The Effects of Vacuum Evaporation on Amino Acid Contents in Pureed *Aloe Chinensis Baker* Gel using HPLC

IOP Conf. Series: Materials Science and Engineering **543** (2019) 012014 doi:10.1088/1757-899X/543/1/012014

The Effects of Vacuum Evaporation on Amino Acid Contents in Pureed *Aloe Chinensis Baker* Gel using HPLC

T Y Hendrawati[1*], R A Nugrahani [1], A I Ramadhan [2], S Susanty [1], A Siswahyu[3]

[1]Chemical Engineering Department, Faculty of Engineering, Universitas Muhammadiyah Jakarta, Indonesia
[2]Mechanical Engineering Department, Faculty of Engineering, Universitas Muhammadiyah Jakarta, Indonesia
[3]Chemical Engineering Department, Industrial Technology Faculty, Institut Sains dan Teknologi Al Kamal, Jl. Kedoya Raya Nomor 2, Kebon Jeruk, Jakarta, Indonesia

Email : yuni.hendrawati@ftumj.ac.id

Abstract. *Aloe Chinensis Baker* is an aloe species that is frequently utilized in Asia, including Indonesia. Aloe vera contains various compounds in its leaves, such as vitamins, enzymes, and amino acids. The largest component of Aloe vera leaves is water. Vacuum evaporation is considered to be a potential method in the process to reduce the water content while maintaining the integrity of the nutrients. The objective of this research is to reduce the water content in pureed Aloe vera gel to obtain extracts. Evaporation using a rotary vacuum evaporator is conducted at an pressure of 110 mBar and temperature of 40 ^{o}C, and a varied time of 30, 60, 90, and 120 minutes. Analyses of amino acid contents are performed with HPLC. The results show that at 120 minutes, the preserved amino acid contents include: L-Isoleucine 29.3 ppm, L-Proline 10.8 ppm, L-Arginine 9.0 ppm, L-Asparagine 243 ppm, L-Threonine 56.9 ppm, L-Leucine 30.9 ppm, L-Methionine 38.4 ppm, L-Histidine 15.4 ppm, Aspartic Acid 24.4 ppm, L-Lysine 108.9 ppm, L-Cysteine 131.0 ppm, L-Alanine 40.2 ppm, and Glycine 230.7 ppm. The resulting Aloe vera extract show the following characteristics, density of 0.99 g/ml and pH of 6.5. Yield of 120 minutes evaporation is 40 %.

1. Introduction

Aloe vera is one of the oldest and well known medicinal plants in the world. Aloe vera gel contains important compounds including 19 out of 20 amino acids needed by human bodies, as well as seven out of eight essential amino acids [1]. One of the species of Aloe vera largely cultivated in Indonesia, especially Potianak, West Kalimantan, is *Aloe chinensis Baker* [2]. In cosmetic and pharmaceutical formulations, Aloe vera extract is needed at a lower water but maintained contents of active compounds [3]. A vacuum evaporation process is hoped to preserve those active compounds, especially amino acids. For organic compounds, the commonly used methods of qualitative and quantitative analysis mainly include such methods of mutual coupling as UV, fluorescence, gas chromatography, liquid chromatography, mass spectrometry, etc. [4]. In previous studies, The HPLC method for determining the blood concentration of nifekalant with Ornidazole as internal standard in chinese people plasma was developed and reported [5]. Quantitative analyses of amino acids are performed in this research using HPLC instruments.

Aloe vera is a very commercial species and its leaves are frequently used in cosmetic and pharmaceutical industries. In the food industry, they are used as a functional food source as well as an ingredient in other products, such as gels in in health drinks. In the cosmetic and hygienic industries, aloe vera becomes one of the main materials in cream, lotion, soap, shampoo, facial wash, and other

products [6]. In the pharmaceutical industry, it is used in the productions for topical applications, such as balms and non-prescription gels, and also for pills and capsules [7].

