*Journal of Food Biosciences and Technology,*
Islamic Azad University, Science and Research Branch, Vol. 12, No. 4, 35-40, 2022
DOI:10.30495/jfbt.2022.20320 https://dorl.net/dor/20.1001.1.22287086.2022.12.4.4.9

# The evaluation of Amino Acid Profiles in Sugar Beet Industry

**F. Sahraei [a], M. Honarvar [b*]**

[a] M. Sc Student of the Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[b*] Associate Professor of the Department Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Received: 11 February 2021 Accepted: 9 May 2021

ABSTRACT: Food should contain enough amino acids to grow and maintain human health. For all practical purposes, the nutritional value of protein food is defined by its basic amino acid composition. In the sugar industry in particular, production and the importance of the final product, the presence of amino acids in each stage of sugar production, that briefly includes diffusion, purification, evaporation, crystallization, results in inappropriate color production and also one must remember that asparagine, is one of the amino acids that produces acrylamide. The aim of this research work is to investigate the profile of amino acids in the sugar industry, including thick juice and molasses. Amino acid profiles in these products were performed by analyzing 20 amino acids, most notably asparagine. In this study, samples of thick juice and molasses were studied during seven day intervals to measure the amino acid profiles. Different physico-chemical tests were performed to measure the quality of each sample. Finally, by employing HPLC, the amount of each amino acid was determined in all samples. The results indicated that samples of thick juice followed by molasses contained high amounts of asparagine.

**Keywords**: Amino Acids, HPLC, Sugar Industry.

## Introduction

Sugar beet as an industrial and strategic plant is one of the main sources of sugar production in Iran. Now more than 200,000 hectares of water lands are allocated to sugar beet. The raw material supplies 35 sugar factories in the country (Abdollahian-Noghabi *et al.*, 2007). According to the reports provided by the Iranian Sugar Association, the annual sugar production capacity in the country is about 2.5 million tons, while the top highest annual beet and sugar canes production has been 1.5 million tons in the country. However, the annual consumption of sugar in Iran is about 2.2 million tons, or, in other words, the amount of sugar consumed per Iranian is about 28-30 kg of sugar a year.

The sugar production process is a continuous operation that aims to extract sugar from the structure of sugar beet cells. Its aim is to obtain the highest purity sugar and to maintain the lowest purity of

[*]Corresponding Author: m-honarvar@hotmail.com

molasses. During the process of producing and extracting sugar, various types of operations and techniques are used, including heat, diffusion, energy consumption, filtration, deposition, evaporation, crystallization and drying (Asadi, 2006).

In recent years, studies have been carried out on the effects of dietary foods containing different percentages of alanine amino acid on growth performance, nutrition and survival rates in fish under the same breeding conditions.

The amino acids are divided into two groups of essential and nonessential amino acids. Essential amino acids include lysine, threonine, histidine, isoleucine, methionine, phenylalanine, valine, tryptophan and leucine. Nonessential amino acids are proline, glycine, alanine, serine, cysteine, aspartic acid, glutamic acid, asparagine, glutamine and tyrosine.

Muttucumaru and colleagues conducted a study in 2016, that showed the release of free asparagine could affect acrylamide formation. (Muttucumaru *et al*., 2016)

Passos and colleagues conducted a study in 2018 that showed the use of polysaccharides such as pectin in the formation of acrylamides in biscuits, therefore the taste and color of the products did not change. (Passos *et al*., 2018)

## Materials and Methods

Sampling was carried out at the sugar factory in Hamedan at the time intervals of seven days for three consecutive weeks. The samples included raw juice, thin juice, thick juice, sugar and molasses.

Samples prepared for the first, second and third weeks are numbered 1, 2 and 3 respectively. After the sampling, all specimens were stored in dark colored containers and analyzed promptly. All solutions and chemicals used in this study were prepared by the Merck Chemical Company, Germany.

Different physico-chemical tests were carried out to evaluate the quality of the samples. Theses consisted of determinations of invert sugar, color, pH and ash.

### - *Invert sugar measurements*

Invert is a mixture of glucose and fructose (50:50) resulting from the acid or enzyme hydrolysis sucrose. In order to measure the Invert samples, the Berlin Institute method was used (Wojtczak, 2003)

### - *pH measurement of the samples*

The pH of the samples was measured using JENWAY pH meter, made in UK according to the ICUMSA standard.

