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Variability of Physicochemical Database and Trypsin Inhibitory Activity of Komak (*Lablab purpureus* (L.) Sweet) Beans with Distinct Colors

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Abstract

Komak (*Lablab purpureus* (L.) sweet) is a highly productive plant used for sustainable cultivation. However, beans are currently underutilized. A complete database including physicochemical characteristics is important for developing new foods derived from komak beans. This study aimed to determine the physical (weight, dimensions and color) and chemical (macromolecules, amino acids, sugars, minerals, phytic acid, tannin and volatile compounds) profiles, as well as the trypsin inhibitory activity of different varieties of komak beans, viz. white and black beans. The levels of the studied parameters differed significantly ($p < 0.05$) between the beans. However, the common profile of komak beans was characterized by a moderate level of protein (26 %), high carbohydrate (66 %) and low fat (2 %), with a weight ranging from 25 to 45 g and low anti-nutritional factor expressed as trypsin inhibitory activity (2 TIU/mg). Additionally, analytical results using HS-SPME-GC/MS revealed that 1-octen-3-ol and benzyl alcohol were the main unpleasant volatile compounds produced upon heating beans. The major mineral compound in black beans is potassium, whereas the white variety contains higher amounts of magnesium, calcium and iron. Interestingly, both beans provided favorable compounds contributing to the umami taste, as the most dominant amino acids were glutamic and aspartic acids. Finally, as white beans were lower in antinutritive compounds (phytic acid and tannin), this measure may encourage further studies to expand the development of this variety to promote underutilized beans as alternative food sources.

Keywords: Amino acid, Antinutrients, Nutrients, Phytic acid, Tannins

Introduction

Komak (*Lablab purpureus* (L.) sweet) bean is an underutilized legume from the Fabaceae family, originating from Africa [1,2], and is distributed in tropical and subtropical areas. This commodity is widely grown in India [3], Bangladesh [4], Indonesia, the Philippines, Papua New Guinea and Central to South America [5]. Their vast distribution is attributed to their high productivity and adaptability to the environment [6]. In Indonesia, komak is commonly cultivated in East Java and West Nusa Tenggara as a backyard or intercrop [7,8]. Within these regions, 2 varieties of komak exist with distinct bean coat colors: Black and white.

Komak beans are harvested at the same time as jack beans (4 months after planting), but earlier than velvet beans (9 - 12 months) [8]. The production volume of dry beans is estimated at 1 - 2.5 tons per hectare [9], which is higher than the average farm yield of soybean (1.25 tons per hectare) [10]. In keeping with the sustainable cultivation of this drought- and heat-tolerant crop, komak beans are available as new raw materials for food diversification. Previous studies have reported on the properties of this commodity as a food source. However, their application in food is limited by the presence of antinutritional factors (ANFs), including trypsin inhibitors, phytic acid and tannins [11-13], which can affect nutrient digestibility.

Apart from antinutrients, the specific odor of komak beans, as well as legumes in general, is an unfavorable aspect that influences consumer preference. The volatile compounds responsible for unpleasant (beany, green and earthy) odors present in a number of legumes are mostly aldehydes, ketones, alcohols and pyrazine [14,15]. The selection of komak beans for consumption by local people also varies because of their physical characteristics such as bean size, weight and color. In addition to consumer preferences,

the physical characteristics of komak beans are crucial in the design of processing machinery [16]. Therefore, the objective of this study was to establish a comprehensive physicochemical database and trypsin inhibitory activity of komak beans with distinct colors and to promote the utilization of the beans as part of a healthy diet.

Materials and methods

Materials

Two varieties of sweet komak (*Lablab purpureus* (L.) sweet) beans, i.e., black and white (**Figure 1**), were collected from North Lombok Province, Lombok Island, Indonesia. The beans were harvested 130 - 135 days after planting.

(a) (b)

Figure 1 Komak beans with distinct coat colors: Black (a) and White (b).

Sample preparation

The komak bean samples were ground using a chopper blender (Philips HR2116, Bogor, Indonesia), resulting in powders passing through a 60-mesh sieve (ABM, Jakarta, Indonesia). The powder was placed in a brown glass bottle and stored in a freezer at 18 °C until analysis.

Dimension, weight and color measurement

The weight, dimension and color of the beans of each komak variety were measured. The sample was placed in an analytical balance (Fujitsu FS-AR.210, Tokyo, Japan), and the weight of every 100 seeds was recorded in triplicates. A digital caliper with an accuracy of 0.01 mm (stainless-steel hardened, Shanghai, China) was used to measure the length, width and thickness of the beans. The coat color of the beans was determined using a chromameter (Minolta CR 400, Osaka, Japan) and expressed in terms of lightness (L), redness/greenness (a*) and yellowness/blueness (b*) [17].

Proximate composition analysis

The levels of moisture, protein, fat and ash in komak beans were determined according to the official methods of AOAC analysis [18]. In contrast, the carbohydrate content was defined by calculating the percentage remaining after all other components were measured.

Moisture content

The weight of the dry bottle dish (M_1) was recorded and 2 g of komak bean powder was added to the dish and weighed (M_2). Subsequently, the samples were dried in an oven (Memmert UN110, Schwabach, Germany) at 105 °C for 6 h. The sample was then placed in a desiccator for 15 min to reach ambient temperature. A constant weight of the dried sample was measured (M_3). Moisture content was calculated using Eq. (1).

$$\text{Moisture } (\%) = \frac{M_2 - M_3}{M_2 - M_1} \times 100\ \%. \quad (1)$$

Protein content

Komak bean powder (1 g) was mixed with catalysts containing 0.8 g $CuSO_4$ and 7.0 g Na_2SO_4 then placed in a Kjeldahl digestion flask containing 98 % sulfuric acid (3 mL). Subsequently, the sample was heated until the solution turned transparent blue green. After reaching ambient temperature, the solution was diluted with 10 mL of distilled water. The solution was then exposed to a distillation process employing 20 mL of NaOH, and the distillate was transferred to an Erlenmeyer flask with a 4 % boric acid solution (5 mL) and a methyl red indicator. The distillates were then titrated with HCl 0.02 N. The total nitrogen

content was calculated using Eq. (2). Subsequently, the percentage of nitrogen was multiplied by a conversion factor of 6.25 to calculate the crude protein percentage.

$$\%N = \frac{(\text{mL HCl sample} - \text{mL blanko}) \times \text{N HCl} \times 14.007}{\text{sample (mg)}} \times 100\ \%. \quad (2)$$

Crude fat content

Briefly, 2 g of sample (M_1) was placed in a thimble and covered with fat-free cotton. The thimble containing the samples was placed in a Soxhlet extractor and 50 mL petroleum ether was added. A cleaned and dried flask was measured (M_2) for the extract recipient. The extraction proceeded for 4 h, followed by 1 h of drying in an oven at 70 °C, and cooling in a desiccator for another hour. The weight of the extract-filled flask was determined (M_3) and the amount of crude fat in the sample was calculated as Eq. (3).

$$\text{Crude fat}\ (\%) = \frac{M_3 - M_2}{M_1} \times 100\ \%. \quad (3)$$

where M_1, M_2 and M_3 are the sample weight, extraction flask, and crude fat content, respectively.

