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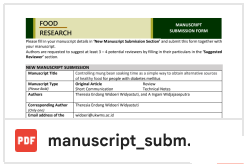
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# Controlling mung bean soaking time as a simple way to obtain alternative sources of healthy food for people with diabetes mellitus

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**Abstract**

This research examines the effect of soaking time on the in vitro digestibility of starch and protein of mung bean, in order to obtain an alternative source of healthy food in a simple way. A single factor of randomized block design used in this study. Each treatment level was conducted in triplicates. The whole mung bean seeds were subjected to five different times of soaking, namely control (without soaking) and soaking for 2, 4, 6 and 8 hrs. Total starch, in vitro starch digestibility, protein content and in vitro protein digestibility of freeze dried and grounded whole mung beans were analyzed. The results showed a trend of the total starch content and starch digestibility decreasing during longer soaking time, in a range of 39.14-33.52 % dry basis and 57.46-49.82% respectively. Based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slow digesting starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking, up to 6 hours of soaking time (in a range of 46.93%-51.29%). Based on these results, the suggested soaking time was 6 hours that gave optimum digestibility of starch and protein for human consumption. The recommendation in order to obtain the benefits as a healthy food source for people with diabetes mellitus.

**Keywords:** in vitro digestibility, starch, protein, mung beans, soaking time

## 1. Introduction

Mung bean (*Vigna radiata*) has been consumed in China for over 2,000 years, in the form of many kinds of food such as sprouts, noodles, cookies and others (Fayyaz *et al*., 2018). Mung bean, as one of the legumes, is being an important part of the dietaries of Indonesian people and contribute substantially to the nutrient intake of human beings, including children. Mung bean is less flatulent and is well tolerated by children (Dahiya *et al*., 2015).

Mung bean is one of the commodities with potency sources of starch and protein. Starch is the most component of carbohydrates in mung beans, about 30.74 ± 3.39% (Widjajaseputra *et al*., 2019a). Since starch is the most significant component in mung bean, the characteristics of starch will determine its

suitability for its end use. Mung bean which its high amylose content can improve the swelling power and gel texture of a starch noodle product. This is as reported by Li *et al*. (2008), that the high-quality starch noodle made from mung bean starch results from its high amylose content. Menon *et al.* (2016) studied gluten-free starch noodles from sweet potatoes and got that fortification with mung bean starch reduced the rate of release of glucose from cooked noodles in vitro condition. The high amylose content showed higher viscosities which could be used in food products that require this property, such as thickeners for creams, sauces, soups and puddings. However, Li *et al.* (2011) declared that starches from different mung bean varieties showed significant differences in the physicochemical, thermal, and pasting properties. So that they may suit diverse applications such as porridge, cakes, snacks, beverages etc.

Besides as a source of starch, mung bean is an excellent source of vitamins, minerals, and protein with ideal essential amino acid profile (Mubarak, 2005). The specific profile of mung bean amino acid allows its usage to supplement cereals and rice in particular. The mung bean flour could be used as a supplement for wheat flour, increasing the nutritional quality of bakery products (Marquezi *et al*., 2016). However, the original properties of the components in the mung bean will change depending on the processing applied.

Soaking is the beginning of legume processing which is usually done in preparation before use. During soaking, water entered the bean, its tissues hydrated, and some enzymes can be activated to break down complex structures such as starch and protein into simpler compounds. The treatment result in alteration of their nutritional quality which could either be reduction in nutrients and antinutrients or improvement in digestibility or availability of nutrients (Kaur *et al*., 2015). Pagar et al. (2021) who studied the horse gram, found that treatment having 6 hrs soaking, 72 hrs germination, and drying at 70˚C was the best where a maximum decrease in the anti-nutritional factors at the same time flour functional properties got enhanced due to the soaking and germination. It is essential to understand these changes to select appropriate techniques to obtain maximum nutritional and health benefits. In a previous study, it was reported that the digestible (total sugar) and indigestible carbohydrates (resistant starch) increased during four hours of soaking (Widjajaseputra *et al*., 2019a). Widjajaseputra *et al*. (2019b) also reported that the soaking treatment improved the protein quality. In relation to the provision of healthy food for people with diabetes, high-quality protein sources are needed, but the type and quality of carbohydrate digestibility need to be considered. The study of the potency of soaked mung bean as a healthy food source, especially for diabetic people, based on the digestibility perspective is needed.