### - *Measuring the color of the samples*

Color is one of the most important parameters in the sugar industry, because the color of the product side of the production process has the greatest impact on the color of the final product (Bahrami *et al*., 2015). The color of the samples was examined using a spectrophotometer device at 420 nm.

### - *Measuring the sugar content of the samples*

The percentage of sugar or Pol indicates the polarimetric sugar content of the samples. In addition it is an indicator for determining the sucrose content of the samples. Sugar content is determined by Saccharomat, made in china, according to the ICUMSA standard.

### - *Measuring the brix or dry matter of the samples*

The dry matter content of the samples was measured by Anton Paar Refractometer, made in china.

- ***Measurement of the ash content***

The ash contents of the samples were examined using conductivity method using Jenway Kentucky made in UK.

- ***Amino acid profile Measurement***

AOAC Official Method (994.12) was employed to determine the amino acid profile.

**Results and Discussion**

The results of examining the qualitative characteristics of the sample are shown in Table 1.

According to the results of Table 1, the physicochemical properties of the obtained samples were determined in different time intervals. The results showed that invert samples showed different values at different times. The results of Table 1 indicate that, among the various samples, the raw juice sample has the highest invert content. Raw juice, due to acidic conditions and the possibility of further decomposition of sucrose into the invert, shows higher values than invert. In relation to the brix or dry matter of the samples, samples with a higher solids content, indicate higher brix rates. Thick juice and molasses contain the highest amount of brix in the sugar beet samples. The pH of the samples, indicates the acidic or alkaline conditions present, was the lowest in raw juice samples and in the samples of thin juice the highest hot might be due to the addition of lime with purification process. As expected, there is the highest level of Pol or percent sugar in sugar samples. However, among juices, thick juice has higher levels of sugar because of higher dry matter. Concerning the color of the samples, the lowest color was found in the samples of sugar and the highest color in molasses samples. Molasses, due to high turbidity and the presence of various dye impurities, usually have the highest levels of color in the sugar industry. Ash, that indicates the presence of solutes in the samples, showed the highest levels in molasses and the least amount in raw and thin juice.

Tables 2, 3 and 4 show the amount of amino acids in thick juice and molasses and in concentrated syrup samples and molasses in the first, the second and the third weeks. The values are based on the percentage and mg / kg amino acids. The results of chemical analysis of amino acids based on the samples are shown in Table 2 and 3, in percentage terms. The results of the chemical analysis of amino acids based on the samples are shown in Table 4 in mg / kg. According to the results obtained from the tables, in relation to amino acid, aspartic acid molasses 3 had the highest rate. Molasses 1 had the lowest levels of glutamic acid. The asparagine amino acid, which is the most important amino acid in the sugar industry, has the least content in thick juice 1 and the highest in thick juice 3. In terms of amino acid serin molasses 2 had the highest concentration and the richest syrup had the least amount. The samples contained 0.00 to 0.005% glutamine. Molasses 2 had the highest levels of histidine amino acid. The lowest amount of glycine amino acid was related to molasses 1. Regarding the amino acid content of citrulline, all three molasses have equal concentrations. Nevertheless, in thick juice samples, thick juice 1 had the highest amount of thick juice 2 and 3. Thick juice 2 has the lowest amount of arginine amino acid. The highest amount of alanine amino acid in molasses 2 was among the samples. All the thick juice samples have equal amounts of methionine amino acids. In addition, samples of molasses 1 and molasses 3 had the same amount of this amino acid. Molasses 2 had the highest amount of methionine amino acid. Thick juice 3 contains the least amount of valine amino acid. Molasses 2 had the highest amount of phenylalanine amino acids. Thick juice 3 contains the least amount of leucine and isouclinate amino acids. Molasses 2 had the highest amount of lysine amino acids.