Ash content

Approximately 2 g of sample (M_2) was placed in a weighed crucible (M_1) and then placed on a hot plate under a fume hood, where it was heated until the sample was charred, and smoking stopped. The sample was placed in a muffle furnace at 550 °C for 3 h to form a gray sample. After cooling in a desiccator, the sample was weighed (M_3) to calculate the ash content.

$$\text{Ash}(\%) = \frac{M_3 - M_1}{M_2} \times 100\ \%. \quad (4)$$

where M_1 - crucible weight, M_2 - sample weight and M_3 - crucible containing sample ash.

Carbohydrate

Carbohydrate content was calculated by difference. The formula is shown in Eq. (5).

$$\text{Carbohydrate}\ (\%) = 100 - (\text{ash}\ (\%) + \text{fat}\ (\%) + \text{moisture}\ (\%) + \text{protein}\ (\%)). \quad (5)$$

Analysis of mineral

The potassium, phosphorus, magnesium, calcium and iron contents were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Agilent Technologies 700 Series ICP-OES, Santa Clara, CA, USA) according to the AOAC [18]. Approximately 0.5 g of the powdered sample was digested and mineralized by adding 10 mL of concentrated HNO_3 and then heated in a microwave at 150 °C for 15 min. After reaching ambient temperature, yttrium (100 mg/L) was added as an internal standard by adjusting the volume to 50 mL using distilled water. The solution was then filtered before the inductively coupled plasma optical emission spectrometry (ICP-OES) analysis.

ICP-OES was conducted using concentric glass as the nebulizer type. The intensity of torch alignment was > 1,000,000, while a torch alignment position was 1 to 1 for both horizontal and vertical. The absorbance of calcium, phosphorus, iron, magnesium, potassium and yttrium was measured at wavelengths of 317, 214, 238, 285, 766 and 371 nm, respectively.

Analysis of glucose and sucrose

The analysis of glucose and sucrose was based on the AOAC method [18]. The powdered sample (0.5 g) was diluted with 10 mL of distilled water in a 25 mL volumetric flask and sonicated for 1 min. The solution was mixed with Carrez I and II solutions (1 mL), and the volume was adjusted using distilled water to the mark. The solution was transferred into a 2 mL tube, centrifuged at 14000 rpm for 3 min and filtered through a 0.45 µm GHP/RC filter before the analysis using HPLC (Waters, Singapore) connected to a Waters 2414 refractive index detector. Separation in the carbohydrate amino (NH_2) column (5 µm, 250×4.6 mm^2) was set in an isocratic mobile phase (80 % acetonitrile) at a flow rate of 1 mL/min at room temperature.

Analysis of amino acid

The amino acid profiles were analyzed according to a method of Waters Corporation [19] using Acquity UPLC H-Class Amino Acid Analysis (Waters, Singapore), however for cystine, methionine and

tryptophan, which were determined using LC-MS/MS (Shimadzu, Kyoto, Japan). Powdered samples (0.1 g) were hydrolyzed with 6 N HCl (10 mL) for 24 h at 105 °C. The hydrolysis solution was transferred into a 50 mL volumetric flask, diluted with distilled water and filtered using a 0.2 µm syringe filter (Sartorius Minisart, Germany). An internal standard (2.5 mM alpha-aminobutyric) was added to the filtrate. The solution was derivatized by adding AccQ·Tag Ultra borate buffer and AccQ·Tag Ultra reagent and then mixed by vortexing for 1 min. The solution (1 µL) was injected into a UPLC system with Acquity UPLC BEH C18 (2.1×100 mm^2, 1.7 µm) column at 49 °C and detected by an AQUITY UPLC photodiode array detector (Waters, Singapore) at 260 nm of wavelength. The flow rate was 0.7 mL/min. The following mobile phases (A - D) were used: A) 100 % AccQ-Tag Ultra eluent A concentrate, B) 10 % AccQ-Tag Ultra eluent B, C) Double distilled water and D) 100 % AccQ-Tag Ultra eluent B. The gradient elution was employed: 0 min 10 % A, 90 % C; 0.29 min 9.9 % A, 90.1 % C; 5.49 min 9.0 % A, 80 % B, 11 % C; 7.10 - 7.30 min 8.0 % A, 15.6 % B, 57.9 % C, 18.5 % D; 7.30 min 8.0 %, 15.6 % B, 57.9 % C, 18.5 % D; 7.69 min 7.8 % A, 70.9 % C, 21.3 % D; 7.99 min 4.0 % A, 36.3 % C, 59.7 D; 8.59 min 4.0 % A, 36.3 % C, 59.7 % D; 8.68 min 10.0 %, A, 90.0 % C; and 10.20 min 10.0 % A, 90.0 % C.

The amino acids cystine, methionine and tryptophan were analyzed using LC-MS/MS. Powdered samples (0.5 g) were stored in a cooling water bath and placed in a freezer at –15 °C (15 min). The sample was diluted with 5 mL of an oxidizing solution (9 mL formic acid and 1 mL 30 % hydrogen peroxide) and incubated at –15 °C for 16 h. Sodium bisulfite (0.84 g) was added to the oxidized sample and incubated at room temperature for 3 h. Subsequently, the sample was hydrolyzed with 5 mL of hydrochloric acid (6 M with 0.1 % phenol) at 110 °C for 24 h. The sample volume was adjusted to 25 mL using distilled water. The pH of the solution was adjusted to 2.20 ± 0.05 in a cooling water bath, and aquabides were added to a 50 mL volumetric flask. The solution was then centrifuged at 14000 rpm for 3 min, filtered (0.2 µm RC membrane filter) and injected (2 µL) into the LC-MS/MS system. Formic acid (0.1 %) in acetonitrile was prepared as mobile phase A, and ammonium formate (100 mM) was prepared as mobile phase B. An initial gradient of 14 % B was maintained for 3 min and then increased to 100 % B at 10 min. Gradient returned to 14 % B at 12 min. The flow rate was 0.4 mL/min passing through an Imtakt Intrada Amino Acid column (50×3 mm^2, 3 µm; Imtakt, Kyoto, Japan) set at 37 °C [20].