Aloe vera leaves contain various compounds such as vitamins, minerals, enzymes, and amino acids [6,8]. The presence of these compounds makes aloe vera a useful plant in many fields including cosmetics, pharmaceuticals, and foods. The largest component in aloe vera leaves is water [9,10]. Vacuum evaporation is hoped to reduce the water contents while maintaining the amount of useful bioactive compounds. In this research, the effects of time, temperature, and pressure during evaporation of pureed aloe vera gel are analyzed. The objective of this study is to reduce the water content in pureed aloe vera gel and produce aloe vera extract.

Amino acids are the main building blocks of proteins and can be categorized into two groups, ie. essential and non-essential amino acids. Essential amino acids cannot be produced in the body, and thus must be added in foods, whereas non-essential amino acids can be produced in the body [11]. Amino acids are carboxylic acids with amino ($-NH_2$) groups. Amino acids found in proteins possess the $–NH_2$ group on the carbon atom at the α position from the –COOH group. The types of amino acids, their sequence in proteins, and spatial relationships among them determine the three dimensional structures and biological characteristics of simple proteins. Amino acids are organic compounds with functional groups of carboxyl (-COOH) and amines (usually $–NH_2$). The carboxyl group is acidic, while the amine group is basic. Amino acids in proteins are connected with peptide bonds, and thus in a dipeptide, there is one peptide bond [12].

All amino acids found in proteins have similar attributes, the carboxyl and amino groups are on the same carbon. The differences lie in the R-groups, in which they vary in structure, size, electrical charge, and solubility in water. Several amino acids have specific reactions based on their R-groups. Using hydrolitic reactions on proteins, 20 amino acids are obtained and can be categorized based on their R-groups. The first group is non-polar amino acids with hydrophobic R-groups, ie. Alanine, Isoleucine, Leucine, Methionine, Phenylalanine, Proline, Tryptophan and Valine. The second group is polar amino acids with no charge on the R-group, which consists of Asparagine, Cysteine, Glutamine, Lysine, Serine, Threonine, and Tyrosine. The third group contains amino acids with positively charged R-group, and the fourth contains amino acids with negatively charged R-groups. From these 20 amino acids, there are eight essential amino acids, and those are Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, and Valine. These essential amino acids cannot be synthesized in human body, and thus must be obtained from food and other supplements. Leucine also has a function in the immune system [13]. Lysine has a function as a precursor in antibodies, strengthens the circulatory system, maintains normal cell growth, forms collagen in collaboration with proline and vitamin C, and lowers excess triglycerides in blood [14]. Amino acids have higher melting points (greater than 200 ^oC) than carboxylic acids or amines [15].

2. Experimental Method

2.1. Material and Instruments

Aloe chinesis Baker leaves from Pontianak Indonesia, pH meter, Pycnometer, Viscometer, HPLC

2.2. Preparation of sample pureed aloe vera gel

Preparation of Pureed Aloe vera gel:

a. Rind is peeled off aloe vera leaves hygienically with a knife.
b. Aloe vera gel is pureed in a blender.
c. Aloe vera gel is filtered using a filter press.
d. Filtrate from the filtration process is dehydrated with vacuum evaporation with an initial volume of 500 ml.

e. Evaporation with a rotary vacum evaporator is carried out at a pressure of 110 mBar and a temperature of 40 ^{0}C with a variable time of 0, 30, 60, 90 and 120 minutes.

2.3. Analysis of pureed Aloe vera Gel

Analyses are performed on yield, pH, density, viscosity, and results of HPLC on the quantitative amino acid analyses. Amino acids are analyzed with HPLC. The amino acids analyzed are those mainly freed from proteins through hydrolyses using HCl 6 N. Hydrolysates are dissolved in sodium citrate buffer, and each amino acids are separated using HPLC.

3. Results and Discussion

Yield is calculated as the percentage of volume of evaporated water divided by the initial volume from the evaporation process in a vacuum evaporator at 110 mBar and 40 ^{0}C. The resulting yields at varied evaporation time are presented in the following Table 1.

Table 1. Yields at varied evaporation times (at pressure of 110 mBar and temperature of 40 ^{0}C)

Evaporation time (minutes)	Volume of Evaporated (ml)	Yield (%)
0	500	100
30	350	70
60	250	50
90	200	40
120	150	30

In addition to yields, pH, density, and viscosity are also measured. The results from analyses of pH, density, and viscosity are presented in the following Table 2.