**Table 1.** Analysis of sample qualities

| Sample name | Invert (%) | Color (IU) | Ash (%) | Pol (%) | pH | Brix (%) |
|---|---|---|---|---|---|---|
| Raw juice1 | 0.055 | 2890.58 | 0.51 | 12.05 | 6.2 | 13.9 |
| Thin juice1 | 0.013 | 1086.942 | 0.412 | 13.9 | 8.3 | 15.5 |
| Thick juice1 | 0.040 | 1259.981 | 1.3263 | 50.10 | 8.6 | 54.3 |
| Molasses1 | 0.270 | 24179.37 | 19.7876 | 41.6 | 8.6 | 77.42 |
| Sugar1 | 0.018 | 47.497 | 15.538 | 99 | 6.12 | - |
| Raw juice2 | 0.071 | 22821.242 | 0.543 | 12.9 | 6.03 | 14.81 |
| Thin juice2 | 0.007 | 3094.156 | 0.477 | 13.05 | 8.63 | 14.48 |
| Thick juice2 | 0.034 | 3691.037 | 1.828 | 52.95 | 8.45 | 58.59 |
| Molasses2 | 0.09 | 22022.69 | 25.171 | 47.1 | 6.7 | 86.2 |
| Sugar2 | 0.07 | 115.328 | 20.117 | 99.1 | 7.7 | - |
| Raw juice3 | 0.076 | 21694.170 | 0.53 | 12.95 | 5.9 | 14.55 |
| Thin juice3 | 0.021 | 2062.170 | 0.48 | 14.2 | 8.03 | 15.72 |
| Thick juice3 | 0.0049 | 2811.403 | 1.480 | 49.80 | 7.7 | 54.71 |
| Molasses3 | 0.049 | 21695.88 | 24.878 | 43.2 | 6.6 | 79.56 |
| Sugar3 | 0.011 | 50.425 | 17.413 | 99.1 | 7.23 | - |

**Table 2.** The results of chemical analysis of amino acids in thick juice and molasses samples

| Number | Sample | (%) Cit | (%)Thr | (%) Gly | (%) His | (%) Gln | (%) Asn | (%) Glu | (%) ASP |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Thick juice1 | 0.004 | 0.016 | 0.023 | 0.005 | 0.003 | 0.001 | 0.274.0 | **0.055** |
| 2 | Molass es1 | 0.001 | 0.005 | 0.006 | 0.068 | 0.003 | 0.002 | 0.163 | **0.040** |
| 3 | Thick juice2 | 0.003 | 0.013 | 0.020 | 0.002 | 0.000 | 0.002 | 0.227 | **0.052** |
| 4 | Molass es2 | 0.001 | 0.005 | 0.013 | 0.128 | 0.005 | 0.002 | 0.291 | **0.082** |
| 5 | Thick juice3 | 0.002 | 0.013 | 0.022 | 0.003 | 0.001 | 0.003 | 0.248 | **0.059** |
| 6 | Molass es3 | 0.001 | 0.005 | 0.012 | 0.125 | 0.004 | 0.002 | 0.306 | **0.085** |

**Table 3.** The results of chemical analysis of amino acids thick juice and molasses samples

| Number | Sample | (%) Lys | (%) Leu | (%) Is-Leu | (%) Phen | (%) Val | (%) Meth | (%) Arg |
|---|---|---|---|---|---|---|---|---|
| 1 | Thick juice1 | 0.006 | 0.024 | 0.030 | 0.006 | 0.028 | 0.001 | 0.006 |
| 2 | Molasses1 | 0.007 | 0.047 | 0.055 | 0.009 | 0.049 | 0.002 | 0.001 |
| 3 | Thick juice2 | 0.004 | 0.020 | 0.028 | 0.005 | 0.029 | 0.001 | 0.003 |
| 4 | Molasses2 | 0.010 | 0.058 | 0.075 | 0.013 | 0.070 | 0.004 | 0.001 |
| 5 | Thick juice3 | 0.004 | 0.019 | 0.027 | 0.005 | 0.025 | 0.001 | 0.004 |
| 6 | Molasses3 | 0.009 | 0.048 | 0.069 | 0.012 | 0.072 | 0.002 | 0.001 |