## 2. Materials and methods

71 *2.1 Materials*

72 The commercial mung bean was obtained from a local market in Surabaya, East Java, Indonesia.
73 All the chemicals, standards, and reagents were of analytical grade.

74

75 *2.2 Soaking procedure*

76 According to Widjajaseputra *et al*. (2019b), the mung bean was sorted. Only intact and sound
77 grains were washed and soaked (1:5 w/v) in distilled water at 30°C for 0 (control), 2, 4, 6 and 8 h,
78 afterward the grains were drained and freeze-dried (Bluewave B-10B Vacuum Freeze Drier; China) to
79 2% - 3% moisture content. The dried grains were ground (Miyako, Indonesia), wrapped in an airtight
80 plastic container and aluminum foil bag as secondary packaging, and then stored in a refrigerator
81 (LG, Indonesia) at 5 ± 1ºC until analyzed.

82

83 *2.3 The experimental design and statistical analysis*

84 The experimental design used in this study was a single factor with a randomized block design.
85 The whole mung bean seeds were subjected to five different times of soaking, namely control
86 (without soaking/P0) and soaking for 2 (P1), 4 (P2), 6 (P3), and 8 hours (P4). Each treatment level
87 was conducted in triplicates. Total starch, in vitro starch digestibility, protein content and in vitro
88 protein digestibility of freeze dried and grounded whole mung beans were analyzed. Water content
89 analysis was used to dry basis calculation. Data were expressed as mean ± standard deviation (SD)
90 for the three in each group (n=3). The data were subjected to one-way ANOVA ($p < 0.05$) with a least
91 significant difference (LSD) test at $p < 0.05$ using SPSS (version 19) for comparative analysis.

92

93 *2.4 Analysis Methods*

94 *2.4.1 Total starch analysis*

95 According to Goni *et al.* (1997), total starch was measured as glucose by incubating the
96 sample suspension in the optimum condition of amyloglucosidase for 45 min measuring activity.
97 The factor conversion of glucose to starch was 0.9.

98 *2.4.2 In vitro starch digestibility analysis*

99 Starch digestibility was determined by measuring digestible starch in vitro (Goni *et al*., 1996
100 and Goni *et al.,* 1997). The principle of digestible starch determination was to analyze total
101 starch with enzymes and measured undigested starch during 180 minutes within a 30 minute

interval, then calculated the percentage of hydrolyzed starch (digestible starch) in equations (a) and (b) as follows:

(a) Digestible starch = (total starch – undigested Starch)

(b) Starch digestibility (%) = (total starch – undigested starch)/total starch x 100%

*2.4.3 Protein content analysis*

The principle of determining protein content using the macro Kjeldahl method (AOAC, 2010). The protein in the sample was determined by measuring the amount of nitrogen (N). The measured nitrogen content was multiplied by the conversion factor resulting in protein content. The conversion factor used was 6.25.

*2.4.4 In vitro protein digestibility*

The principle of determining in vitro protein digestibility is to compare the total nitrogen content after the sample is treated with protein digestive enzymes (pepsin) with the total N of the initial sample. Nitrogen content was measured using the Kjeldahl micro method. Calculation of protein digestibility as stated in equation (c).

(c) Protein digestibility $= \frac{\text{N total filtrate}}{\text{N total ingredients}}$ x diluting factor x $100\%$

## 3. Results and discussion

*3.1. Effects of soaking time on total starch, undigested starch and starch digestibility*

The total starch of mung bean seeds decreased from 41.13% dry to 33.52% dry basis during 8 hours of soaking, because more intensive hydrolysis occurred during the longer soaking time (Table 1). The decrease of total starch was due to the leaching out of the soluble part of starch in soaking water and hydrolysis process to simpler compounds such as sugars and dextrin. The same thing was also found by Grewal and Jood (2009), that a significant decrease in starch content was followed by increased sugar content due to soaking and cooking of green gram.

