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Abstract. Nagara bean planting is very dependent on a wetland environment, where the land is only dry for a few months. Conditions like this affect the sustainability of the existence of nagara beans. The aim of this research is to analyze the feasibility of sustaining the existence of nagara beans based on the potential protein present in various forms. The protein content of whole nagara beans was 14.22%, when roasted it was 18.42%, when peeled and dried into flour it was 24.16%, when the fat is removed it was 22.54%, concentrate flour was 17.58%, the protein isolate was 61.31%, when fermented it becomes tempeh at 9.58%, tempeh defatted flour was 26.09%, tempeh flour concentrate was 21.28% and tempeh flour protein isolate was 38.4%, sprout flour on a small scale was 31.06%, and sprout flour on a big scale becomes 19.83%. The amino acid composition that was often found in nagara beans was glutamic acid, aspartic acid, lysine, phenylalanine, threonine and leucine. Nagara bean protein is mostly in the form of globulin and albumin. Based on its protein potential, nagara beans are feasible to maintain their continued existence.

1. Introduction

Nagara beans are a local superior bean from South Kalimantan which grow in the swampy area of lebak and belongs to the cowpea group. North Daha and South Daha subdistrict, Hulu Sungai Selatan district are producers of nagara beans. Nagara bean planting is very dependent on a wetland environment, where the land is only dry for a few months. Conditions like this affect the sustainability of the existence of nagara beans.

The planting season in the villages of North Daha and South Daha is only carried out during the dry season, whereas during the rainy season the soil is flooded and cannot be planted. This condition causes the nagara bean to not grow rapidly, because planting is limited and their utilization is also lacking. Nagara bean is usually used by traditional communities as an additional part of a substitute for vegetables in curry dishes. Apart from that, nagara beans can also be used in the produce of kuku peanuts or fried peanuts which come from nagara beans which have had their skin removed. Nagara bean is also processed into magali cakes, which are cakes made from nagara beans which have been removed from the skin, mashed, and mixed with brown sugar.

There are several cowpea cultivars found in South Kalimantan. There are four cultivars of cowpea germplasm, namely paddy, plank, yellow and arabic. Paddy cultivars have longer pod, small seeds and yellowish white with dark brown hilum. Plank cultivars have large pods, large yellowish-white or greenish seeds with a large dark brown triangular hilum. Yellow cultivars have large pods, when young the pods are green and yellowish white when ripe, the seeds are yellowish white with a dark brown

hilum. Arabic cultivars have large pods, with relatively large seed sizes, the seeds are slightly yellowish white with a black hilum.

The yellow cowpea cultivar that grows in the North Daha and South Daha areas became the national superior cowpea under the name of nagara bean or cowpea nagara cultivar in 1994. This yellow cowpea is called *Vigna unguiculata* ssp Cylindrica or nagara bean. The cowpea plants can generally be found in several places in the world, such as California - United States, most countries in sub-Saharan Africa, and several other Asia countries. As also written by [1].

Nagara bean is also different from similar cowpeas, namely white beans which are commonly found in Java. Nagara beans have physical characteristics of small size, white bones and wrinkled surface. If the cowpeas are white, the skin is smooth and not wrinkled, and the size tends to be larger, then these cowpeas come from Java and not nagara beans.

Nagara bean is a nut for which the protein, carbohydrates, and micronutrients such as vitamins and minerals can be utilized [1] [2] [3]. Based on [4], world production of cowpea in 2021 will be 8.9 million tons. Most of the cowpea producers are in Africa (95.3%) and Asia, only 2.9%, one of them is nagara beans. One that has the potential to be developed from nagara beans is protein which is contained in various forms.

The availability of nagara beans as a source of protein means that nagara beans can provide the protein needs that mothers and children really need, especially in preventing stunting, malnutrition and child death at birth. This is in accordance with goal 3 of the United Nation SDGs program. Apart from that, the availability of nagara bean protein must continue. Therefor there needs to be a balance between consumption and production (goal 12 SDGs program) [5]. So far, the obstacle to sustainability is the planting of nagara beans which is very dependent on the wetland environment. In fact, people in the area and those around it need nagara beans as a source of protein. Moreover, at this time, nagara bean production is decreasing. Therefore, it is necessary to conduct research on the potential of nagara bean protein to strengthen the existence of nagara beans. The aim of this research is to analyze the feasibility of sustaining the existence of nagara beans based on the potential protein present in various forms

2. Materials and methods

The materials used were the nagara bean (*Vigna unguiculata* ssp Cylindrica) originating from North Daha or South Daha subdistricts, Hulu Sungai Selatan district, South Kalimantan province and several chemicals for analysis originating from Merck.