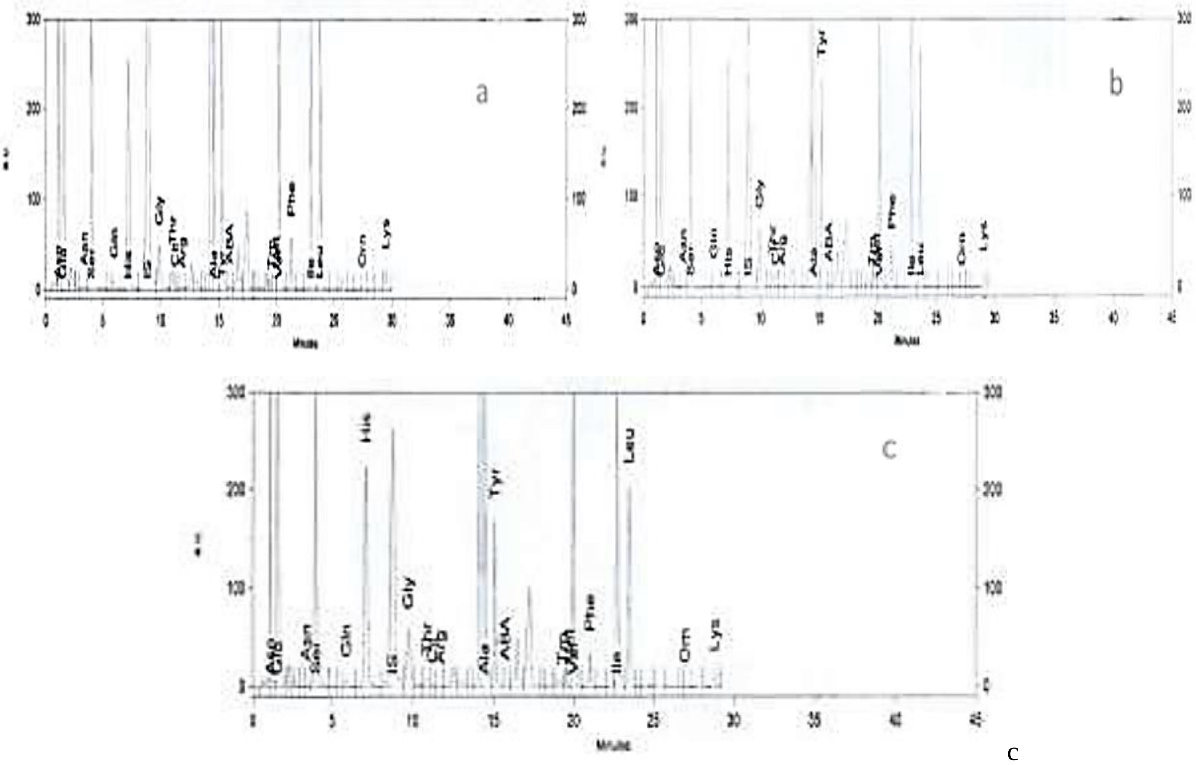
**Table 4.** The results of chemical analysis of amino acids in concentrated syrup samples and molasses in ppm

| Number | Sample | Lys | Leu | Is-Leu | Phen | Val | Meth | Ala | Arg |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Thick juice1 | 62.66 | 239.26 | 300.55 | 62.01 | 279.63 | 11.44 | 579.87 | **62.92** |
| 2 | Molasses1 | 65.36 | 465.53 | 546.26 | 95.00 | 486.24 | 22.22 | 851.46 | **10.16** |
| 3 | Thik juice2 | 44.45 | 201.38 | 281.16 | 50.87 | 288.65 | 8.41 | 588.85 | **34.81** |
| 4 | Molasses2 | 101.93 | 581.77 | 752.63 | 129.95 | 700.91 | 37.61 | 1436.00 | **13.78** |
| 5 | Thick juice3 | 41.40 | 185.90 | 269.15 | 49.75 | 245.63 | 7.00 | 664.28 | **40.53** |
| 6 | Molasses3 | 87.03 | 481.49 | 691.41 | 115.45 | 715.95 | 23.42 | 1410.34 | **12.37** |

| Number | Sample | Cit | Thr | Gly | His | Glen | Ser | Asn | Glu | ASP |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Thick juice1 | 41.95 | 161.44 | 231.92 | 46.77 | 28.53 | 269.48 | 14.31 | 2736.64 | 553.93 |
| 2 | Molasses1 | 10.14 | 51.90 | 58.30 | 684.71 | 30.96 | 362.70 | 17.88 | 1629.20 | 404.55 |
| 3 | Thick juice2 | 25.24 | 126.45 | 203.75 | 17.50 | 3.05 | 258.66 | 24.86 | 2267.04 | 522.96 |
| 4 | Molasses2 | 13.05 | 51.65 | 127.82 | 1276.61 | 46.29 | 694.47 | 24.02 | 2910.66 | 821.43 |
| 5 | Thick juice3 | 21.81 | 126.70 | 216.52 | 33.85 | 5.29 | 292.93 | 27.89 | 2482.53 | **590.56** |
| 6 | Molasses3 | 12.89 | 45.23 | 123.18 | 12454.01 | 42.67 | 657.50 | 2029 | 3056.56 | **849.80** |