Legume starch contains amylose higher than cereal or tuber starch. This starch has a lower bioavailability than most other starches when it is raw or retrograded (Guillon and Champ, 2002). Widjajaseputra *et al.* (2019a) stated about amylose level of mung beans (32.56 ± 0.31%) is higher than cereal (around 25%) and tuber starch (around 17%-19%). Singh *et al.* (2003) state that the factors which could influence starch retrogradation were the amylopectin content, intermediate materials, size and

shape of the granules, the botanical source, and the amylose content. A higher proportion of amylose content being linked to a higher tendency to retrogradation would affect the level of digestibility.

The starch digestibility decreases (from 57.46% to 49.82%) with the longer soaking time, along with the decreasing of total starch during the soaking treatment up to 8 h of soaking from 39.14% to 33.52% (Table 1). If compared with the control, soaking for 2 hours resulted in a significant increase in starch digestibility. This is because there were starch granules that were more readily hydrolyzed by enzymes that become active due to the imbibition of water in the soaked mung bean seeds. After 4 hours of soaking time, the decrease in starch digestibility was not significantly different. Figure 1 shows that based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slow digesting starch.

The increasing of undigested starch up to 3 hours of digestion (in vitro) can be affected by the number of resistant starch (RS). RS is defined as the portion of starch and starch products that resist to digestion, passing directly through the small intestine (Fabbri *et al.*, 2016). Widjajaseputra *et al.* (2019a) stated that RS of mung bean was increased from 11.12% db to 18.49% db if soaked for 4 hours, although it was decreased significantly (13.65% db) if soaking was continued for up to 8 hours. This phenomenon is in line with the statement of Lang et al. (1999) that mung bean starch contains RS 11% db and has a long absorption period, which within 4.5 hours after consumption has not been completely digested. It was due to the high level of amylose of mung bean starch (around 32 %– 34%). The difference in RS can be caused by differences in starch structure in various amylose-amylopectin ratio among of different starch granules which affects the RS level in processed food, included during seeds soaking and freeze-drying treatment in samples preparation. Retrogradation of amylose can be occurred during soaking and freeze drying treatment in sample preparation, and part of starch was being to exist as RS3. RS3 is starch that has been retrograded into more highly stabile crystalline structures. In addition, Fabbri *et al.* (2016) found that the cooling process of legumes can increase RS as a result of retrogradation. This phenomena caused a decrease in the level of starch digestibility. The different process following the soaking would give the different effect on starch digestibility. As an example, if soaking was followed by dehulling and cooking, the level of starch digestibility would increase as obtained by Grewal and Jood (2009).

*3.2. Effects of soaking time on protein content and protein digestibility*

The values of protein digestibility were significantly different on 6 hours (P3) and 8 hours (P4) of treated soaking time compared to Po, P1, and P2 as shown on Table 2. Protein digestibility increased during soaking treatment, up to 6 hours of soaking time, and then the protein digestibility slightly

decreased on 8 hours of seed soaking.

This phenomenon was affected by increasing the soluble protein of mung bean seeds from 108.96 mg/g of dry weight in raw seeds without soaking to 159.81 mg/g of dry weight in seeds with 6 hours of soaking time (Widjajaseputra *et al*., 2019b). The higher of soluble protein indicated the readiness of proteins to be digested. Based on protein digestibility shown in Table 2, the recommended soaking time for mung bean seed was 6 hours, because a germination process has taken place longer than 6 hours of soaking. During germination periods there were hydrolyzing processes that would effect on protein and starch degradation to produce energy for the new plant. This phenomena was revealed by Grewal and Jood (2009) that germination process decreased starch content, thereby increasing soluble sugars and improved starch digestibility to be 49% and 48% in two different of green gram (*Vigna radiata L.)* cultivars respectively.

