



An Analysis of Essential Amino Acid Contents of Canistel Fruit (*Pouteria campechiana*) as A Source of Vegetable Protein in An Effort of Food Diversification

A Ifriany Harun*, Risya Sasri, Nyemas Firda Diannisa, Yunita Puspasari

Tanjungpura University Chemistry Education Study Program, Pontianak, Indonesia

* Corresponding Author e-mail: andi.ifriani@fkip.untan.ac.id

Article History

Received: 10-01-2024

Revised: 29-01-2024

Published: 29-02-2024

Keywords: canistel fruit, amino acid, food diversification

Abstract

Canistel fruit is a fruit that is not cultivated and is not known to many people because of limited information about this fruit. In the field of biochemistry, analysis of the essential amino acids contained in canistel fruit has never been carried out. The aim of this research is to determine the type and levels of essential amino acids in Canistel fruit as a source of vegetable protein in an effort to diversify food. The sample used was Canistel fruit. The method used was chromatography using UPLC with a C18 column, PDA detector, gradient pump system, and column temperature of 49°C. 15 types of amino acids were obtained, consisting of 7 essential amino acids and 8 non-essential amino acids. Essential amino acids are L-Fenilalanin (994,64 mg/kg), L-Isoleusin (704,58 mg/kg), L-Valin (876,69 mg/kg), L-Lisin 1.068,36 mg/kg), L-Leusin (1.199,45 mg/kg), L-Treonin (857,16 mg/kg), and LHistidin (421,03 mg/kg) and 8 non-essential amino acid are L-Serin (957,38 mg/kg), L-Asam glutamat (2.137,7 mg/kg), L-Alanin (1.070,01 mg/kg), LArginin (838,15 mg/kg), L-Glisin (1.101,56), L-asam aspartate (1.900,63 mg/kg), L-Tirosin (141,90 mg/kg), and L-Prolin (1.146,84 mg/kg).

How to Cite: Harun, A., Sasri, R., Diannisa, N., & Puspasari, Y. (2024). An Analysis of Essential Amino Acid Contents of Canistel Fruit (*Pouteria campechiana*) as A Source of Vegetable Protein in An Effort of Food Diversification. *Hydrogen: Jurnal Kependidikan Kimia*, 12(1), 35-43. doi:<https://doi.org/10.33394/hjkk.v12i1.10491>



<https://doi.org/10.33394/hjkk.v12i1.10491>

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INTRODUCTION

Protein is one of the main nutrients, apart from carbohydrates and lipids, which is very necessary for humans. Protein is a group of macronutrients (nutrients needed in large quantities), and has a more important role in the formation of biomolecules than energy sources (building up the body's structure) (Rismayanthi, 2015). Protein is useful for cell development and differentiation which is important for growth and increasing body immunity. Protein is used as an energy source if carbohydrates and fat in the body are reduced (Azhar, 2016). Protein also regulates metabolic processes in the form of enzymes and hormones to protect the body from toxic or dangerous substances and maintain cells and body tissues (Anissa & Dewi, 2021).

Based on the source, protein can come from vegetable and animal sources. Protein from animal sources is a complete protein containing all types of essential amino acids in appropriate amounts for growth (Damayanti, 2017). Sources of animal protein are still very diverse, coming from eggs, meat, various types of fish, milk and dairy products, while vegetable protein can be found in various sources, including vegetables, fruit and nuts. In particular, soybeans dominate as one of the main choices for obtaining vegetable protein.

Food diversification is an effort that is closely related to improving the quality of human resources (Gifary et al., 2022). One of the efforts is to diversify food sources of protein other than the protein currently consumed. The goal is to meet protein needs from various protein

sources, especially vegetable protein. The Ministry of Agriculture directs the food diversification program towards diversifying food sources of protein by increasing protein consumption among people who are considered to be still low. Diversification of vegetable protein in Indonesia is very important, because Indonesia is still very dependent on soybean imports from various countries. According to CNBC Indonesia data, soybean imports as of January 2023 amounted to 224.33 million kg.