Analysis of anti-nutritional components

Analysis of phytic acid

Phytic acid was analyzed using a spectrophotometry technique according to a previously described method [21]. The powdered sample (0.1 g) was extracted with 0.5 M HNO_3 (20 mL) in a water bath shaker for 4 h at room temperature (28 - 30 °C) and filtered. The extract (1 mL) was added to distilled water (0.4 mL) and 0.005 M $FeCl_3.6H_2O$ (1 mL), mixed, and heated in boiling water (100 °C) for 20 min. The solution were cooled down and mixed with n-amyl alcohol (5 mL) and 0.1 M ammonium thiocyanate (0.1 mL). The solution was then centrifuged for 10 min at 3000 rpm. The intensity of the color was measured by UV-Vis spectrophotometer (Genesys 10S UV-VIS, Thermo Scientific, Waltham MA, USA) at 495 nm against an n-amyl alcohol blank. The phytic acid standard curve was prepared using a mixture of Na-phytate and HNO_3 solutions. The results were presented as mg/g dry matter.

Analysis of tannin

Tannin levels were determined as previously reported [22] with slight modifications. Powdered samples (0.3125 g) were diluted in distilled water (62.5 mL) and boiled (100 °C) for 2 h. After filtration (Whatman filter paper no. 1), the solution was mixed with Folin-Ciocalteu reagent (0.5 mL) and 20 % Na_2CO_3 (2 mL). The mixture was then incubated at room temperature for 30 min. The absorbance was measured using UV-Vis spectrophotometer at 748 nm. The tannin content was calculated from the standard curve using a linear regression equation and expressed as mg/100 dry matter.

***Analysis of trypsin inhibitory activity* (*TIA*)**

TIA was determined according to the method of Olu *et al*. [23] and Coscueta *et al*. [24]. TIA analysis was performed by preparing an extract from the sample, a substrate (BAPNA solution), and a trypsin solution. The extraction of the sample (1 g) was conducted using 0.01 M NaOH (50 mL) in a water bath shaker at room temperature for 3 h. The extract was subsequently centrifuged at 3500 rpm for 10 min. The substrate was freshly prepared before the assay by adding 0.05 M tris-buffer (100 mL) pH 8.2 to BAPNA (40 mg) in dimethyl sulfoxide (DMSO) (1 mL), and the mixture was kept at 37 °C. Trypsin solution was prepared by diluting trypsin (4 mg) in 0.001M HCl (200 mL) and incubating at 4 °C.

The extract (2 mL) was mixed with BAPNA (5 mL) and trypsin solution (2 mL). The mixture was then incubated in a water bath shaker at 37 °C for 10 min. The reaction was terminated by adding 30 %

acetic acid (1 mL) and centrifugation at 3500 rpm for 10 min. Absorbance was measured at 410 nm using a UV-Vis spectrophotometer. The control solution was prepared using distilled water (2 mL) and the blank was prepared using 30 % acetic acid (1 mL). The TIA was calculated as follows:

$$\frac{\text{TIU}}{\text{mg}}\ \text{sample} = \frac{\text{A} \times \left(\frac{100}{df}\right)}{\frac{S}{df}}. \quad (6)$$

where A was the difference between sample and blank measurement, S was the weight of the sample (mg), and df was the dilution factor (mL).

Analysis of headspace solid phase microextraction (HS-SPME/GC-MS)

Volatile compounds released in the headspace of komak beans headspace were analyzed using headspace solid-phase microextraction (HS-SPME/GC-MS). The powdered sample (5 g) was weighed and placed in a headspace vial (22 mL) fitted with polytetrafluorethylene-silicone septa. A fiber (DVB/CAR/PDMS) was inserted into the vial and incubated for 45 min (80 °C) in a water bath. Thereafter, the fiber was immediately injected into the injector port of the GC-MS for desorption at 250 °C for 5 min. The analysis was performed using an Agilent 7890A GC (Agilent Technologies, Inc., Santa Clara, CA, USA) coupled with an Agilent 5975C XL EI/CI MS equipped with A DB-Wax capillary column (30×250×0.25 m^3). Initially, the temperature of the oven was programmed at 40 °C, which was followed by an increase of 5 °C/min to 120 °C, and then increased to 240 °C at 9 °C/min and maintained for 5 min. Helium was used as the carrier gas at a 1 mL/min flow rate. The ion source and quadrupole temperatures were 250 °C and 150 °C, respectively. Volatile compounds were identified by their mass spectra based on NIST 14.0. The linear index (LRI) was calculated using the retention data of the standard alkane series (C9 - C31). Relative quantification of the peak area percentage was obtained by comparing the peak area of each compound to the total peak area in the chromatogram [25].

Statistical analysis

Data are presented as mean ± standard deviation. An independent t-test was used to compare the means, which were calculated using SPSS version 25 software (SPSS Inc., Chicago, USA). Principal component analysis (PCA) was performed using Statgraphics Centurion 19 software (Statgraphics Technologies, Inc., Virginia, USA).

Results and discussion

Physical properties

The physical characteristics of the 2 komak bean varieties were measured (**Table 1**), as the size and shape of the seeds play an important role in processing. Variability in size and shape could affect the cooking time and uniformity of the result. In this study, the different varieties of komak beans did not vary in weight or size.

Table 1 Physical characteristics of komak beans (*Lablab purpureus* (L.) sweet).

Physical parameters	White komak bean	Black komak bean
100 seed weight (g)	35.27 ± 0.06[a]	35.70 ± 0.09[a]
Length (mm)	10.11 ± 0.01[a]	10.10 ± 0.04[a]
Width (mm)	7.73 ± 0.05[a]	7.71 ± 0.09[a]
Thickness (mm)	6.11 ± 0.18[a]	6.22 ± 0.02[a]
Color	L = 59.05 ± 0.32[a] a = 2.13 ± 0.36[a] b = 19.78 ± 0.67[a]	L = 30.29 ± 0.16[b] a = 0.47 ± 0.07[b] b = 2.27 ± 0.27[b]

Note: Values with different notations indicate significant differences within the same row ($p < 0.05$).