### *3.3. Perspective of mung bean as healthy food source for people with diabetes mellitus*

The high protein digestibility of mung beans as a result of soaking for 6 hours can support its usage as a good food source of protein. Combination of mung bean protein and rice protein in 3:4 ratio respectively can increase the chemical amino acid score to be 72 (Dahiya *et al*., 2015). Based on this recommendation, an increase in the protein bioavailability can be obtained. Consumption of legumes provides quality protein along with other micronutrients without adding extra energy or fat. According to Mak *et al.* (2018), in fact a diet high in protein-low starch was associated with a lower risk for gestational diabetes mellitus among women who were overweight at pre-pregnancy. Mung bean seeds are an affordable source of not only protein but also the starch, which has an advantage of consisting higher resistant starch compared to cereal, root, and tuber starch. Based on these characteristics, mung bean can be used as a good source of resistant starch with a high protein content in various food applications.

Mung beans as one kind of variety of pulses, are high in fiber and have a low glycemic index, making them particularly beneficial to people with diabetes by assisting in maintaining healthy blood glucose and insulin levels (Dipnaiki and Bathere, 2017). Previously, the same thing was also reported by Rebello *et al.* (2014), that mung bean like other legumes had a medium glycemic index (GI) and high content of dietary fibers, which makes benefit to be a healthy food source. High resistant starch content combined with medium GI is positive attributes that could promote the product as better food choice not only for diabetes mellitus patients but also for people which is suffered from celiac disease, obesity and other

malnutrition symptoms. In particular for the nutrition management of gestational diabetes mellitus, it is important to focus on quality of carbohydrates and encourage consumption of vegetables, fruits, complex carbohydrates, and high-fibre foods (Kapur *et al*., 2020). A balanced diet consisting healthy carbohydrate sources with adequate proteins and fats based on individual and cultural food preferences as well as based on physical activity and physiological status will results in weight control as well as diabetes management (Devi *et al*., 2021).

## 4. Conclusion

Based on the rate of starch hydrolysis of soaked mung beans, the mung bean starch is a slow digesting starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking up to 6 hours of soaking time (in a range of 46.93%-51.29%). Based on the obtained data, the recommended soaking time was 6 hours which revealed the optimum digestibility of starch and protein for people with diabetes mellitus. The further investigation will be needed to provide to get better food choice not only for diabetes mellitus patients, but also for those suffering from celiac disease, obesity and other malnutrition symptoms.

## Conflict of interest

The authors declare no conflict of interest.


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Table 1. Effects of soaking time on total starch, undigested starch and starch digestibility

| Soaking time (Hour) | Total starch (% dry basis ± SD)* | Undigested starch (% dry basis ± SD)* | Starch digestibility** (% ± SD)* |
|---|---|---|---|
| P0 | 41.13 ± 0.19 c | 19.16 ± 0.45 b | 53.42± 1.31 b |
| P1 | 39.14 ± 0.72 b | 16.65 ± 0.13 a | 57.46± 1.12 c |
| P2 | 38.85 ± 0.18 b | 18.70 ± 0.56 b | 51.87± 1.67 ab |
| P3 | 38.18 ± 0.44 b | 18.53± 0.34 b | 51.47± 0.31 a |
| P4 | 33.52 ± 1.29 a | 16.82± 0.21 a | 49.82 ± 1.30 a |

Values are means ± standard deviations (n=3).
P0 = control (without soaking), P1 = soaked 2 h , P2 = soaked 4 h, P3= soaked 6 h, P4= soaked 8 h.
* Different superscripts in the same column showed a significant difference based on the LSD Test ($p < 0.05$).
** Starch Digestibility (%) = (Total Starch – Undigested Starch)/(Total Starch) x 100%

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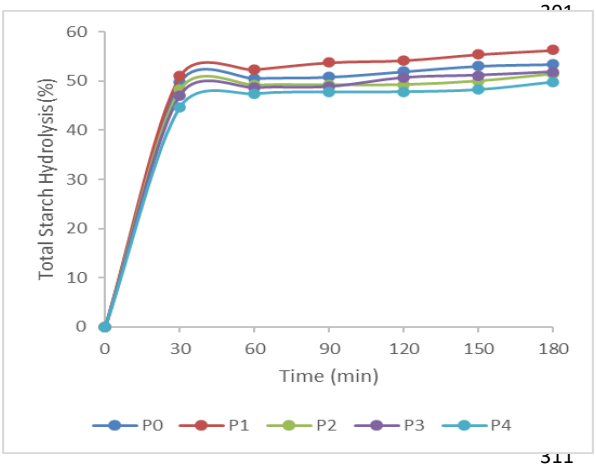


Figure 1. Mung bean starch hydrolysis rate. P0 = control (without soaking), P1 = soaked 2 h , P2 = soaked 4 h, P3= soaked 6 h, P4= soaked 8 h.