Amino acids are precursors of protein, consisting of essential and non-essential amino acids. Because the body cannot produce essential amino acids, essential amino acids must be met from the food consumed daily. Unlike carbohydrates and fats, our bodies cannot store amino acids, including essential ones. There are 10 types of essential amino acids, namely histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, arginine and valine (Poedjiadi, 1994). A food ingredient is said to have high protein nutritional value if it contains a complete range of essential amino acids at high levels. So not only in terms of the high amount of protein, but especially in terms of the essential amino acids that make up the protein.

Canistel fruit (*Pouteria campechiana*) belongs to the sapotaceae family (Azurdia, 2006; Silva et al., 2009) which originates from Central America and is distributed in tropical and sub-tropical areas (Mehraj et al., 2015). Other names for this fruit are sapodilla, alkesah, kanistel, and campoleh (campolay). The Canistel tree has a height of 12-20m with a trunk diameter of 25-60cm (Alia Tejacal et al., 2007). Sapodilla is a seasonal fruit that bears fruit around August – October. Ripe fruit has a strong, sweet aroma, yellow color and soft flesh. This fruit is rarely found in the market due to lack of public interest (Puspita et al., 2019).

In Indonesia, canistel fruit is not cultivated, so this fruit is rarely found anymore. Not many people know about this fruit because information about this fruit is also very limited. canistel fruit has quite a lot of benefits, including maintaining eye health, as a source of antioxidants, can reduce the risk of hypertension, reduce the risk of diabetes, maintain healthy joints and cartilage and several other benefits.

Apart from its benefits in the health sector, this fruit is also often combined to produce various kinds of dishes, because it tastes sweet and has flesh that has a dry, rough, soft texture, like it contains flour, so this canistel fruit can be made into various types of food, such as it can be mixed with complementary ingredients and food flavor enhancers, namely salt, pepper, orange juice or mayonnaise before consumption. Sometimes the flesh of the fruit is mashed and made into a milkshake by mixing it with ice cream or milk. This fruit can also be used as an alternative to pumpkin, it can be made into pudding, pancakes, pies and jam (Costa et al., 2010). Another use of canistel fruit in food products is combining it with dragon fruit for quick bread products such as madeleines, pancakes and scones (Gusnadi & Suryawardani, 2022). But this fruit can also be consumed directly or roasted first (Aseervatham G. et al., 2019).

Apart from being a food ingredient, canistel fruit also produces natural antioxidants. The contents of canistel fruit are phenolics, flavonoids, ascorbic acid, thiamine, riboflavin and niacin as secondary metabolites (Mehraj et al., 2015). Fruit and fruit flesh contain the highest antioxidants (Kong et al., 2013). Apart from that, this fruit contains carotenoids and is very good for making butter (Muliawati et al., 2002). Antioxidants in the form of vitamin C and β -carotene can be found in canistel fruit (Kubola et al., 2011). Apart from that, canistel fruit also contains polyphenolic antioxidants such as gallic acid, gallocatechin, catechin, epicatechin, dihydromyricetin, and myricitrin (Aly et al., 2016).

Because the Canistel fruit has begun to be used to make food, but in the field of biochemistry, no one has researched whether this fruit contains essential amino acids so that it can be used as a source of vegetable protein, encouraging researchers to conduct research with the aim of

determining the type and levels of amino acids. essential in canistel fruit as a source of vegetable protein in efforts to diversify food.

METHOD

This research uses the SIG (Saraswanti Indogenetech) amino acid analysis procedure method. This research uses several tools, namely a set of UPLC tools, C18 Column, PDA Detector, gradient pump system, column temperature 49°C, analytical balance, 0.2 µm syringe filter, dropper pipette, 5 ml volume pipette, glass funnel, spray bottle, flask measuring 100 mL, tongs, static pole, clamp, stirring rod, spatula, vernier caliper, measuring cup, stirring rod, beaker, porcelain cup and 125 ml Erlenmeyer.

The materials used include: canistel fruit, HNO₃, H₂SO₄, HCl, Wattman paper (0.2 µm), Na₂SO₄, aquabides, Accq eluent, 15 types of standard amino acids, consisting of 7 essential amino acids (L-Phenylalanine, L-isoleucine, L-Valine, L-Lysine, L-Leucine, L-Threonine and L-Histidine), and 8 types of non-essential amino acids (L-Serine, L-glutamic acid, L-Alanine, Glycine, L- Aspartic Acid, L-Tyrosine, L-Proline, L-Arginine,).

