Nutritional Contents and Bioactive Compounds among Several Variants of Dolichos lablab: Fundamental Facts for Functional Food Development

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Short Communications

Nutritional Contents and Bioactive Compounds among Several Variants of *Dolichos lablab*: Fundamental Facts for Functional Food Development

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ABSTRACT
To date, the data describing various nutritional and secondary metabolites content of Lablab beans is incomplete. Therefore, this study evaluated the nutritional value, secondary metabolites, and antioxidant activity of three different variants of Lablab beans, i.e., brown, black, and cream beans. The results showed that the brown Lablab beans had outperformed other variants according to their nutritional value and flavonoid content with outstanding DPPH scavenging activity. However, the black beans also showed good bioactive contents through their total phenolic percentage with decent reducing activity via the FRAP assay. Those who are keen in developing functional food from Lablab beans should consider this data as a reference.

Legumes have provided nutritional value for years, contributing to the development of agriculture and food security (Considine et al. 2017). Not only are they a staple food in several regions of the globe, but legumes also provide valuable nutritional and health benefits (Polak et al. 2015; Piergiovanni 2021). The consumption of legumes has been reported to have protective roles against modern society's health problems, such as diabetes mellitus, hyperlipidemia, and cardiovascular diseases (Polak et al. 2015; Hermanto et al. 2022a; Hermanto et al. 2022b). Furthermore, the bioactive compounds in legumes also provide numerous biological activities to achieve physiological homeostasis (Çakir et al. 2019). Those facts are more than enough to describe the vital role of legumes in developing social health status. There are many species of legumes worldwide, but not all beans are known in the society. One of the underutilized legumes is Lablab beans (*Dolichos lablab*), also known as Koro Komak in Indonesia (Purwanti et al. 2019b). Natively grown in the African continent and Indian subcontinent, Lablab beans have become the primary source of energy due to their rich fibre and carbohydrate contents (Maass et al. 2010; Purwanti et al. 2019a). Moreover, Lablab beans also have superior environmental adaptation due to their ability to grow in drought areas (Missanga et al. 2021). Their innate nature may benefit the maintenance of food security, particularly in lands with low water supply. Thus, the cultivation of Lablab beans provides a promising means in maintaining primary food stock in dry areas.

Three primary accessions or variants of Lablab beans have been identified in Indonesia (Purwanti et al. 2019b). Those accessions are commonly identified based on the beans' colour, i.e., brown, black, and cream (figure 1). Although other variants may exist, these three are commonly found in several regions in Indonesia, such as East Java (Probolinggo and Madura Island) and West Nusa Tenggara (Purwanti et al. 2019b). The previous study reported the bioactivity of bioactive compounds and nutritional values of Lablab beans (Purwanti et al. 2021; Purwanti et al. 2022). Nevertheless, no report addresses the nutritional differences among the variants of Lablab beans. Lablab beans have numerous bioactivities including antioxidant (Maheshu et al. 2013), antidiabetic (Purwanti et al. 2022), antivirus (Purwanti et al. 2021), antimicrobial (Bai-Ngew et al. 2021), and anti-inflammatory properties (An et al. 2020). These bioactivities make it a promising and excellent candidate for functional food development. The details on the nutritional comparison among Lablab beans will provide a fundamental guideline for determining suitable variants for functional food development, and it will be addressed by this study.

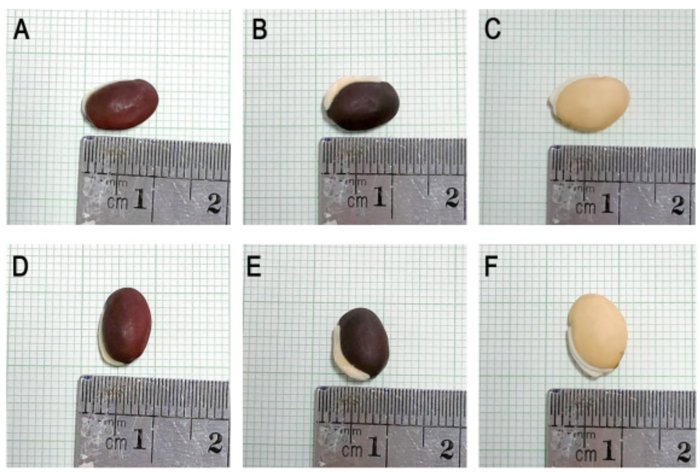

Figure 1. The most popular Lablab beans variant found in Indonesia are brown (A, D), black (B, E), and cream (C, F). The beans have been positioned to obtain the length (A-C) and the width (D-F).