Table 2. Effects of soaking time on protein content and protein digestibility

| Soaking time (hour) | Protein content (% dry basis ± SD) | Protein digestibility* (% ± SD) |
|---|---|---|
| P0 | 26.29 ± 0.27 | 46.93 ± 1.53[a] |
| P1 | 25.88 ± 0.40 | 48.41 ± 0.38 [ab] |
| P2 | 25.90 ± 0.25 | 48.56 ± 0.54 [ab] |
| P3 | 25.68 ± 0.33 | 51.29 ± 1.20 [c] |
| P4 | 26.10 ± 0.82 | 49.83 ± 3.00 [bc] |

Values are means ± standard deviations (n=3)
P0 = control (without soaking), P1 = soaked 2 h , P2 = soaked 4 h, P3= soaked 6 h, P4= soaked 8 h.
* Different superscripts in the same column showed a significant difference based on the LSD Test ($p < 0.05$)

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# Controlling mung bean soaking time as a simple way to obtain alternative sources of healthy food for people with diabetes mellitus

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**Abstract**

This research examines the effect of soaking time on the *in vitro* digestibility of starch and protein of mung bean, in order to obtain an alternative source of healthy food in a simple way. A single factor of randomized block design used in this study. Each treatment level was conducted in triplicates. The whole mung bean seeds were subjected to five different times of soaking, namely control (without soaking) and soaking for 2, 4, 6 and 8 hrs. Total starch, in vitro starch digestibility, protein content and in vitro protein digestibility of freeze dried and grounded whole mung beans were analyzed. The results showed a trend of the total starch content and starch digestibility decreasing during longer soaking time, in a range of 39.14-33.52 % dry basis and 57.46-49.82% respectively. Based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slow digesting starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking, up to 6 hours of soaking time (in a range of 46.93%-51.29%). Based on these results, the suggested soaking time was 6 hours that gave optimum digestibility of starch and protein for human consumption. The recommendation in order to obtain the benefits as a healthy food source for people with diabetes mellitus.

**Keywords:** *In vitro* digestibility, Starch, Protein, Mung beans, Soaking time

## 1. Introduction

Mung bean (*Vigna radiata*) has been consumed in China for over 2,000 years, in the form of many kinds of food such as sprouts, noodles, cookies and others (Fayyaz *et al*., 2018). Mung bean, as one of the legumes, is being an important part of the dietaries of Indonesian people and contribute substantially to the nutrient intake of human beings, including children. Mung bean is less flatulent and is well tolerated by children (Dahiya *et al*., 2015).

Mung bean is one of the commodities with potency sources of starch and protein. Starch is the most component of carbohydrates in mung beans, about 30.74 ± 3.39% (Widjajaseputra *et al*., 2019a). Since starch is the most significant component in mung bean, the characteristics of starch will determine its suitability for its end use. Mung bean which its high amylose content can improve the swelling power and gel texture of a starch noodle product. This is as reported by Li *et al*. (2008), that the high-quality starch noodle made from mung bean starch results from its high amylose content. Menon *et al.* (2016) studied

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gluten-free starch noodles from sweet potatoes and got that fortification with mung bean starch reduced the rate of release of glucose from cooked noodles in vitro condition. The high amylose content showed higher viscosities which could be used in food products that require this property, such as thickeners for creams, sauces, soups and puddings. However, Li *et al.* (2011) declared that starches from different mung bean varieties showed significant differences in the physicochemical, thermal, and pasting properties. So that they may suit diverse applications such as porridge, cakes, snacks, beverages etc.

Besides as a source of starch, mung bean is an excellent source of vitamins, minerals, and protein with ideal essential amino acid profile (Mubarak, 2005). The specific profile of mung bean amino acid allows its usage to supplement cereals and rice in particular. The mung bean flour could be used as a supplement for wheat flour, increasing the nutritional quality of bakery products (Marquezi *et al*., 2016). However, the original properties of the components in the mung bean will change depending on the processing applied.