Simplisa canistel fruit is made by cleaning and separating canistel fruit from the skin and seeds. The fruit flesh is then finely sliced and dried. The drying process is carried out in a place that is not exposed to direct sunlight for approximately 7 days or until the fruit flesh is completely dry (water content less than 10%). The method for determining essential amino acid levels in canistel fruit is as follows:

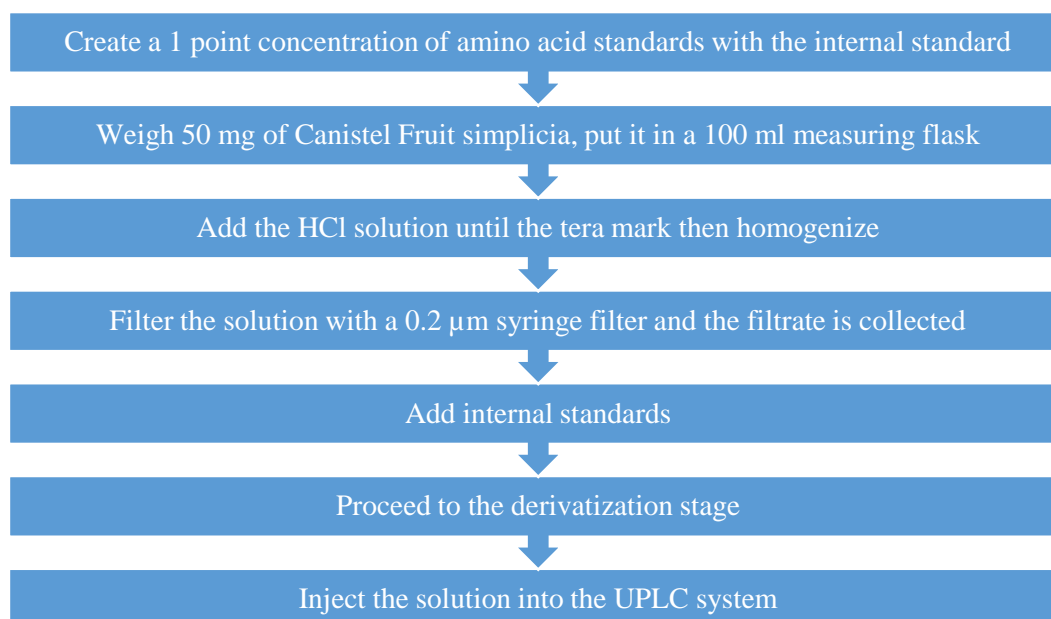


Figure 1. Determination of essential amino acid levels

RESULTS AND DISCUSSION

Canistel fruit is a fruit characterized by a yellow to orange color, sweet and fresh taste with a distinctive aroma. This fruit is a seasonal fruit and until now, it is not cultivated, so it can only be found at certain times. This fruit also has the property of being easily damaged, so to maintain the quality of the fruit it is best stored at 15°C in a plastic container (Muhammad et al., 2021). This fruit sample was obtained from Cianjur district, West Java. The selection of

fruit used for research was fruit with a medium level of ripeness, characterized by bright yellow skin color and sufficient fruit hardness (not too hard and not too soft).

Testing for the types and levels of amino acids carried out on canistel fruit included 15 types of amino acids, 7 of which were essential amino acids and 8 were non-essential amino acids, while the 2 essential amino acids that were not tested were methionine and tryptophan. This is due to difficulties in obtaining standard amino acids. The 15 amino acids tested in Dayak onion samples can be seen in the following table:

Table 1. Types of amino acids tested in canistel fruit by UPLC

No.	Name of Amino Acid Tested	Types of Amino Acids
1.	L- Fenilalanin	Esensia
2.	L - Isoleusin	Esensial
3.	L-Valin	Esensial
4.	L-Lisin	Esensial
5.	L-Leusin	Esensial
6.	L-Threonin	Esensial
7.	L-Histidin	Esensial
8.	L-Serin	Non Esensial
9.	L-Asam Glutamat	Non Esensial
10.	L-Alanin	Non Esensial
11.	L-Arginin	Non Esensial
12.	Glisin	Non Esensial
13.	L-Asam Aspartat	Non Esensial
14.	L-tirosin	Non Esensial
15.	L-Prolin	Non Esensial

The types of amino acids contained in canistel fruit were obtained by comparing the retention time (RT) between standard amino acids and the RT from the results of separating canistel fruit. The following is a chromatogram of the retention time of canistel fruit carried out in duplicate with an injection volume of 1.00 µl and a run time of 15.0 minutes.

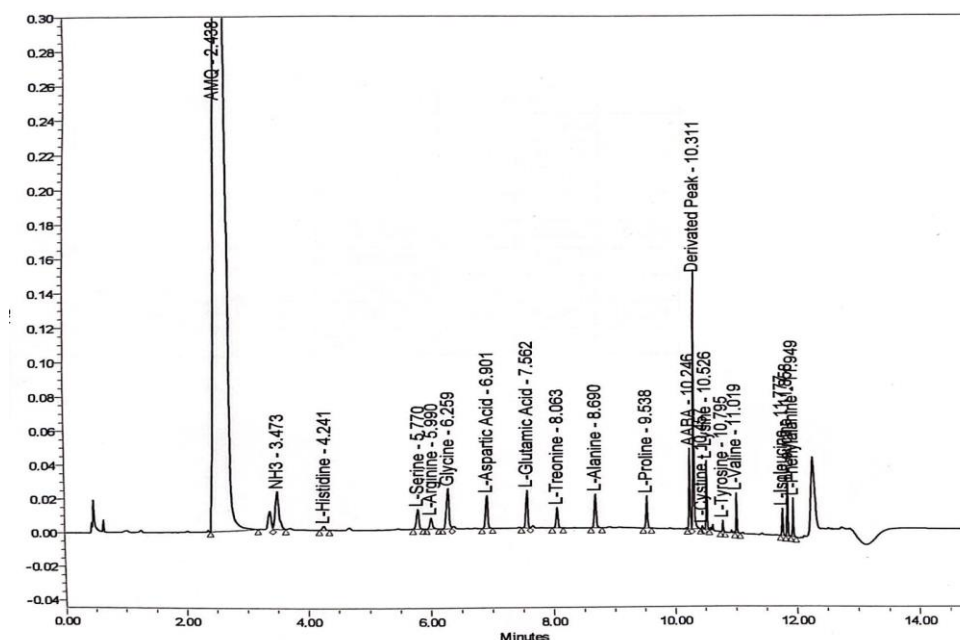


Figure 2. Chromatogram of retention times for 15 amino acids in canistel fruit

With UPLC and the same injection volume, measurement of the retention times of standard amino acids gives the following chromatogram (Figure 3).