The studied komak bean varieties were larger than those from Kenya; hence, their weight was also higher. The width and thickness of Kenyan komak beans were reported as 6.8 - 7.4 and 5.2 - 5.5 mm, respectively. While the weights varied from 26.1 to 29.2 g/100 bean [12]. The classification of seed size based on 100-seed weight is 1) large (greater than 40 g), 2) medium (25 - 45 g) and 3) small (less than 25

g) [17]. Hence, the komak beans used in this study (35.27 - 35.70 g/100 bean) were classified as medium-sized seeds in comparison with soybeans (14.65 - 19.53 g/100 bean), canavalia beans (137 - 184 g/100 bean) and mucuna beans (95.80 g/100 bean) [26-28].

The color parameters (L, a* and b*) of the 2 types of komak bean were significantly different. The seed colors of the 2 beans were brownish-yellow and black. The white komak beans have a higher L value, demonstrating a lighter color than the black komak beans. Additionally, the a* and b* values for both bean types were in the positive part of the scale, with white beans having a lower a*, indicating less redness, and a higher b*, indicating more yellowness than black beans. Variation in color indicates the amount of antinutrients present in the seeds. Dark bean seeds have more antinutrient compounds than white, cream and brown beans [28]. In this research, the anti-nutritional content of the black beans was greater than that of the cream beans.

Proximate composition

The proximate compositions of the 2 komak bean varieties (**Table 2**) showed no significant difference ($p < 0.05$). Moisture content is important for defining the shelf life of beans [29,30]. Komak beans with 10 % moisture content and airtight aluminum packaging stored at ≤ 5 °C for 6 months showed no deterioration in bean quality (maintained germination, vigor and less lipid peroxidation) [31]. According to SNI 01-3922-1995, the standard moisture content of legumes is 13 %. The studied komak beans contained 9 % moisture and thus had a relatively long shelf life.

The oil content, enzyme activity (α-amylase, lipase and protease) and mineral content (Cu, Mn and Zn) of groundnut seeds with 8 % moisture can be maintained for 18 months in cold storage (4 °C) [32]. Komak bean is low in fat but rich in proteins and carbohydrates, which contribute uniquely to the diet [33]. The fat content of komak bean (1.8 %) is equal to that of pigeon pea (1.60 %) [34], black bean (1.9 %) and navy bean (1.8 %) [35] but less than that of soybean (18.50 %) [36].

The 2 komak bean varieties contained 26 % protein and 66 % carbohydrate. Compared to other legumes, the komak bean has more protein than the jack bean (24.94 %) [37], velvet bean (24 %) [38] and pigeon pea (21.20 %) [39], but less protein than soybeans (37.10 - 44.40 %) [26-40]. Owing higher protein content compare to the aforemention beans, komak beans may be a viable option for nutrient supplementation in areas where other legumes are not readily accessible. Similarly, the high carbohydrate content of komak beans (66 %) may be beneficial for preventing protein-energy malnutrition [41,42].

Table 2 Proximate analysis of komak (*Lablab purpureus* (L.) sweet) beans.

Compounds	White Bean	Black Bean
Moisture (%)	8.91 ± 1.01	9.02 ± 1.00
Protein (%db)	26.00 ± 0.62	26.23 ± 0.43
Fat (%db)	1.78 ± 0.09	1.84 ± 0.51
Ash (%db)	5.26 ± 1.18	5.84 ± 1.27
Carbohydrate (%db)	66.95 ± 1.36	66.13 ± 1.08

The complete amino acid profiles of the 2 komak bean varieties are shown in **Table 3**. The levels and composition of amino acids in the studied samples were lower than those in black komak beans from Kenya [43]. The amino acid content of black beans from Kenya expressed per 100 g were L-alanine (1.16 g), L-arginine (1.13 g), L-aspartic acid (1.91 g), L-cysteine (0.18 g), L-glutamic acid (2.51 g), L-glycine (1.61 g), L-histidine (0.54 g), L-leucine (1.64 g), L-isoleucine (0.68 g), L-lysine (0.96 g), L-methionine (0.09 g), L-phenylalanine (0.92 g), L-proline (1.00 g), L-serine (1.51 g), L-threonine (0.91 g), L-tyrosine (0.59 g) and L-valine (0.91 g). The compounds L-serine, L-alanine, L-glycine and L-proline showed lower levels in the black komak beans studied. This difference could be due to differences in varieties and cultivation sites [44,45]. Shaahu *et al.* [46] demonstrated that the amino acid composition of 3 varieties of lablab bean, i.e., highworth, rongai brown and rongai white, varied, with highworth having the highest concentration of amino acids. In terms of protein, Purwanti *et al*. [47] reported that lablab beans grown in Madura had higher protein levels than those grown in the West Nusa Tenggara and Probolinggo regions of Indonesia. Furthermore, Assefa *et al*. [48] also found a significant impact on amino acid concentration in soybeans grown in different regions of the United States, where the Southeast had a higher amino acid concentration than the Northwest.

Table 3 Amino acid compositions of komak bean (*Lablab purpureus* (L.) sweet).

Amino acid (g/100 g)	Komak Beans	
	White	Black
L-Serine	1.19 ± 0.01[a]	1.44 ± 0.00[b]
L-Glutamic acid	3.47 ± 0.02[a]	3.70 ± 0.02[b]
L-Phenylalanine	1.40 ± 0.01[a]	1.54 ± 0.00[b]
L-Isoleucine	0.99 ± 0.01[a]	1.01 ± 0.00[a]
L-Valine	1.12 ± 0.01[a]	1.17 ± 0.01[b]
L-Alanine	0.85 ± 0.00[a]	0.97 ± 0.00[b]
L-Arginine	1.66 ± 0.01[a]	1.49 ± 0.01[b]
L-Glycine	0.86 ± 0.01[a]	0.98 ± 0.00[b]
L-Lysine	1.29 ± 0.01[a]	1.39 ± 0.01[b]
L-Aspartic acid	2.15 ± 0.02[a]	2.47 ± 0.01[b]
L-Leucine	1.87 ± 0.01[a]	1.97 ± 0.01[b]
L-Tyrosine	0.77 ± 0.01[a]	0.73 ± 0.00[b]
L-Proline	0.91 ± 0.01[a]	0.90 ± 0.00[a]
L-Threonine	0.94 ± 0.01[a]	1.06 ± 0.00[b]
L-Histidine	0.71 ± 0.00[a]	0.81 ± 0.00[b]
L-Cysteine	0.23 ± 0.00[a]	0.78 ± 0.00[b]
L-Methionine	0.01 ± 0.00[a]	0.17 ± 0.00[b]
L-Tryptophan	0.20 ± 0.00[a]	0.21 ± 0.00[a]

Note: Values with different notations indicate significant differences within the same row ($p < 0.05$).