2.1. Skinless nagara bean flour

Before soaking in an alkaline solution for 3 hours, the dirt and damaged parts of the nagara beans are removed and washed with water [6]. The nagara bean skins are peeled after the soaking process and dried until dry using an oven at 50^{o}C. Dried beans are ground and sifted through a 60mesh sieve.

2.2. Roasted nagara bean flour

As stated by [6], nagara beans are sorted and washed with water for cleaning. After that, 1 kg of nagara beans are put into a frying pan and roasted while stirring for 50 minutes. The roasted beans are ground and sifted through a 60mesh sieve.

2.3. Nagara bean tempeh

As stated by [6] that the processing of nagara bean tempeh is done by soaking the nagara beans for 1 night. Nagara beans are washed with running water. Nagara beans are ground to separate the shell from the nut and the flesh of the nut as well as. The nut skins are separated and washed with water, and then boiled for 1 hour. Nuts without skin are drained and added with commercial tempeh yeast as much as 1 g for every 100 g of beans. The beans are stirred and put in plastic, and then fermented for 1 night at room temperature until nagara beans become tempeh.

2.4. Nagara bean tempeh flour

Nagara bean tempeh cut into pieces and dried using an oven dryer at 50°C until dry with a moisture content of 10-12%. The dried nagara bean tempeh is then mashed and sieved through a 60mesh sieve.

2.5. Defatted flour

The part of fat nagara bean flour and nagara bean tempeh flour were removed by a percolation method using hexane solvent. The mixture of nagara bean flour and hexane was then shaken at 180 rpm for 1 hour at room temperature. The mixture is filtered using filter paper. The filter results were then dried in an oven at 50°C until dry with a moisture content between 10 – 12%.

2.6. Protein concentrate

Nagara bean defatted flour and nagara bean tempeh defatted flour were then made into protein concentrate by dissolving 60 g of nagara bean defatted flour and nagara bean tempeh defatted in 300 ml of 80% ethanol. This mixture was stirred at room temperature for 30 minutes. Then the mixture is filtered. The filter results were then added with 300 ml of distilled water and adjusted to a pH of 4.5 using 2N HCl solution. This mixture was then centrifuged at 2000 rpm for 15 minutes. The centrifugal results are then separated between the precipitate and the supernatant. The supernatant was discarded and the precipitate was added with 300 ml of distilled water and neutralized to a pH of 6.5 – 7 with 2N NaOH solution. This mixture was then centrifuged again at 2000 rpm for 15 minutes. The precipitate was collected and the supernatant was discarded. This precipitate is then dried in the oven until dry at 50°C until dry with a moisture content between 10 – 12%. The dried precipitate is called nagara bean protein concentrate or nagara bean tempeh protein concentrate.

2.7. Protein isolate

Protein isolate can be prepared by adding 200 g of nagara bean defatted flour or nagara bean tempeh defatted flour by adding 1 liter of distilled water (1: 5) and adjusting the pH to 8.5 – 8.7 using 2 N NaOH solution. Then extracted at 60°C for 30 minutes using a water bath shaker. After extraction, this mixture was then centrifuged at 2000 rpm for 15 minutes. The supernatant was collected which is called supernatant 1. The precipitate was then added with 400 ml of distilled water and centrifuged again at 2000 rpm for 15 minutes. The supernatant is collected which is called supernatant 2. The precipitate is discarded. Supernatants 1 and 2 were mixed and then the pH was adjusted to 4.5 with 2N HCl solution. The solution was centrifuged at 2000 rpm for 15 minutes. The supernatant was discarded and the precipitate was added with 200 ml of distilled water and neutralized to pH 7 with 2N NaOH solution. This mixture was then centrifuged again at 2000 rpm for 15 minutes. The supernatant was discarded and the precipitate was dried in an oven at 50°C until dry with a moisture content between 10 – 12%. The dried precipitate is called nagara bean protein isolate or nagara bean tempeh protein isolate.