Table 2. pH, density, and viscosity at varied evaporation times (at pressure of 110 mBar and temperature of 40 ^{0}C)

Evaporation time (minutes)	pH (Temp. 25 °C)	ρ (g/ml) (Temp. 25 °C)	η (cP)
0	6.5	1.004	129.6
30	6.5	1.005	133.8
60	6.5	1.003	402.3
90	6.5	0.99	10,474
120	6.5	0.99	13,349

Based on the results in Table 2, pH stays the same at 6,5 as time increases. However, density decreases from 1,005 g/ml to 0,99 g/ml, and viscosity increases from 129.6 cP to 13,349 cP as the evaporation time increases.

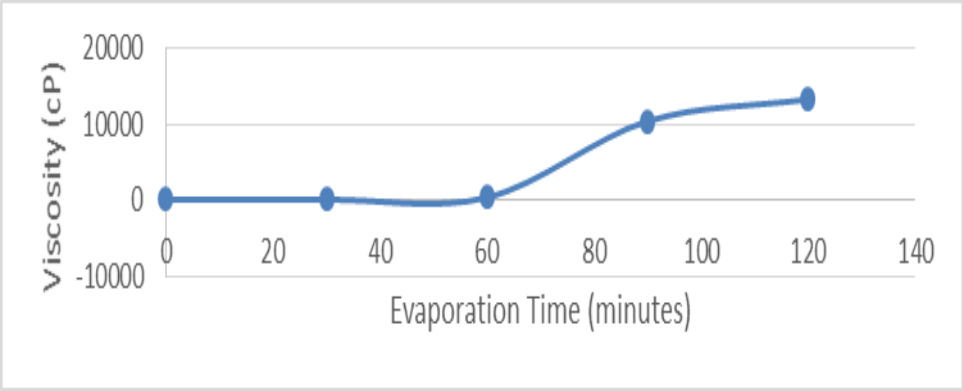

Figure 1. Effects of time of evaporation on Aloe vera gel viscosity in vacuum evaporation process at pressure of 110 mBar and temperature 40 ^{0}C

The analyses of amino acid contents in Aloe vera gel prior to evaporation result in L-Isoleucine 3.72 ppm, L-Valine 6.85 ppm, L-Proline 0.07 ppm, L-Phenylalanine 4.74 ppm, L Arginine 4.81 ppm, L-Threonine 5.68 ppm, L-Leucine 8.53 ppm, L-Serine 6.35 ppm, L-Methionine 1.83 ppm, L-Histidine 5.92 ppm, Aspartic Acid 14.37 ppm, L-Tyrosine 3.34 ppm, L-Lysine 8.27 ppm, Glutamic Acid 14.27 ppm, L-Cysteine 0.02 ppm, L-Alanine 1.09 ppm, and Glycine 7.80 ppm.

The HPLC analysis of amino acid content after 30 minutes of vacuum evaporation results in separation of amino acid components as shown in the chromatogram presented in Figure 2 .

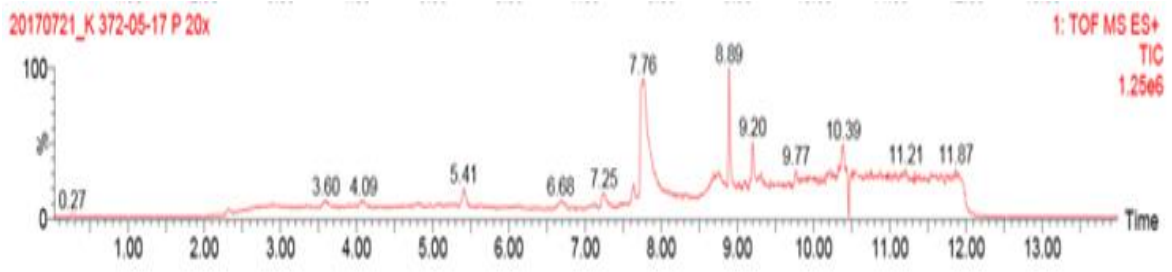

Figure 2. HPLC Chromatogram of Aloe vera gel vacuum evaporated for 30 minutes at pressure of 110 mBar and temperature 40 ^{0}C