The sample of the beans for this study was obtained from Sumenep, Madura Island, East Java, Indonesia during dry season in 2019. Details of the sample profiles and precise locations are as described in the previous study (Purwanti et al. 2019b). The beans were stored in 4°C until used. The beans were processed as per the extraction method previously mentioned in (Purwanti et al. 2022). Briefly, grounded beans were soaked in 96% ethanol with 3:1 ratio (volume in L and weight in kg) for 24 hours. The soaked beans powder then filtered to obtain the filtrate and homogenate. The filtrate then rotary evaporated to separate solvent and solute followed by freeze drying process to obtain Lablab beans' extract. The extract then processed to the subsequent analysis.

The crude fibre, total protein, and crude fat content were determined according to the previous protocol (Thiex 2009). Amylose and amylopectin content were also measured colorimetrically using a previously described method (McGrance et al. 1998). On the other hand, IKA C2000 Calorimeter System (IKA Works, Germany) was employed to calculate the total calories as per manufacturer's protocol.

To determine the secondary metabolites content, total phenol and total flavonoid was employed. Total flavonoid was performed as per a previous protocol with minor modifications (Pratami et al. 2018). Quercetin was used as the standard flavonoid compound. The extract was dissolved in water, then 50 µL of the dissolved extract was mixed with 10 µL of 5% $NaNO_2$, followed by the addition of 150 µL of water and 10 µL of 1 M CH_3COONa, consecutively. The sample was then incubated at room temperature for 40 minutes. After incubation, the sample was quantified using a spectrophotometer at 415 nm wavelength. The total flavonoid concentration was described as percent (%) of Quercetin Equivalent (QE) according to the standard curve.

Total phenol was measured according to the previous study with minor modifications (Hyun et al. 2014), with gallic acid as the standard. The sample was diluted in water, and 100 µL of the sample was added to 1 mL of Folin Ciocalteu reagent and incubated for 5 minutes at room temperature with minimum light ambiance. 1 mL of 7.5% Na_2CO_3 was added to the mixture, followed by incubation for 90 minutes in the same condition mentioned beforehand. Upon incubation, the sample was then quantified spectrophotometrically at 725 nm. The total phenol was defined as percentage of Gallic Acid Equivalent (GAE) as per the build standard curve.

The antioxidant activity was measured by DPPH scavenging and Ferric Reducing Antioxidant Potential (FRAP) assays. The method for DPPH scavenging and FRAP reducing power was performed as described in the earlier work (Irshad et al. 2012). All data were analysed by one-way ANOVA followed by Least Significant Difference (LSD) post-hoc analysis. The data was determined as significantly different if the p-value is < 0.05. The data was then visualised as mean $\pm$ standard deviation.

All Lablab bean variants have good nutritional content according to the caloric, total fibre, protein, crude fat, amylose, and amylopectin content. The fibre content of black Lablab bean was the highest, with 8% fibre content, followed by the cream and brown variants (table 1). The considerable content of dietary fibre in Lablab beans displayed an immense potential to be developed as a functional food. As commonly known, fibre consumption can improve physiological homeostasis, particularly in relations to lipid and glucose metabolism (Jahan et al. 2020). High fibre content is also suitable for dietary intervention to prevent obesity (Dayib et al. 2020). With regards to total protein content, cream beans had the same protein content as brown beans, while black beans had the lowest content of total proteins (table 1). The high percentage of total protein content in Lablab beans would be valuable as a candidate for functional foods since plant-based protein have broad health benefits, such as antioxidant, antiviral, antidiabetic, and anticancer properties (Maphosa et al. 2017; Liu et al. 2020; Sipahli et al. 2021; Roy et al. 2022; Purwanti et al. 2022). Nevertheless, specific treatment, such as isoelectric preparation, was suggested to obtain a protein isolate with adequate quality and good functional properties (Subagio 2006).