Komak beans are composed of essential and nonessential amino acids. Glutamic acid was the most abundant amino acid, followed by aspartic acid. Both are the most common non-essential amino acids found in legumes [49]. In contrast, the essential amino acids tryptophan and methionine were the least abundant in beans. Hence, to achieve a well-balanced and complete amino acid intake, komak beans can be consumed together with cereals to provide sufficient tryptophan and methionine [50,51].

Sugars, including glucose and sucrose, can contribute to sweet taste and are easily digestible [52]. **Table 4** shows the contents of glucose (0.55 - 0.58 %) and sucrose (2.05 - 2.25 %) in komak beans, which were not significantly different between the 2 varieties. These values were higher than those previously reported for komak beans [53]. Although legumes typically contain low levels of sucrose (1 - 3 %) [54], the level of sucrose is commonly higher than that of glucose.

Table 4 Glucose and sucrose contents of komak (*Lablab purpureus* (L.) sweet) bean.

Sugar (%)	Komak beans	
	White	Black
Glucose	0.58 ± 0.01	0.55 ± 0.01
Sucrose	2.25 ± 0.06	2.05 ± 0.01

Mineral composition

The mineral composition of the komak (black and white) bean samples is listed in **Table 5**. The 2 varieties showed significant differences in phosphorus, magnesium, calcium and iron, while potassium levels in the bean varieties were comparable. The results demonstrated that different varieties cause variations in mineral content, which is consistent with formerly reported results [12,46]. Differences in mineral composition can be affected by the variety, origin, geographic source and level of soil fertility [55, 56]. According to Shaahu *et al*. [46], the mineral compositions of the 3 varieties of lablab beans, namely highworth, rongai brown and rongai white, were found to differ. The highworth variety demonstrated the highest levels of Mg, Zn, P and Fe, while the levels of Na, Ca and Mn were similar. Furthermore, research conducted in Eastern India during winter and summer seasons of 2020 - 2022 revealed differences in the content of Ca, Fe and Zn in soybeans grown during these periods. These variations in annual conditions led

to changes in climatic factors, such as temperature, moisture and soil nutrients, which in turn affected the growth of soybeans an resulted in distinct compositions in the beans [48].

Table 5 Mineral content of Komak (*Lablab purpureus* (L.) sweet) bean.

Mineral (mg/100 g)	Komak beans	
	White	Black
Potassium	1686.95 ± 2.46[a]	1692.25 ± 1.64[a]
Phosphorus	358.05 ± 0.91[a]	494.26 ± 0.92[b]
Magnesium	150.26 ± 2.03[a]	124.40 ± 1.37[b]
Calcium	123.07 ± 0.00[a]	111.74 ± 0.99[b]
Iron	4.71 ± 0.01[a]	4.30 ± 0.08[b]

Note: Values with different notations indicate significant differences within the same row ($p < 0.05$).

Potassium was the mineral with the highest concentration in the sample beans. High potassium levels in the body can promote iron utilization and are beneficial to treating hypertension [57]. The phosphorus content of komak beans was higher than that of soybeans (245 mg/100 g), white beans (113 mg/100 g), and black beans (140 mg/100 g) [58], but lower than those of velvet beans (592.1 mg/100 g) [59]. Phosphorus deficiency in the human body is associated with symptoms including fatigue and anxiety [60,61]. An intake of beans as part of a regular diet could contribute to the prevention of these symptoms. Magnesium, a cofactor of about 300 enzymes, is involved in enzymatic activities, glucose metabolism and cardiac function [62,63].

Komak beans have a higher calcium composition than mungbean (81 mg/100g) [64] and pigeon peas (77.4 mg/100 g) [65]. Calcium is needed to build and fix bones and teeth, as well as for blood clotting [66]. The iron levels in komak beans are similar to those in jack beans (4.4 mg/100 g) [67]. Iron is essential for the production of red blood cells in all organs of the body and for the development of fetuses and children [68]. The studied komak beans were rich in potassium, phosphorus, magnesium, calcium and iron. Because of their high mineral content, komak beans could potentially be used as an alternative vegetable for mineral sources.

Anti-nutritional component

Komak beans, like other legumes, contain antinutritional compounds that are important for plant defence mechanisms [69]. In terms of bioavailability, antinutritional compounds have negative effects on human health. As demonstrated in **Table 6**, various levels of antinutrients are present in komak beans due to their variety, location, climate and soil conditions [70].

Table 6 Anti-nutritional compounds of komak bean (*Lablab purpureus* (L.) sweet).

Compounds	Komak beans	
	White	Black
Phytic acid (mg/g dry matter)	11.77 ± 0.97[a]	14.08 ± 0.88[b]
Trypsin inhibitor (TIU/mg dry matter)	2.14 ± 0.02[a]	2.12 ± 0.01[a]
Tannin (mg/g dry matter)	11.94 ± 0.23[a]	12.99 ± 0.20[b]

Note: Values with different notations indicate significant differences within the same row ($p < 0.05$).

White komak beans exhibit a lower phytic acid content than their black counterparts. Additionally, it has been noted that lablab bean types from Nigeria contain more phytic acid both in their white (20.4 mg/g) and brown (18.8 mg/g) varieties [46], as well as other legumes, including fava bean (19.65 mg/g) and soybean (22.91 mg/g) [71]. In general, the phytic acid contained in beans ranges from 14.8 to 23.4 mg/g [72]. Phytic acid is an antinutrient because it inhibits the absorption of certain minerals, such as calcium, copper, zinc and iron, by binding them [73,74]. This can be eliminated by using processing methods. Soaking decreased phytic acid by 22 % in lablab beans, while autoclaving for 20 min at 121 °C removed 52 % phytic acid from those beans [75]. The combination of soaking, germination, skin peeling, and continued steaming renders phytic acid undetectable in common beans [76]. However, low levels of phytic acid, regarded as an antinutrient, are beneficial for health, such as for diabetes prevention [77].