In the 30 minutes evaporation. the amino acids that are preserved in the Aloe vera extract are L-Isoleucine 13.4 ppm, L-Valine 455.1 ppm, L-Proline 14.7 ppm, L-Phenylalanine 7.3 ppm, L-Arginine 12.2 ppm, L-Asparagine 73.7 ppm, L-Leucine 118.7 ppm, L-Histidine 21.7 ppm, Aspartic Acid 82.4 ppm, and L-Tyrosine 5.4 ppm. The essential amino acids retained are L-Isoleucine. L-Valine. L-Phenylalanine and L-Leucine.

The HPLC analysis of amino acid content after 60 minutes of vacuum evaporation results in separation of amino acid components as shown in the chromatogram presented in Figure 3.

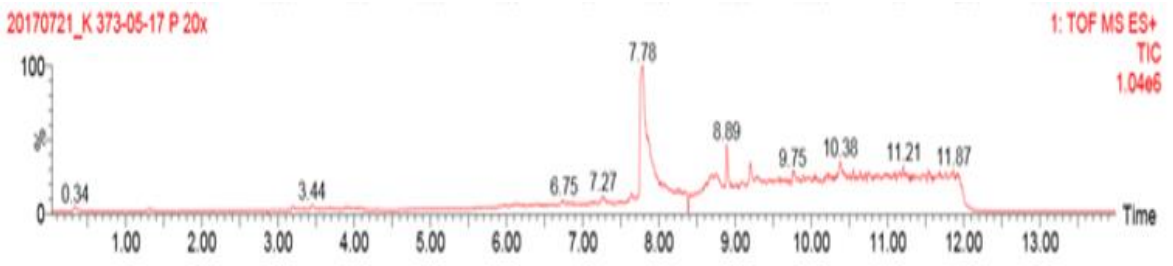

Figure 3. HPLC Chromatogram of Aloe vera gel vacuum evaporated for 60 minutes at pressure of 110 mBar and temperature 40 ^{0}C

In the 60 minutes evaporation. the amino acids that are preserved in the Aloe vera extract are L-Isoleucine 41.2 ppm. L-Valine 16.9 ppm, L-Proline 12.9 ppm, L-Phenyllalanine 3.3 ppm, L-Arginine 10.3 ppm, L -Asparagine 16.8 ppm, L-Threonine 59.7 ppm, L-Leucine 49.6 ppm, L-Histidine 19.3 ppm, and Aspartic Acid 43.9 ppm. The essential amino acids retained are L-Isoleucine. L-Valine. L-Phenylalanine. L-Threonine and L-Leucine.

The HPLC analysis of amino acid content after 90 minutes of vacuum evaporation results in separation of amino acid components as shown in the chromatogram presented in Figure 4.

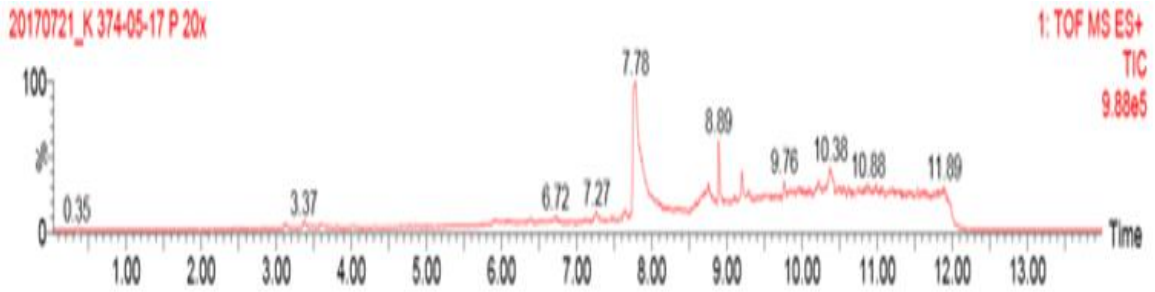

Figure 4. HPLC Chromatogram of Aloe vera gel vacuum evaporated for 90 minutes at pressure of 110 mBar and temperature 40 ^{0}C