Soaking is the beginning of legume processing which is usually done in preparation before use. During soaking, water entered the bean, its tissues hydrated, and some enzymes can be activated to break down complex structures such as starch and protein into simpler compounds. The treatment result in alteration of their nutritional quality which could either be reduction in nutrients and antinutrients or improvement in digestibility or availability of nutrients (Kaur *et al*., 2015). Pagar et al. (2021) who studied the horse gram, found that treatment having 6 hrs soaking, 72 hrs germination, and drying at 70˚C was the best where a maximum decrease in the anti-nutritional factors at the same time flour functional properties got enhanced due to the soaking and germination. It is essential to understand these changes to select appropriate techniques to obtain maximum nutritional and health benefits. In a previous study, it was reported that the digestible (total sugar) and indigestible carbohydrates (resistant starch) increased during four hours of soaking (Widjajaseputra *et al*., 2019a). Widjajaseputra *et al*. (2019b) also reported that the soaking treatment improved the protein quality. In relation to the provision of healthy food for people with diabetes, high-quality protein sources are needed, but the type and quality of carbohydrate digestibility need to be considered. The study of the potency of soaked mung bean as a healthy food source, especially for diabetic people, based on the digestibility perspective is needed.

## 2. Materials and methods

### *2.1 Materials*

The commercial mung bean was obtained from a local market in Surabaya, East Java, Indonesia. All the chemicals, standards, and reagents were of analytical grade.

77
78 *2.2 Soaking procedure*

79 According to Widjajaseputra *et al*. (2019b), the mung bean was sorted. Only intact and sound
80 grains were washed and soaked (1:5 w/v) in distilled water at 30°C for 0 (control), 2, 4, 6 and 8 h,
81 afterward the grains were drained and freeze-dried (Bluewave B-10B Vacuum Freeze Drier; China) to
82 2% - 3% moisture content. The dried grains were ground (Miyako, Indonesia), wrapped in an airtight
83 plastic container and aluminum foil bag as secondary packaging, and then stored in a refrigerator
84 (LG, Indonesia) at 5 ± 1ºC until analyzed.

85

86 *2.3 The experimental design and statistical analysis*

87 The experimental design used in this study was a single factor with a randomized block design.
88 The whole mung bean seeds were subjected to five different times of soaking, namely control
89 (without soaking/P0) and soaking for 2 (P1), 4 (P2), 6 (P3), and 8 hours (P4). Each treatment level
90 was conducted in triplicates. Total starch, in vitro starch digestibility, protein content and in vitro
91 protein digestibility of freeze dried and grounded whole mung beans were analyzed. Water content
92 analysis was used to dry basis calculation. Data were expressed as mean ± standard deviation (SD)
93 for the three in each group (n=3). The data were subjected to one-way ANOVA ($p < 0.05$) with a least
94 significant difference (LSD) test at $p < 0.05$ using SPSS (version 19) for comparative analysis.

95

96 *2.4 Analysis Methods*

97 *2.4.1 Total starch analysis*

98 According to Goni *et al.* (1997), total starch was measured as glucose by incubating the
99 sample suspension in the optimum condition of amyloglucosidase for 45 min measuring activity.
100 The factor conversion of glucose to starch was 0.9.

101 *2.4.2 In vitro starch digestibility analysis*

102 Starch digestibility was determined by measuring digestible starch in vitro (Goni *et al*., 1996
103 and Goni *et al.,* 1997). The principle of digestible starch determination was to analyze total
104 starch with enzymes and measured undigested starch during 180 minutes within a 30 minute
105 interval, then calculated the percentage of hydrolyzed starch (digestible starch) in equations (a)
106 and (b) as follows:

107 (a) Digestible starch = (total starch – undigested Starch)

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(b) Starch digestibility (%) = (total starch – undigested starch)/total starch x 100%

*2.4.3 Protein content analysis*

The principle of determining protein content using the macro Kjeldahl method (AOAC, 2010). The protein in the sample was determined by measuring the amount of nitrogen (N). The measured nitrogen content was multiplied by the conversion factor resulting in protein content. The conversion factor used was 6.25.