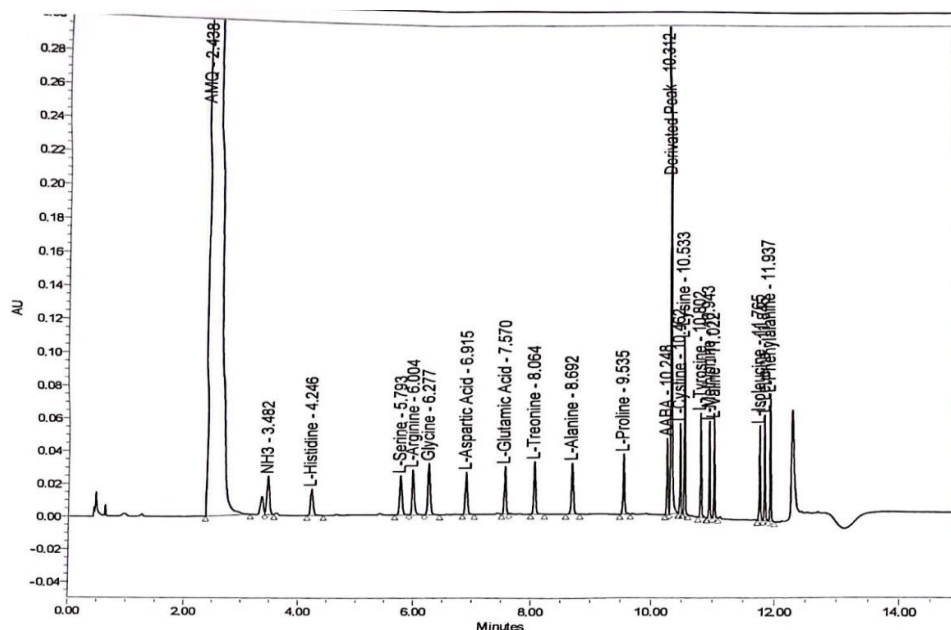


Figure 3. Retention time chromatogram of 15 standard amino acids

Determining the types of amino acids contained in canistel fruit was carried out by comparing the average retention time of the chromatogram results of canistel fruit with the retention time of standard amino acids. The results can be seen in table 2 as follows:

Table 2. Comparison of retention times for 15 types of standard amino acids with canistel fruit amino acids

No.	Amino Acid	Type AA	RT Canistel fruit	RT AA Standard
1.	L- Fenilalanin	Esensial	11,949	11,937
2.	L - Isoleusin	Esensial	11,777	11,765
3.	L-Valin	Esensial	11,019	11,022
4.	L-Lisin	Esensial	10,526	10,533
5.	L-Leusin	Esensial	11,858	11,845
6.	L-Threonin	Esensial	8,063	8,064
7.	L-Histidin	Esensial	4,241	4,246
8.	L-Serin	Non Esensial	5,770	5,793
9.	L-As.Glutamat	Non Esensial	7,562	7,570
10.	L-Alanin	Non Esensial	8,690	8,692
11.	L-Arginin	Non Esensial	5,990	6,004
12.	Glisin	Non Esensial	6,259	6,277
13.	L-As. Aspartat	Non Esensial	6,901	6,915
14.	L-tirosin	Non Esensial	10,795	10,802
15.	L-Prolin	Non Esensial	9,538	9,535

Based on the results in table 2, it can be seen that in canistel fruit there are 7 types of essential amino acids and 8 types of non-essential amino acids. Essential amino acids are a type of amino acid that cannot be produced by the body, so it is necessary to consume foods that contain essential amino acids to meet these needs. Lack of essential amino acid intake can affect the body's growth and metabolism (Nurbaiti et al., 2023)

Determination of the concentration of amino acids in canistel fruit was carried out using data on the area under the curve and the amino acids contained were calculated using the formula:

$$[AA] (kg/mg : mg/L) = \frac{\left(\frac{\text{sample ratio}}{\text{std ratio}}\right) \times \left(\frac{[\text{std injection}]}{10^6}\right) \times BM \times FP \times VA}{\text{BPU or sample vol}}$$

While, [AA] = amino acid concentration; Std = Standard; BM = Molecular Weight of amino acids; FP = Dilution Factor; VA = final volume of sample; BPU = test portion weight (g); and Vol = Volume. Meanwhile, the percentage of amino acid content is obtained using the formula:

$$AA \text{ levels } (\%) = \frac{\text{amino acid levels (mg/kg)}}{10000}$$

Based on the area under the curve, the levels of 15 amino acids found in canistel fruit are:

Table 3. Amino acid levels contained in canistel fruit

No.	Amino acid	AA type	Units	AA level		Mean AA Levels
				Simplo	Duplo	
1.	L- Fenilalanin	Esensial	mg/kg	993,43	995,84	994,64
2.	L - Isoleusin	Esensial	mg/kg	704,23	704,92	704,58
3.	L-Valin	Esensial	mg/kg	875,97	877,41	876,69
4.	L-Lisin	Esensial	mg/kg	1067,99	1068,73	1068,36
5.	L-Leusin	Esensial	mg/kg	1198,48	1200,41	1199,45
6.	L-Threonin	Esensial	mg/kg	856,52	857,79	857,16
7.	L-Histidin	Esensial	mg/kg	420,11	421,95	421,03
8.	L-Serin	Non Esensial	mg/kg	957,69	957,07	957,38
9.	L-As.Glutamat	Non Esensial	mg/kg	2138,19	2137,21	2137,7
10.	L-Alanin	Non Esensial	mg/kg	1070,30	1069,72	1070,01
11.	L-Arginin	Non Esensial	mg/kg	836,67	839,63	838,15
12.	Glisin	Non Esensial	mg/kg	1101,77	1101,34	1101,56
13.	L-As. Aspartat	Non Esensial	mg/kg	1900,95	1900,30	1900,63
14.	L-tirosin	Non Esensial	mg/kg	353,83	355,65	141,90
15.	L-Prolin	Non Esensial	mg/kg	1147,15	1146,53	1146,84

From table 3, you can see the content of 7 types of essential amino acids in canistel fruit. The essential amino acid with the highest concentration in canistel fruit is L-leucine (1,195.45 mg/kg). L-Leucine has a role in regulating blood sugar levels, growth and repair of muscles and bones. L-Leucine has been proven to prevent muscle damage (Khofifah & Lestari, 2022).

Leucine also plays an important role in the wound healing process and growth hormone production. The essential amino acid that is least found in canistel fruit is L-Histidine (401.23 mg/kg). The function of histidine is to support growth, blood cell formation, tissue repair, and protect nerve cells (Thalacker-Mercer, 2020). Histidine also participates in the formation of histamine which is involved in the immune system (Solichah, 2022).

Table 4. Shows daily essential amino acid requirements for men and women.

AA Essential	Minimum Requirements (grams/day)	
	Men	Women
Isoleusin	0,70	0,45
Leusin	1,10	0,62
Lisin	0,80	0,50
Fenilalanin	1,10	0,22
Metionin	1,10	0,29
Treonin	0,50	0,31
Tryptophan	0,25	0,61
Valin	0,8	0,65

(Nuraini, 1991)

Essential amino acid requirements in healthy individuals are generally used as a reference for assessing the quality of protein sources used in medical nutrition. A chemical score can be calculated for each amino acid by comparing the protein's amino acid profile with the recommended amino acid profile (Liu et al., 2019; Ochoa Gautier et al., 2017; Wolfe et al.,

2018). From table 4, it can be seen that the essential amino acid content in canistel fruit meets the minimum daily requirements for men and women for 1 kg of canistel fruit. In order to add flavor when consumed, Dutch sapodilla fruit can be processed into several foods, for example pies, pudding, and yogurt (Adyas et al., 2022; Rejeki Retna Pertiwi et al., 2022; Vasco A.H. Goeltom et al., 2022).

CONCLUSION

So far, canistel fruit has only been studied for its use but has never been studied as an alternative food source of protein. Canistel fruit contains 7 types of essential amino acids, namely L-Phenylalanine, L- Isoleucine, L-Valine, L-Lysine, L- Leucine, L-Threonine, and L- Histidine, while L-methionine and L-Tryptophan were not tested. because of the difficulty in obtaining standards for these amino acids.

The levels of each essential amino acid are L-Phenylalanine (994.64 mg/kg), L-Isoleucine (704.58 mg/kg), L-Valine (876.69 mg/kg), L-Lysine 1,068, 36 mg/kg), L- Leucine (1,199.45 mg/kg), L-Threonine (857.16 mg/kg), and L- Histidine (421.03 mg/kg).

Apart from essential amino acids, canistel fruit also contains non-essential amino acids, namely L-Serine (957.38 mg/kg), L-Glutamic acid (2,137.7 mg/kg), L-Alanine (1,070.01 mg /kg), L-Arginine (838.15 mg/kg), L-Glycine (1,101.56), L-aspartic acid (1,900.63 mg/kg), L-Tyrosine (141.90 mg/kg), and L-Proline (1,146.84 mg/kg).

RECOMMENDATIONS

After knowing the essential amino acid content in canistel fruit, research should be carried out on the use of alkesa fruit in the form of food that can be consumed by the public as a source of vegetable protein in an effort to diversify food.

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