Among the amino acids in the sugar industry, the amino acids of asparagine and glutamine are more important. The presence of amino acids in the sugar industry can lead to inappropriate color in the products. Asparagine amino acid also produces acrylamide. Acrylamide is a carcinogenic compound, that is formed by the reaction of the amino acid with reduced sugars as a carbonyl compound during the millard reaction. A study by Muttucumaru *et al*. In 2016 showed that free asparagine release could affect acrylamide formation. (Muttucumaru *et al*., 2016) Acrylamide is formed by the reaction of millard and the reaction between the asparagine amino acid and the reducing sugars at a temperature above 120 ° C. In fact, the addition of reducing sugars to the amino acid of asparagine generates a large amount of acrylamide. Therefore, reducing sugars and amino acids of asparagine are the main precursors of acrylamide. However, the effect of acrylamide formation will depend on the relative levels of precursors. The primary Millard reaction is the main source of acrylamide formation. In addition, asparagine is decarboxylated, i.e., 1-amino propionamide, an unstable intermediate in the formation of acrylamide like acrylamide; it can be formed at high temperatures in the presence of glucose. The raw juice extracted from sugar beet slices contains sucrose and various non-sugar impurities such as amino acids. These compounds are extracted from sugar beet by water at high-temperature used in diffusion and can have a significant impact on the ability to produce sugar in products like thick juice and molasses. Therefore, the amino acids (including Glycine (Gly), Alanine (Ala), Valine (Val), Leucine (Leu), Isoleucine (IS-Leu), Serine (Ser), Threonine (Thr), Methionine (Meth), Aspartic acid (Asp), Glutamic acid (Glu), Glutamine (Gln), Asparagine (Asn), Lysine (Lys), Arginine Arg), Phenylalanine (Phen), Threonine (Thr), Histidine ,Citrine (Cit)) thick juice and Molasses were analyzed.

Figure l presents the chromatograms of amino acid profiles in the samples.




c

**Fig. 1.** Chromatogram of molasses sample 1(a), Molasses sample 2(b), molasses sample 3(c)

## Conclusion

According to the results, the amount of asparagine amino acid in the thick juice was relatively high and this can lead to the formation of acrylamide that might be harmful to the health. Moreover, almost all amino acids are present in the samples that indicate the nutritional value of the products.

## References

Abdollahian-Noghabi, M., Mirzaei, M. R., Moharamzadeh, M., Hosainpour, M., Niromand-Jahromi, M., Javaheri, M. A. & Pourghasemi, A. (2007). Determination of some physical and technological characteristics of sugar beet varieties. www.agris.fao.org

Asadi, M. (2006). Beet-sugar handbook. John Wiley & Sons.

Bahrami, M. E., Ansari, K. & Honarvar, M. (2015). CIE color coordinates measurement of imported raw cane sugar samples using spectrophotoradiometer and correlation with their certain quality parameters. *Food Science and Technology*, 13(55), 69-79.

Muttucumaru, N., Powers, S. J., Elmore, J. S., Dodson, A., Briddon, A., Mottram, D. S. & Halford, N. G. (2017). Acrylamide-forming potential of potatoes grown at different locations, and the ratio of free asparagine to reducing sugars at which free asparagine becomes a limiting factor for acrylamide formation. *Food Chemistry*, 220, 76-86.

Passos, C. P., Ferreira, S. S., Serôdio, A., Basil, E., Marková, L., Kukurová, K. & Coimbra, M. A. (2018). Pectic polysaccharides as an acrylamide mitigation strategy– Competition between reducing sugars and sugar acids. Food hydrocolloids, 81, 113-119.

Wojtczak, M. (2003). ICUMSA-International commission for uniform methods of sugar analysis. Gazeta Cukrownicza, 111(06), 191-192.