Cream beans have the highest percentage of crude fat among all variants (table 1). The low-fat content of Lablab beans exhibit a great potential as functional food compared to other beans since most legumes contain around 1,5% crude fat total (Etiosa et al. 2017). Low-lipid food provides more health benefits with deleterious high-energy intake, particularly in areas with high level of famine cases (Delaš 2011; Robson 2013). For instance, West Nusa Tenggara province in Indonesia has the highest occurrence of hunger cases (Mone & Utami 2021). Interestingly, this region also founds a large distribution of Lablab beans (Jayanti et al. 2011). The utilization of Lablab beans to reduce the incidence of famine should be considered. Thus, the low-fat content of Lablab beans displayed their potential as a functional food candidate.

This study also measured the amount of amylose and amylopectin as part of its functional properties and energy source. The black Lablab bean has the highest amylose content, with 15% amylose content, followed by brown and cream (table 1). In contrast, black beans had the lowest amylopectin content compared to the other analysed variants (table 1). Similarly, black beans also had the lowest calorie per gram compared to other variants of Lablab beans (table 1). A food source with high amylopectin induces a better glycemic response, especially during fasting (Singhania & Senray 2012). This starch also provides higher energy intake than low amylopectin sources (Singhania & Senray 2012). Moreover, the increasing ratio of amylopectin/amylose reflects better nutrient digestibility (Gao et al. 2020). A diet containing large portion of amylopectin positively associated with the postprandial insulin response resulted in more efficient nutrient uptake and glucose metabolism (Gao et al. 2020). Therefore, brown and cream beans may become potential candidates as functional food.

This study demonstrated that Lablab beans have been found to have comparable levels of total protein with *Vigna radiata* and *Pisum sativum*, and even higher levels than *Glycine max* and *Lens culinaris* (Singh et al. 2022). In addition, Lablab beans have a favourable nutritional profile with higher dietary fibre and lower fat content compared to *Phaseolus vulgaris*, *L. culinaris*, *P. sativum*, and Edamame (Dhingra et al. 2012; Mullins & Arjmandi 2021; Didinger & Thompson 2021). The amylose content in Lablab beans was higher than *Cicer arietinum* and *G. max* (Tayade et al. 2019). Moreover, the amylose and amylopectin content in Lablab beans also similar with *V. angularis*, a "red pearls" that has good nutrients and hypoglycemic activity (Zhang et al. 2022).

The total phenolic and flavonoid contents evaluation demonstrated that brown beans exhibited the greatest content of flavonoid ($p < 0.01$), while the cream beans had the most negligible flavonoid content (table 2). On the other hand, the phenolic content was highest in black beans compared to other Lablab variants ($p < 0.05$, table 2). This result showed that Lablab beans have many phenolic compounds, with the flavonoid group being the most abundant in brown beans. In other words, the other variants may comprise of other phenolic compounds like phenolic acids, tannins, and other phenolic compounds (Purwanti et al. 2022). The current result was also higher than several edible beans, such as *P. vulgaris*, *P. lunatus*, *V. radiata* and *C. arietinum* (Zhao et al. 2014). Nonethe-

Table 1. The comparison of primary metabolites and nutritional content among Lablab bean variants.

Variant	Fiber (%)	Protein (%)	Crude Fat (%)	Amylose (%)	Amylopectin (%)	Calorie (kcal/g)
Brown	7.02 ± 0.015^a	24.91 ± 0.06^a	0.36 ± 0.01^a	14.41 ± 0.095^a	87.18 ± 0.030^a	3.86 ± 0.005^a
Black	8.16 ± 0.040^b	23.43 ± 0.23^b	0.45 ± 0.03^b	15.46 ± 0.515^b	85.79 ± 0.015^b	3.83 ± 0.002^b
Cream	8.11 ± 0.005^c	24.82 ± 0.15^a	0.55 ± 0.04^c	13.52 ± 0.120^c	87.90 ± 0.025^c	3.85 ± 0.002^a

Note: The data was presented as mean ± standard deviations (n = 3). Different alphabetical notation indicates significant difference with $p < 0.05$ based on LSD test.