2.8. Small scale nagara bean sprout flour

The nagara beans are first sorted from defects and foreign objects. The nagara beans used are still mostly empty and light and split, and there is still a lot of dirt in the form of leftover pods, twigs and stones. These dirt materials will interfere during the germination process. Nagara beans are then washed with water and soaked in water with a ratio between nagara beans and water is 1: 3. 500 grams of nagara beans are used. Soaking was carried out for 12 hours. After soaking, the soaking water is removed and drained. Furthermore, the nagara beans are wrapped in banana leaves, so that the nagara beans are still moist during the germination process. The germination process was carried out for 48 hours. After germination is complete, the nagara bean sprouts are cleaned of the skin and dried at 50°C. After drying, the sprouts were crushed to 80 mesh.

2.9. Scale up nagara bean sprouts flour

The nagara beans are first sorted from the defects and dirt of the nagara beans. Then the nagara beans are washed and soaked with the ratio between the nagara beans and water is 1: 3. Soaking time and

germination on scale up are 12 hours and 48 hours. In this scale up, the amount of nagara beans used is 3 kg or 6 times more than the small scale. Nagara beans that have been soaked and drained are then put in a plastic basket with small holes and covered with black plastic. During the germination process, the nagara beans are sprinkled periodically with a little water. After 48 hours of germination, the skin of the nagara bean sprouts was removed and dried at 60 – 70°C. The dried sprouts were then crushed to 80 mesh.

2.10. Protein content analysis

The Kjeldahl micro method was used to determine the crude protein content contained in various forms of nagara beans [7].

2.11. Amino acid composition analysis

The sample containing 3 mg of protein was put into an ampoule and added with 1 ml of 6 N HCl. The sample mixture was frozen using dry ice-acetone. The air in the sample that has been frozen is completely removed. If there are still air bubbles, then 1 or 2 drops of n-octyl alcohol are added to the sample as an anti-bubbling. The ampoule was vacuumed again for 20 minutes, then the center of the tube was closed by heating it over a fire. The closed ampoules were placed in the oven at 110°C for 24 hours. Samples that have been hydrolyzed are cooled at room temperature. Then the contents were transferred to a 50 mL evaporator flask and the ampoule was rinsed with 2 mL of 0.01 N HCl. The rinse liquid was also put into the evaporator flask. Rinsing is done 2 to 3 times. The samples were then frozen using a freeze dryer in a vacuum. To convert cysteine into cystine, 10-20 mL of water is added to the sample and the sample is dried again using a freeze dryer. This is repeated 2 to 3 times. The dried sample was added with 5 mL of 0.01 N HCl and ready to be analyzed. Samples were filtered using millipore paper. Potassium borate buffer pH 10.4 was added to the sample in a ratio of 1: 1. Into a clean empty vial, 10 µl of sample was added and 25 µl of OPA reagent (orthophthaldehyde) was added. This sample mixture was left for 1 minute so that the derivatization was complete. Samples were injected into the HPLC column as much as 5 µl. HPLC used with ultra techsper columns and fluorescence detectors. The mobile phases used were buffer A (a mixture of Na-acetate, Na-EDTA, methanol and tetrahydrofuran (THF)) and buffer B (a mixture of 95% methanol and distilled water) with a flow rate of 1 ml/minute using the gradient method.

Calculation of amino acids concentration (expressed in µmol of amino acids) in nagara bean is:

$$\text{Amino acid concentration} = \frac{\text{Sample peak area}}{\text{Standard peak area}} \text{x concentration standard} \quad (1)$$

After that, the percent of amino acids in nagara bean is determined as follows:

$$\text{Percent of amino acids} = \frac{\text{µmol amino acid x molecular weight of the amino acid x 100}}{\text{µg sample}} \quad (2)$$

2.12. Protein types analysis with the SDS-PAGE method

The type of protein analyzed was defatted flour and protein concentrate from nagara bean and nagara bean tempeh. The protein contained in the flour was precipitated using the [8] method with a concentration of 90% v/v. By means of 10 ml of sample added 90 ml of acetone. Protein electrophoresis based on the [9] method using the SDS-PAGE (Sodium dodecyl sulfate–polyacrylamide gel electrophoresis) method with a vertical slab gel. Slab gel was made with a concentration of 4% collecting gel and 12.5% separating gel. The marker used was from Fermentas: Unstained Protein Molecular Weight Marker with 7 protein bands (Table 1).