The presence of anti-nutritional factor trypsin inhibitors can reduce the activity of digestive enzymes (trypsin and chymotrypsin), thereby inhibiting protein digestion, enlargement of the pancreas and muscle loss [78,79]. Komak beans exhibited a low trypsin inhibitor content (2 TIU/mg) compared to jack (23.58 TIU/mg) [80] and velvet (42.02 TIU/mg) beans [81], which contain 20× higher. The trypsin inhibitory activity of these compounds can be reduced by autoclaving the beans for 30 min at 121 °C which has been successfully applied in soybeans [82] and chickpeas [83].

The tannin contents of the studied komak beans were 12 - 13 mg/g, lower than Indian lablab bean (20 - 22 mg/g) [13], black bean (21 mg/g) [84], soybean (11 - 18 mg/g) [85], but higher than jack bean (8.25 mg/g) [86] and faba bean (1.51 mg/g) [87]. Excessively tannin-rich diets can inactivate enzymes that aid protein uptake [88]. Tannins can form complexes with enzymes, thereby inhibiting protein digestibility [89]. Boiling for 20 min reduced tannin by 31 % in lablab beans [90]. Sprouting for 24 h and autoclaving at 121°C for 30 min reduced kidney bean tannins by 97 % [91].

Despite having certain undesirable effects, tannin also provides health benefits, such as minimizing diabetes risk by increasing glucose absorption and lowering blood sugar levels [92]. Tannin is safe for consumption and does not cause any side effects at intake levels below the range of 1.5 - 2.5 g per day, but consumption above this range is responsible for low iron absorption from the diet [93].

Headspace volatile profiles

The composition and levels of volatile compounds detected in the headspace of the 2 types of komak beans were found to vary (**Table 7**). Different varieties of beans have also been reported to affect headspace volatile profiles [94]. The volatile compounds detected in the headspace included aldehydes, alcohols, phenols, hydrocarbons, ketones, pyrazine, acids, esters, terpene, pyrrole, naphthalenes and furan. The identified volatile compounds were similar to those reported in Lablab beans from Kenya [95]. Other studies have also discovered hydrocarbons, aldehydes, alkanes, alkenes, alcohols, ketones, furans and other compounds in varying amounts in faba beans [96], common beans [97] and soybeans [98].

Table 7 Volatile compounds of komak (*Lablab purpureus* (L.) sweet) bean by HS-SPME/GC-MS.

No	Volatile compounds	Code	Peak area (%)		Odor description*
			White komak beans	Black komak beans	
	Aldehyde				
1	Benzaldehyde	Ald1	0.68 ± 0.08[a]	0.81 ± 0.08[a]	strong sharp sweet bitter almond cherry
2	(E)-2-Tridecenal	Ald2	0.13 ± 0.02	nd	waxy citrus rind tangerine fatty creamy soapy watery cilantro
3	(E)-Cinnamaldehyde	Ald3	0.07 ± 0.01	nd	sweet spice candy cinnamon red hots warm
4	α-Hexylcinnamaldehyde	Ald4	nd	0.01 ± 0.01	fresh floral green jasmine herbal waxy
5	Furfural	Ald5	1.18 ± 0.24[a]	0.95 ± 0.21[a]	sweet woody almond fragrant baked bread
	Alcohol				
6	1-Octen-3-ol	Alc1	1.15 ± 0.03[a]	1.46 ± 0.34[a]	mushroom earthy green oily fungal raw chicken
7	6-methyl-5-Hepten-2-ol	Alc2	0.14 ± 0.25[a]	0.53 ± 0.07[a]	sweet oily green coriander
8	cis-Linalool oxide	Alc3	0.61 ± 0.03[a]	0.85 ± 0.31[a]	earthy floral sweet woody
9	2-Methylthioethanol	Alc4	0.04 ± 0.04[a]	0.11 ± 0.04[a]	sulfurous meaty
10	2-Octen-1-ol	Alc5	nd	0.35 ± 0.13	green vegetable
11	1-Nonanol	Alc6	0.64 ± 0.06[a]	1.21 ± 0.80[a]	fresh clean fatty floral rose orange dusty wet oily
12	2-Nonen-1-ol	Alc7	nd	0.18 ± 0.07	sweet fatty, melon cucumber vegetable
13	(E)- 2-Nonen-1-ol	Alc8	0.16 ± 0.01[a]	0.32 ± 0.07[b]	waxy green, violet melon
14	Epoxylinalol	Alc9	nd	0.07 ± 0.01	floral honey
15	1-Phenylethanol	Alc10	nd	0.11 ± 0.02	floral earthy green honeysuckle
16	Benzyl alcohol	Alc11	2.00 ± 0.24[a]	6.86 ± 2.86[b]	floral rose phenolic balsamic
17	Phenylethyl Alcohol	Alc12	0.55 ± 0.06[a]	0.58 ± 0.14[a]	floral rose dried rose flower rose water
	Phenol				
18	2-Formylphenol	Phe1	0.64 ± 0.06[a]	0.32 ± 0.19[b]	medical spicy cinnamon wintergreen cooling
19	o-Guaiacol	Phe2	0.78 ± 0.03[a]	0.67 ± 0.14[a]	phenolic smoke spice vanilla woody
20	2-methoxy-4-methylphenol	Phe3	0.04 ± 0.01[a]	0.30 ± 0.09[b]	spicy clove vanilla phenolic medicinal leathery woody
21	2-methyl-Phenol	Phe4	nd	0.03 ± 0.05	musty phenolic plastic medicinal herbal leathery
22	Phenol	Phe5	0.17 ± 0.04[a]	0.09 ±0.04[a]	phenolic plastic rubber
23	2,5-dimethyl-Phenol	Phe6	nd	0.01 ± 0.00	sweet naphthyl phenolic smoke bacon