In the 90 minutes evaporation. the amino acids that are preserved in the Aloe vera extract are L-Isoleucine 64.3 ppm, L-Proline 12.1 ppm, L-Arginine 10.5 ppm, L-Asparagine 18.5 ppm, L-Threonine 65.1 ppm, L-Leucine 65.8 ppm, L-Histidine 17.5 ppm, Aspartic Acid 39.9 ppm, L-Lysine 112.9 ppm, and L-Cystine 209.9 ppm. The essential amino acids retained are L-Isoleucine. L-Valine. L-Phenylalanine. L-Threonine and L-Leucine and L-Lysine.

The HPLC analysis of amino acid content after 120 minutes of vacuum evaporation results in separation of amino acid components as shown in the chromatogram presented in Figure 5.

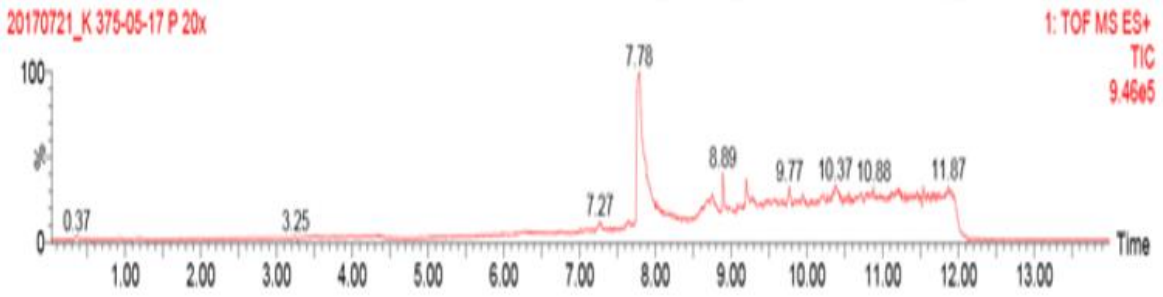

Figure 5. HPLC Chromatogram of Aloe vera gel vacuum evaporated for 120 minutes at pressure of 110 mBar and temperature 40 ^{0}C

In the 120 minutes evaporation, the amino acids that are preserved in the Aloe vera extract are L-Isoleucine 29.3 ppm, L-Proline 10.8 ppm, L-Arginine 9.0 ppm, L-Asparagine 243 ppm, L-Threonine 56.9 ppm, L-Leucine 30.9 ppm, L-Methionine 38.4 ppm, L-Histidine 15.4 ppm, Aspartic Acid 24.4 ppm, L-Lysine 108.9 ppm, L-Cystine 131.0 ppm, L-Alanine 40.2 ppm and Glycine 230.7 ppm. The essential amino acids retained are L-Isoleucine, L-Threonine, L-Leucine, L-Methionine and L-Lysine.

Tabel 3. The results of HPLC analysis on amino acid content of vacuum evaporation of Aloe vera gel at evaporation time 0 minutes until 120 minutes at pressure of 110 mBar and temperature 40 ^{0}C

No	Amino Acid content (ppm)	Evaporation time 0 minutes	Evaporation time 30 minutes	Evaporation time 60 minutes	Evaporation time 90 minutes	Evaporation time 120 minutes
	Essential amino acids					
1	L-Isoleucine	3.72	13.4	41.2	64.3	29.3
2	L-Valine	6.85	455.1	16.9	13.3	0.0
3	L-Phenylalanine	0.07	7.3	3.3	4.7	0.0
4	L-Threonine	5.68	0.0	59.7	65.1	56.9
5	L-Tryptophan	0.0	0.0	0.0	0.0	0.0
6	L-Leucine	8.53	118.7	49.6	65.8	30.9
7	L-Methionine	1.83	0.0	0.0	0.0	38.4
8	L-Lysine	8.27	0.0	0.0	112.9	108.9
	Non Essential amino acid					
9	L-Proline	0.07	14.7	12.9	12.1	10.8
10	L-Arginine	4.81	12.2	10.3	10.5	9.0
11	L-Asparagine	0.00	73.7	16.8	18.5	243.0
12	L-Serine	6.35	0.0	0.0	0.0	0.0
13	L-Glutamine	nil	0.0	0.0	0.0	0.0
14	L-Histidine	5.92	21.7	19.3	17.5	15.4
15	Aspartic acid	14.37	82.4	43.9	39.9	24.4
16	L-Tyrosine	3.24	5.4	0.0	0.0	0.0
17	L-Glutamic acid	14.27	0	0.0	0.0	0.0
18	L-Cysteine	0.02	0	0.0	209.9	131.0
19	L-Alanine	1.09	0	0.0	0.0	40.2
20	Glycine	7.80	0	0.0	0.0	230.7