Table 1. Marker composition from fermentas: unstained protein molecular weight marker.

Molecular Weight (kDa)	Protein	Source
116.0	beta-galactosidase	E. coli
66.2	bovine serum albumin	bovine plasma
45.0	ovalbumin	chicken egg white
35.0	Lactate dehydrogenase	porcine muscle
25.0	REase Bsp981	E. coli
18.4	beta-lactoglobulin	bovine milk
14.4	Lysozyme	chicken egg white

3. Results and discussion

3.1. Protein content

Various forms of nagara beans have the potential to produce protein at different levels depending on the process carried out. The protein content of whole nagara beans is 14.22% and when roasted it was 18.42% (Table 2). The increased protein content in roasted nagara beans is caused by a decrease in water content due to roasting. Apart from that, the roasting process can also inactivate the lipoxygenase enzyme which plays a role in the formation of unpleasant and beany flavors.

Nagara bean protein content is increased by 24.16% (Table 2) by removing the skin of the bean which is part of the epidermis. Nagara bean epidermis is a carbohydrate, namely fiber. Nagara beans can also be processed into tempeh through a fermentation process, so it can hydrolyze protein into amino acids. The protein content of nagara bean tempeh is 9.58% with a total solid of 32.75% (Table 2), because the nagara bean tempeh is still wet.

Table 2. Protein content of various forms of nagara beans and processed nagara beans[a].

Nagara Bean Forms	Protein Content (%)	Dry Matter (%)	Nagara Bean Forms	Protein Content (%)	Dry Matter (%)
Whole nagara bean	14.22±0.43	86.35	Nagara bean concentrate	17.58±1.63	87.91
Roasted nagara bean	18.42±0.05	94.27	Nagara bean tempeh concentrate	21.28±0.33	86.91
Nagara bean flour without skin	24.16±0.23	91.10	Nagara bean isolate	61,31±0.51	87.00
Nagara bean tempeh	9.58±0.47	32.75	Nagara bean tempeh isolate	38.40±2.57	87.00
Nagara bean defatted flour	22.54±0.34	90.67	Small-scale nagara bean sprout flour	31.06±0.89	89.49
Nagara bean tempeh defatted flour	26.09±1.25	88.30	Scale up nagara bean sprout flour	19.83±0.24	90.12

[a]2 replication

Nagara bean and nagara bean tempeh can increase the amount of protein by making defatted flour, protein concentrate and protein isolate [10]. Defatted flour is nagara bean flour or nagara bean tempeh flour which has had the fat part remove. This removed fat functions to be able to increase the amount of protein in the nagara beans. Only nagara bean defatted tempeh flour could increase its protein content, while nagara bean defatted flour decreased its protein content.

The increase in the amount of protein in nagara bean tempeh defatted flour can be caused by an increase in ammonia nitrogen and amino nitrogen [11] during the fermentation process, thereby

increasing the nitrogen and amino acid content. As for the decrease in protein content in defatted flour, it is suspected that the non-polar amino acids that make up the protein are dissolved with hexane.

In nagara bean and nagara bean tempeh protein concentrate, there was a decrease in protein content compared to their defatted flour. This is because during the extraction process to produce protein concentrate, proteins and amino acids become dissolved. Even so, the protein produced in protein concentrate is purer than defatted flour. According to [12] defatted soybean tempeh flour reduced its total ash, moisture content, fat content, total carbohydrates and crude fiber. In defatted soybean flour, only the protein increases.

In protein isolates, the solvent used for the production of protein isolates only uses aqueous solvents, resulting in an increase in protein content. The protein content of nagara bean protein isolate can reach 61.31% (Table 2). In contrast to the protein content of nagara bean tempeh protein isolate, which was only 38.4% (Table 2). This is because the protein isolate of nagara bean tempeh contains many polar amino acids, so it is easily dissolved in water during extraction. Even though the amount of amino acid protein isolate of nagara bean tempeh can reach 78.95% (Table 3). The protein content in nagara bean tempeh protein isolate is lower than that of soybean tempeh protein isolate by 50.5% [12]. This is because the nagara beans are very hydrophilic, while the soybeans are more hydrophobic. As a result, when the nagara beans are extracted with aqueous solvents, many of the proteins and amino acids are dissolved.