No	Volatile compounds	Code	Peak area (%)		Odor description*
			White komak beans	Black komak beans	
24	2,6-dimethyl-Phenol	Phe7	nd	0.02 ± 0.01	sweet medicinal phenolic rooty coffee
25	m-Xylenol	Phe8	0.04 ± 0.01	nd	weak smoky roasted dark
26	m-Cresol	Phe9	0.03 ± 0.00[a]	0.10 ± 0.02[b]	medicinal woody leather phenolic
27	2,6-Dimethoxyphenol	Phe10	0.03 ± 0.00[a]	0.06 ± 0.02[b]	smoky phenolic balsamic bacon powdery woody
28	m-Eugenol	Phe11	0.74 ± 0.11[a]	0.12 ± 0.01[b]	spicy carnation
29	4-Vinylguaiacol	Phe12	2.72 ± 0.35[a]	0.71 ± 0.08[b]	sweet spicy clove carnation phenolic peppery smoky woody powdery
30	2,4-Di-tert-butylphenol	Phe13	1.72 ± 0.44[a]	0.33 ± 0.12[b]	phenolic
31	4-tert-Butylphenol	Phe14	0.06 ± 0.05	nd	oakmoss leather
32	Butylated hydroxytoluene	Phe15	0.20 ± 0.03[a]	0.09 ± 0.02[b]	mild phenolic camphor
	Hydrocarbon				
33	Dodecane	Hyd1	2.27 ± 0.29[a]	0.80 ± 0.26[b]	alkane
34	Pentadecane	Hyd2	1.63 ± 0.18[a]	0.76 ± 0.04[b]	waxy
35	Tetradecane	Hyd3	1.65 ± 0.19[a]	0.95 ± 0.15[b]	alkane
36	Nonadecane	Hyd4	0.24 ± 0.03[a]	0.18 ± 0.01[b]	bland
37	2-Methoxyanisole	Hyd5	1.71 ± 0.12[a]	0.28 ± 0.04[b]	sweet creamy vanilla phenolic musty
38	Anethole	Hyd6	nd	0.08 ± 0.03	sweet anise licorice medicinal
39	Quinoline	Hyd7	0.02 ± 0.01[a]	0.06 ± 0.05[a]	medical musty tobacco rubber earthy
40	Biphenyl	Hyd8	nd	0.13 ± 0.06	pungent rose green geranium
41	Methyleugenol	Hyd9	0.04 ± 0.01[a]	0.09 ± 0.09[a]	sweet fresh warm spicy clove carnation cinnamon
42	Diphenylmethane	Hyd10	nd	0.03 ± 0.03	sweet green wet plastic geranium
	Ketone				
43	Isophorone	Ket1	0.40 ± 0.04[a]	0.27 ± 0.21[a]	cooling woody sweet green camphor fruity musty cedarwood tobacco leather
44	γ-Caprolactone	Ket2	0.20 ± 0.02[a]	0.17 ± 0.11[a]	herbal coconut sweet coumarin tobacco
45	cis-Geranylacetone	Ket3	0.22 ± 0.00[a]	0.17 ± 0.04[a]	fatty metallic
46	2-Tridecanone	Ket4	0.16 ± 0.02	Nd	fatty waxy dairy milky coconut nutty herbal earthy
47	cis-Jasmone	Ket5	0.05 ± 0.01[a]	0.02 ± 0.00[b]	woody herbal floral spicy jasmin celery
48	Maltol	Ket6	0.07 ± 0.01[a]	0.06 ± 0.02[a]	sweet caramel cotton candy jam fruity baked bread
49	γ-Nonalactone	Ket7	0.32 ± 0.04[a]	0.26 ± 0.09[a]	coconut creamy waxy sweet buttery oily
50	1-(1-Naphthalenyl)-Ethanone	Ket8	nd	0.02 ± 0.01	sweet neroli orange blossom powdery
51	Perhydrofarnesyl acetone	Ket9	0.15 ± 0.04[a]	0.19 ± 0.12[a]	oily herbal jasmin celery woody
52	Dihydroactinidiolide	Ket10	nd	0.01 ± 0.00	musk coumarin
53	Farnesyl acetone	Ket11	0.06 ± 0.04	nd	flower ether
	Pyrazine				
54	2-methoxy-3-(1-methylpropyl)-Pyrazine	Pyr1	0.11 ± 0.10[a]	0.35 ± 0.07[b]	musty green pea galbanum bell pepper
	Acid				
55	Caprylic acid	Acd1	0.03 ± 0.01	nd	fatty waxy rancid oily vegetable cheesy
56	Lauric acid	Acd2	0.05 ± 0.01	nd	mild fatty coconut bay oil
57	Hexadecanoic acid	Acd3	0.12 ± 0.00[a]	0.09 ± 0.02[b]	slightly waxy fatty
	Ester				
58	Isopropyl myristate	Est1	nd	0.05 ± 0.02	faint oily fatty
59	Methyl palmitate	Est2	0.12 ± 0.02[a]	0.03 ± 0.01[b]	oily waxy fatty orris
60	Methyl salicylate	Est3	0.12 ± 0.01[a]	0.17 ± 0.05[a]	wintergreen mint
	Terpene				
61	α-Copaene	Ter1	nd	0.39 ± 0.04	woody spicy honey
62	β-Linalool	Ter2	0.80 ± 0.05[a]	0.78 ± 0.22[a]	citrus floral sweet bois de rose woody green
63	cis-Geraniol	Ter3	nd	0.19 ± 0.03	blueberry sweet natural neroli citrus magnolia
	Furan				
64	2 pentylfuran	Fur1	nd	0.72 ± 0.47	fruity green earthy beany vegetable metallic
	Pyrrole				
65	2-Acetylpyrrole	Prl1	0.14 ± 0.04[a]	0.09 ± 0.05[a]	musty nut skin maraschino cherry coumarinic licorice walnut bready
	Naphthalene				
66	Naphthalene	Nap1	1.65 ± 0.53[a]	0.38 ± 0.10[b]	pungent dry tarry

No	Volatile compounds	Code	Peak area (%)		Odor description*
			White komak beans	Black komak beans	
67	1,1,6-trimethyl-2H-naphthalene	Nap2	0.21 ± 0.02[a]	0.32 ± 0.02[b]	licorice
68	β-Methylnaphthalene	Nap3	nd	0.39 ± 0.18	sweet floral woody
69	2,6-dimethyl-Naphthalene	Nap4	nd	0.06 ± 0.03	grass
	Others				
70	sec-Butylamine	Oth1	0.39 ± 0.19[a]	0.44 ± 0.05[a]	fishy ammonia
71	Methyl nerolate	Oth2	0.02 ± 0.03	nd	floral herbal citrus fruity green geranium
72	Dibutyl phthalate	Oth3	nd	0.08 ± 0.06	faint odor

Note: nd, not detected.