Polar amino acids include serine, threonine, asparagine, glutamine, histidine and tyrosine. The hydrophobic amino acids include alanine, valine, leucine, isoleucine, proline, phenylalanine,

tryptophane, cysteine and methionine. In this research, the content remain in Aloe vera gel extract was essential amino acid. The essential amino acid content was changed because the vacuum evaporation conducted at pressure of 110 mBar and temperature 40 ^{0}C showed that the amino acid content increased and then decreased along the evaporation time. This is due to the polar essential amino acid content dissolved in water was evaporated. This can be seen in Figure 6. Hence the effect of evaporation time on hydrophobic essential amino acid content in Aloe vera gel can be seen at Figure 7.

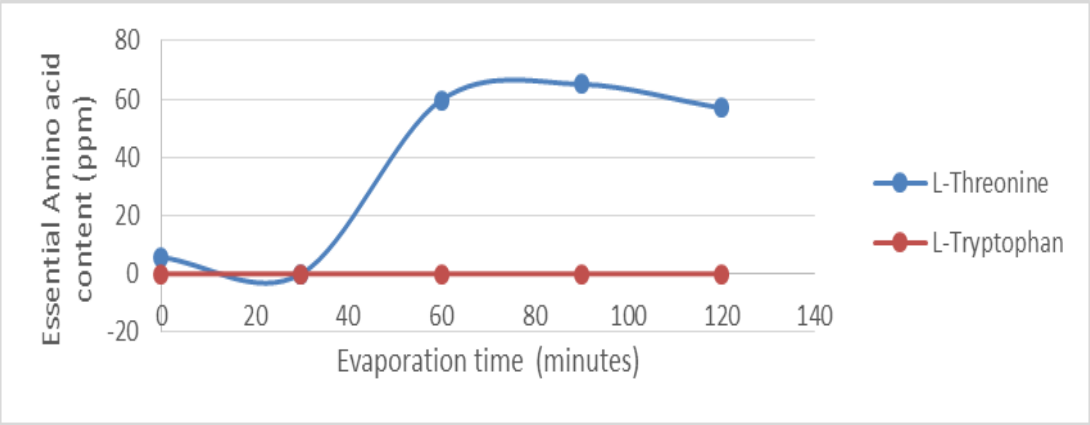

Figure 6. The effect of evaporation time on polar essential amino acid content in Aloe vera gel extract