less, the total flavonoid contents of Lablab beans were lower compared to *P. sativum*, *C. arietinum*, *V. radiata*, *P. vulgaris*, *P. lunatus*, *L. culinaris*, *Vicia faba*, and *G. max* (Sharma & Giri 2022). Although flavonoids are the most abundant phenolic compounds with various biological activities (Kumar & Pandey 2013), other phenolic compounds, either simple phenols or polyphenols other than flavonoids, have also been reported to have bioactivities to improve physiological homeostasis, mainly through their antioxidant activity (Shahidi & Ambigaipalan 2015; Singh et al. 2017).

The high flavonoid content was positively correlated with antioxidant activity through DPPH scavenging activity, where brown beans had the highest scavenging activity compared to the others (table 2). However, the ferric reducing activity was stronger in variant with higher phenolic contents (table 2). These results were supported by a structure-activity relationship between radical scavenging from different phenolic compounds and the radical scavenging mechanism in DPPH and FRAP assay. Flavonoids have an *ortho*-dihydroxyl structure that plays a role in radical scavenging during DPPH assay by forming an intramolecular hydrogen bond and more stable *ortho*-hydroxyl phenoxyl radical during the oxidation process of radical scavenging (Zheng et al. 2010). Alternatively, other phenolic compounds, such as phenolic acids, have *ortho* or *para* position of the hydroxyl group in its benzene ring (Spiegel et al. 2020). Those structural differences influence the radical scavenging mechanism of flavonoids and other phenolic compounds in different antioxidant assay. Hydrogen Atom Transfer (HAT), Single-Electron Transfer followed by Proton Transfer (SET-PT), and Sequential Proton-Loss Electron Transfer (SPLET) are taking place during the DPPH assay. Contrary, SPLET is the main mechanism during the electron transfer enthalpy in the FRAP reaction system (Chen et al. 2020). Ferulic Acid, Hydroxycinnamic Acid, Sinapinic Acid, Coumaric Acid, and Isovanillic Acid are identified phenolic acids in Lablab beans. Also, Rutin and Isoquercetin are flavonoids that also found in Lablab beans (Purwanti et al. 2022). Those compounds were identified in adequate abundance in Lablab beans and may perform as radical scavenger during this study. Nevertheless, future studies are required to compare the secondary metabolites among different variant of Lablab beans to comprehend the phytochemical content differences better. Despite the different mechanisms and types of bioactive compounds in performing the antioxidant activity, it has been displayed that brown and black beans exhibit solid antioxidant properties.

This study shows that all Lablab bean variants have good amount of nutritional value, total phenol and flavonoid contents, and antioxidant activity. Lablab beans have adequate nutritional values surpassed other types of edible beans. Despite having lower flavonoid contents compared to commonly consumed beans, the phenolic compounds in these beans still exhibit superior performance. Finally, the cream variant shows slightly lower nutritional contents and bioactive compound compared to

Table 2. The total flavonoid, phenolic, and antioxidant capacity according to the DPPH scavenging capacity and FRAP analysis.

Variant	Total Flavonoid (%)	Total Phenol (%)	DPPH Scavenging (%)	FRAP (μM $FeSO_4$/mg)
Brown	26.25 ± 0.34^a	40.56 ± 0.69^a	78.87 ± 1.59^a	1154.58 ± 4.17^a
Black	12.99 ± 0.31^b	45.28 ± 2.56^b	40.01 ± 0.16^b	2398.05 ± 4.81^b
Cream	12.15 ± 0.06^c	40.32 ± 3.06^a	24.53 ± 0.16^c	1061.53 ± 2.41^c

Notes: The data was presented in mean ± standard deviations (n = 3). Different alphabetical notation indicates significant difference with $p < 0.05$ based on LSD test.

the other analysed Lablab beans. Still, some varieties possess a promising characteristic as a functional food candidate owing to their nutritional value.

AUTHORS CONTRIBUTION

EP designed the research and acquired the project funding, FE collected and analysed the data, WP and TIP wrote the manuscript, and IGNAW performed critical review and revision.

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CONFLICT OF INTEREST

There is no conflict of interest raised in this study.

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