Values with different notations indicate significant differences within the same row ($p < 0.05$). *The Good Scents Company Information System http://www.thegoodscentscompany.com/search2.html. These volatile compounds are generally derived from carbohydrates, triglycerides, proteins and free amino acids [94]. As shown in **Table 7**, the percentage area of alcohol was the most abundant volatile compound detected in the headspace in both types of komak beans, followed by hydrocarbons, phenols, aldehydes and ketones. Alcohols and aldehydes are chemical compounds formed by the oxidation reaction of lineic and linolenic acids in the presence of lipoxygenase and alcohol reductase or by fatty acid autoxidation decomposition [99,100]. The degradation of amino acids also contributes to the formation of alcohols [101]. The levels of benzyl alcohol (floral rose phenolic balsamic) and 1-octen-3-ol (oily green mushroom) in black komak beans were higher than those in white varieties. A previous study reported that 1-penten-3-ol and 1-octen-3-ol are the dominant volatile alcohol compounds in legumes and are derived from enzymatic reactions that produce an unpleasant odor [98]. The volatile compounds 1-octen-3-ol and 2-pentyl furan are indicators of beany flavor [102]. The different alcohol levels contributed to the distinct aromas of the 2 komak bean varieties.

Similarly, isophorone compounds (aromatic woody sweet green) (ketone classes) were predominant in both types of komak beans. The dominant volatile hydrocarbon compound in white komak beans was 2-methoxy anisole (sweet creamy vanilla phenolic musty), whereas tetradecane (alkane) was found in black komak beans. Furfural was the most abundant volatile aldehyde compound in both types of komak beans. In contrast to alcohols, ketones, aldehydes and hydrocarbons, which are derived from the oxidation of fatty acids, terpenes are naturally present in beans [98].

Principal Analysis Component (PCA)

The utilization of a PCA serves to offer an overview of the komak bean varieties by elucidating their proximate composition, color and anti-nutrient content (**Figure 3(a)**) and the volatile compounds detected in the headspace of komak beans (**Figure 3(b)**). The detection of 41 variable volatile compounds in the headspace was found to be significant in differentiating the various types of komak beans, and thus contributed to the PCA analysis. However, minerals and amino acid compositions were not included in the PCA as they failed to demonstrate a discernible separation of the sample groups.

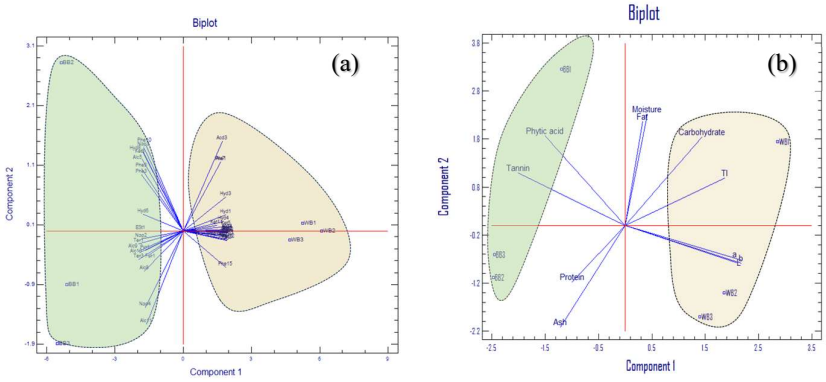

Figure 3 Principal component analysis of nonvolatile compounds (proximate, color, anti-nutrient compounds) (a), and volatile compounds detected in headspace (b) of white and black komak beans.

The sample of white komak bean was found to be located in the positive coordinate of PC1 and was characterized by its specific levels of carbohydrates, trypsin inhibitors and color (Lab). Additionally, 19 volatile compounds were detected in the headspace of the sample (2-octen-1-ol, (E)-2-nonen-1-ol, epoxylinalol, 1-phenylethanol, benzyl alcohol, 2-methoxy-4-methylphenol, m-cresol, 2,6-dimethoxyphenol, 2-methoxyanisole, biphenyl, 1-(1-naphthalenyl)-ethanone, 2-methoxy-3-(1-methylpropyl)-pyrazine, isopropyl myristate, α-copaene, cis-geraniol, 2-pentylfuran, 1,1,6-trimethyl-2H-naphthalene, β-methylnaphthalene, 2,6-dimethyl-naphthalene).

Furthermore, the black komak bean exhibited negative coordinates along the PC1 axis, characterized by the presence of phytic acid, tannin and 22 volatile compounds ((E)-2-tridecenal, (E)-cinnamaldehyde, 2-nonen-1-ol, 2-formylphenol, m-eugenol, 4-vinylguaiacol, 2,4-di-tert-butylphenol, 4-tert-butylphenol, butylated hydroxytoluene, dodecane, pentadecane, tetradecane, nonadecane, anethole, 2-tridecanone, cis-jasmone, farnesyl acetone, caprylic acid, lauric acid, hexadecanoic acid, methyl palmitate, naphthalene). The variations were influenced by the different bean varieties. Black komak beans were found to have higher tannin levels compared to white komak beans, which is consistent with previous studies on black-colored lima beans [28]. Additionally, black komak beans are characterized by the presence of 2-pentylfuran, a compound associated with the beany flavor, which is derived from lipid degradation through an enzymatic reaction [102].

Conclusions

The physical characteristics, nutrient and anti-nutrient compounds and mineral content, as well as volatile compounds detected in the headspace, were measured in both varieties of komak beans. The dimensions of the beans were similar. However, different varieties of komak bean were found to have significantly different nutrient, anti-nutrient and volatile compound profiles detected in the headspace. Both types of komak beans were found to be a good source of carbohydrates and proteins. Additionally, komak beans are a potent source of minerals. The white komak bean was found to contain fewer anti-nutrients (phytic acid, tannin and trypsin inhibitor) than the black komak bean. The predominant volatile compounds in the beans were alcohols, aldehydes, hydrocarbons and ketones, with 1-octen-3-ol and benzyl alcohol being the primary constituents. These findings highlight the importance of komak beans as a valuable source of nutrition, particularly in rural areas and in preparing these legumes for consumption.

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