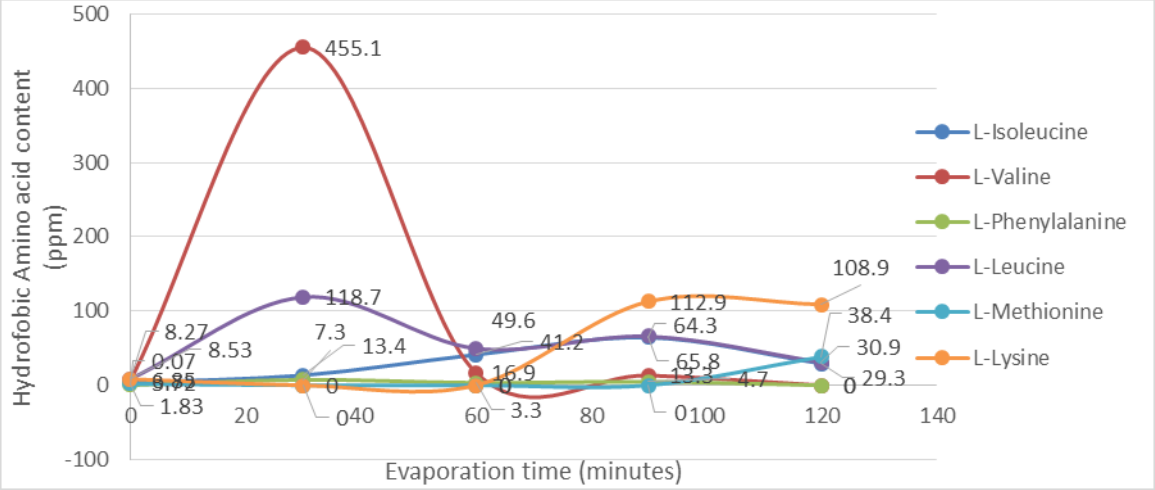

Figure 7. The effect of evaporation time on hydrophobic essential amino acid content in Aloe vera gel extract

4. Conclusion

The processes of vacuum evaporation at the pressure of 110 mBar temperature of 40 ºC and variable time of 30, 60, 90 and 120 minutes can maintain the presence of amino acids in the Aloe vera gel extract. Increasing time produces constant pH at 6.5, decreasing densities from 1.005 g/ml to 0.99 g/ml and increasing viscosities from 129.6 cP to 13,349 cP. After 120 minutes of evaporation, the amino acids that are preserved in the Aloe vera gel extract are L-Isoleucine 29.3 ppm. L-Proline 10.8 ppm. L-Arginine 9.0 ppm. L-Asparagine 243 ppm. L-Threonine 56.9 ppm. L-Leucine 30.9 ppm. L-Methionine

38.4 ppm. L-Histidine 15.4 ppm. Aspartic Acid 24.4 ppm. L-Lysine 108.9 ppm. L-Cystine 131.0 ppm. L-Alanine 40.2 ppm. and Glycine 230.7 ppm. The essential amino acids retained are L-Isoleucine. L-Threonine. L-Leucine. L-Methionine and L-Lysine.

Acknowledgment

Thanks to Universitas Muhammadiyah Jakarta and Directorate of Research and Community Service, Directorate General for Research and Development, Ministry of Research and Technology Higher Education on the Research Grant PTUPT in 2017-2019 contract number 03/E/KPT/2018, 03/01/2018.

References

[1] Rajeswari R, Umadevi M, Sharmila R C, Pushpa R, Selvavenkadesh S, Sampath K P and Debjit B 2012 *J. Pharmaco. Phytochem.***1** 118 – 24.
[2] Hendrawati T Y 2015 *J. Eng. Sci. Tech*. **2015** 47-59
[3] Boudreau M D and Beland F A 2006 *J. Environ. Sci. Health Part C: Environ. Carcinog. Ecotoxicol. Rev.* **24** 103-54.
[4] Qiong H 2017 *Chem. Eng. Trans. 62* 439 – 44.
[5] Xin X, Changbin L and Yali W 2015 *Chem. Eng. Trans.* **46** 1375-80.
[6] Hamman J H 2008 *Molecules* **13** 1599-16.
[7] Eshun K and He Q 2004 *Food Sci. Nutr.* **44** 91-96.
[8] He Q, Changhong L, Kojo E and Tian Z 2005 *Food Control* **16** 95-104.
[9] Grindlay D and Reynolds T 1986 *J. Ethnopharmacol.* **16** 117-51.
[10] Josias H and Hamman 2008 *Molecules* **13** 1599-16.
[11] Sitompul S 2004 *Buletin Teknik Pertanian* **9** 33-37.
[12] Hans D J and Nobert S 2008 *Peptides from A-Z A Concise Encyclopedia* Wiley VCH Verlag GmbH & Co. KGaA.
[13] Edison T 2009 *Amino acid: Esensial for our bodies*. http://livewellnaturally.com, Accessed 27.07.2018.
[14] Harli M 2008 *Asam amino esensial* http://www.suparmas.com. Accessed 27.07.2018.
[15] United States Patent and Trademark Office, 1989, Official Gazette of the United States Patent and Trademark Office: Patents, Volume 1108, 3-4, U.S. Department of Commerce, Patent and Trademark Office, University of California.