content in the lemon juices, but the study

showed that there was a difference of the amount of two amino acids, the Serine the average and the Arginine: the average of the former was 0.7 -2.6 while the average of latter was 6.7-10.2.

A study was conducted to show impact of storing lemon for several months prior to juice extraction on the free amino acids. This study showed that there was not any storage effect on the free amino acids concentrations. But there was an evident effect on the concentration of amino acids between December and May. There was a decrease in the concentrations of Serine, Arginine, and Aspartic amino acids in Jaffa oranges whereas there was an increase in the Alinine amino acid. In the storage period between May and October the Valencia type witnessed an increase in the amounts of the Asparagine acid and the Aspartic acid.

Table-9 shows the concentration of the amino acids in orange and Lemon juice according the place of origin:

Orange Juice	California	Arizona	Florida
Number of samples	13		14
Asparagines	7.9		5.9
Serine	5.3		4.4
α -amino-butiric Acid	0.17		20.0
Histidine	0.26		0.08
Lemon Juice			
Number of samples	13	15	17
Asparatic	12.5	29.0	24.0
Proline	35.0	29.0	24.0

Conclusion:

- 1- The study has shown that the content of the free amino acids in the orange juice stored at low temperature ranges between 2.5 and 3.5. While the amounts of proline, butiric, arginine, aspartic acids increase, the orange juice stored at a low temperature increases, the amounts of serine and glutamic acids decrease.
- 2- There are changes attributed to errors in the manufacturing processes. The greatest mistake was in the dehydration process that is the freezing dehydration. These changes, however, are not calculated or taken into account.
- 3- There are changes in the orange juice that are attributed to errors in the manufacturing processes. The greatest mistake was in the dehydration process that is the freezing dehydration.
- 4- shows and proves the fraud in the commercial grapefruit juice as the analysis results has confirmed
- 5- There were amounts of ash, potassium, and phosphate naturally distrusted but evident. This indicates that salts are added to the grapefruit juice. However, the non-organic materials change.
- 6- Outwardly, we cannot prove that there is anything which is not natural in the sample. But through the chemo-graphic analysis of the free amino acids we can discover the fraud. the results can show whether there is an increase or decrease in the concentration of the amino acids existing in the sample. we can also detect any added salts to the juice.
- 7- Pasteurization of the sample at a high temperature degree we can detect the existence of ammoniac, because during this process a rise in the amount of ammoniac occurs.
- 8- For lemon: the study showed that there was a difference of the amount of two amino acids, the Serine the average and the Arginine: the average of the former was 0.7 -2.6 while the average of latter was 6.7-10.2.
- 9- This study showed that there was not any storage effect on the free amino acids concentrations. But there was an evident effect on the concentration of amino acids between December and May.
- 10- The study also showed that there was a decrease in the concentrations of Serine, Arginine, and Aspartic amino acids in Jaffa oranges whereas there was an increase in the Alinine amino acid. In the storage period between May and October the Valencia type witnessed an increase in the amounts of the Asparagine acid and the Aspartic acid.

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