Protein isolate is very necessary for humans. When compared between soy protein isolate and casein, soy protein isolate can reduce greater to atherosclerosis lesion area compared to casein consumed in the same amount [13]. Soy protein isolate and soy protein concentrate can also lower blood cholestero [14]. Apart from that, the presence of cowpea protein in food can increase the number of good bacteria in digestio [15]. This is the advantage of legume protein.

Nagara beans can also be processed into sprouts. On a small scale, the protein content of nagara bean sprouts increased to 31.06% (Table 2). This is caused by the formation of growth hormone and increased activity of enzymes during the growth process. However, when the production scale for nagara bean sprouts was increased, the protein content decreased to 19.83% (Table 2). This is caused by an increase in the number of sprouts made and the thickness of the pile of sprouts increases, so the activity of enzymes becomes inhibited [16]. As a result, the amount of protein in the nagara bean sprouts decreased.

The protein content in nagara beans is lower than soybeans, namely 34%. However, the existence of nagara beans as a source of protein can also be taken into account, especially for areas that are not soybean producers. Therefore, it is feasible to sustain the production of nagara beans in providing protein to the community.

3.2. Amino acid composition

The amino acid composition of nagara beans was determined using the HPLC-derivatization orthophthaldehyde method. Total amino acids were highest in protein isolate compared to protein concentrate and defatted flour (Table 3). Protein isolates contain amino acids ranging from 77.58 – 78.95 %, protein concentrates ranging from 24.27 – 27.71 % and defatted flour ranging from 24.28 – 26.17 %. The amino acid content found in defatted flour, protein concentrate, and protein isolate tends to be higher than the protein content (Table 3).

The amino acids found in defatted flour, protein concentrate, and protein isolate from nagara bean and nagara bean tempeh are mostly polar amino acids, namely amino acids with a negative charge, namely glutamic and aspartic acids. This is the same as what was stated by [17] which states that cowpea in Ethiopia contain also many glutamic acid and aspartic acid.

In addition, there are several essential amino acids, such as lysine, phenylalanine, threonine, and leucine, which are also found in abundance in nagara beans (Table 3). Essential amino acids are also found in cowpea (*Vigna unguiculata* L. Walp) in Sudan in the form of defatted flour and protein isolate in greater amounts. However, cowpea in Sudan did not find aspartic acid [18]. In addition, cowpea found in Nigeria is almost the same as the amino acid composition of nagara beans. Most of the amino acids found are glutamic acid and aspartic acid [19]. The amino acid composition of the nagara bean is almost

the same as that of the cowpea (*Vigna unguiculata* L. Walp) found in California. The content of polar amino acids is also more than non-polar amino acids in cowpea [20].

If the nagara beans are made into sprouts, then the number of amino acids is reduced [16]. This is due to the washing process on the nagara bean sprouts, the amino acids which are soluble in water become dissolved together with the water. The types of amino acids found in nagara beans are almost the same as those found in soybeans, that is, there are more polar amino acids than non-polar amino acids as stated by [21].

3.3. Protein type

SDS-PAGE method is a method that can be used to determine the type of protein based on its molecular weight (Figure 1). Only the nagara bean protein concentrate was identified and the protein was well separated based on its molecular weight. As for nagara bean defatted flour, nagara bean tempeh defatted flour and nagara bean tempeh concentrate cannot separate the protein properly. The protein in defatted nagara flour cannot be separated due to the large amount of carbohydrates, so the protein cannot be separated properly. The protein in the defatted flour and the nagara bean tempeh concentrate did not separate very well, because the nagara bean tempeh protein had experienced the breaking of peptide bonds into amino acids.

Table 3. Amino acid composition in defatted flour, protein concentrate and protein isolate in nagara bean and nagara bean tempeh.

Amino Acid Type	Amino Acid Concentration (% w/w)					
	Nagara Bean Isolate	Nagara Bean Tempeh Isolate	Nagara Bean Concentrate	Nagara Bean Tempeh Concentrate	Nagara Bean Defatted Flour	Nagara Bean Tempeh Defatted Flour
Alanine	2.53	2.85	0.94	1.23	1.00	1.23
Isoleucine	3.32	3.48	1.14	1.51	1.13	1.36
Leucine	5.31	6.14	1.95	2.60	1.93	2.19
Methionine	1.61	1.89	0.74	1.02	0.73	0.78
Phenylalanine	6.40	6.32	1.50	1.92	1.51	1.71
Tryptophan	0.82	0.85	0.47	0.36	0.39	0.57
Valine	3.73	3.91	1.32	1.71	1.31	1.56
Total	23.72	25.44	8.06	10.35	8.00	9.40
Glycine	2.35	2.42	0.77	1.04	1.00	0.93
Serine	4.10	3.92	1.45	0.21	0.34	0.59
Threonine	5.23	3.34	0.97	1.30	1.09	1.22
Tyrosine	2.67	2.83	0.81	1.11	0.85	0.98
Total	14.35	12.51	4.00	3.66	3.28	3.72
Aspartic Acid	9.23	9.65	2.75	3.37	2.82	3.11
Glutamic Acid	15.70	13.87	4.67	5.12	4.75	4.70
Total	24.93	23.52	7.42	8.49	7.57	7.81
Arginine	2.44	6.15	2.03	2.33	2.30	2.23
Histidine	4.69	3.86	1.23	1.44	1.35	1.45
Lysine	7.45	7.47	1.53	1.44	1.78	1.56
Total	14.58	17.48	4.79	5.21	5.43	5.24
Amino acid total	77.58	78.95	24.27	27.71	24.28	26.17

In the nagara bean protein concentrate, the polypeptides that were mostly found were at a molecular weight of 64.90; 50.86; and 34.77 kilodaltons (kDa). Based on the research of [15], in cowpea (*Vigna unguiculata* L. Walp) found in California, especially in the globulin fraction, the majority of polypeptides found were with molecular weights of 65, 60, 56 and 50 kDa and the minority of polypeptides found were with molecular weight 42 – 28 kDa. In the albumin fraction, the majority of polypeptides are with molecular weights of 90, 91, 32 and 30. In the glutelin fraction there are several bands with molecular weights of 101, 68, 31 and 29 kDa. As for the prolamin fraction, there are 4 dominant bands, namely 105, 62, 59 and 54 kDa. Based on this, the nagara beans are also thought to contain globulin and albumin.

Most of the protein found in cowpea is globulin. According to [22] *Vigna unguiculata* bean (L.) Walp contains 51% globulins, 45% albumins, 3% glutelin, and 1% prolamin. As for [12] stated that the protein content in cowpea in Ethiopia was dominated by the globulin and albumin fractions of 38.4–49.1% and 19.6–22.5%, while the glutelin fraction was 6.4 to 10.4% and prolamin of 1.0–1.14%.

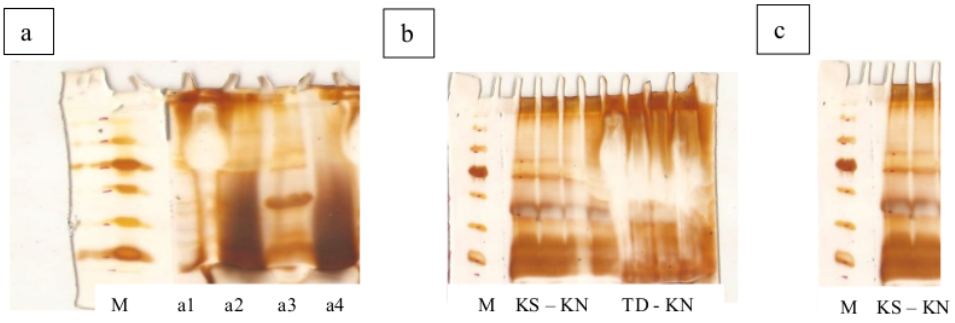

Figure 1. Types of protein in concentrate and defatted nagara flour and nagara bean tempeh (a) a1. TD-KN (nagara bean defatted flour), a2. TD-MPE (nagara bean tempeh defatted flour), a3. KS-KN (nagara bean protein concentrate), a4. KS-MPE (nagara bean tempeh protein concentrate), (b)TD-KN and KS-KN and (c) KS-KN.

4. Conclusion

Nagara beans, South Kalimantan's superior cowpeas, in various forms have the potential to produce protein ranging from 9.58% - 61.31%. The amino acid composition is dominated by polar amino acids, especially glutamic acid and aspartic acid. In addition, nagara beans also contain lysine, phenylalanine, threonine and leucine. Most of the protein in nagara beans is globulin and albumin. Based on its protein potential, nagara beans are feasible to maintain their continued existence, especially in the swampy area of lebak and South Kalimantan.

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