*2.4.4 In vitro protein digestibility*

The principle of determining in vitro protein digestibility is to compare the total nitrogen content after the sample is treated with protein digestive enzymes (pepsin) with the total N of the initial sample. Nitrogen content was measured using the Kjeldahl micro method. Calculation of protein digestibility as stated in equation (c).

$$\text{(c) Protein digestibility} = \frac{\text{N total filtrate}}{\text{N total ingredients}} \times \text{diluting factor x } 100\%$$

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## 3. Results and discussion

*3.1 Effects of soaking time on total starch, undigested starch and starch digestibility*

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The total starch of mung bean seeds decreased from 41.13% dry to 33.52% dry basis during 8 hours of soaking, because more intensive hydrolysis occurred during the longer soaking time (Table 1). The decrease of total starch was due to the leaching out of the soluble part of starch in soaking water and hydrolysis process to simpler compounds such as sugars and dextrin. The same thing was also found by Grewal and Jood (2009), that a significant decrease in starch content was followed by increased sugar content due to soaking and cooking of green gram.

Legume starch contains amylose higher than cereal or tuber starch. This starch has a lower bioavailability than most other starches when it is raw or retrograded (Guillon and Champ, 2002). Widjajaseputra *et al.* (2019a) stated about amylose level of mung beans (32.56 ± 0.31%) is higher than cereal (around 25%) and tuber starch (around 17%-19%). Singh *et al.* (2003) state that the factors which could influence starch retrogradation were the amylopectin content, intermediate materials, size and shape of the granules, the botanical source, and the amylose content. A higher proportion of amylose content being linked to a higher tendency to retrogradation would affect the level of digestibility.

The starch digestibility decreases (from 57.46% to 49.82%) with the longer soaking time, along with the decreasing of total starch during the soaking treatment up to 8 h of soaking from 39.14% to 33.52% (Table 1). If compared with the control, soaking for 2 hours resulted in a significant increase in starch

digestibility. This is because there were starch granules that were more readily hydrolyzed by enzymes that become active due to the imbibition of water in the soaked mung bean seeds. After 4 hours of soaking time, the decrease in starch digestibility was not significantly different. Figure 1 shows that based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slow digesting starch.

The increasing of undigested starch up to 3 hours of digestion (in vitro) can be affected by the number of resistant starch (RS). RS is defined as the portion of starch and starch products that resist to digestion, passing directly through the small intestine (Fabbri *et al.*, 2016). Widjajaseputra *et al.* (2019a) stated that RS of mung bean was increased from 11.12% db to 18.49% db if soaked for 4 hours, although it was decreased significantly (13.65% db) if soaking was continued for up to 8 hours. This phenomenon is in line with the statement of Lang *et al.* (1999) that mung bean starch contains RS 11% db and has a long absorption period, which within 4.5 hours after consumption has not been completely digested. It was due to the high level of amylose of mung bean starch (around 32 %– 34%). The difference in RS can be caused by differences in starch structure in various amylose-amylopectin ratio among of different starch granules which affects the RS level in processed food, included during seeds soaking and freeze-drying treatment in samples preparation. Retrogradation of amylose can be occurred during soaking and freeze drying treatment in sample preparation, and part of starch was being to exist as RS3. RS3 is starch that has been retrograded into more highly stabile crystalline structures. In addition, Fabbri *et al.* (2016) found that the cooling process of legumes can increase RS as a result of retrogradation. This phenomena caused a decrease in the level of starch digestibility. The different process following the soaking would give the different effect on starch digestibility. As an example, if soaking was followed by dehulling and cooking, the level of starch digestibility would increase as obtained by Grewal and Jood (2009).

*3.2 Effects of soaking time on protein content and protein digestibility*

The values of protein digestibility were significantly different on 6 hours (P3) and 8 hours (P4) of treated soaking time compared to Po, P1, and P2 as shown on Table 2. Protein digestibility increased during soaking treatment, up to 6 hours of soaking time, and then the protein digestibility slightly decreased on 8 hours of seed soaking.

This phenomenon was affected by increasing the soluble protein of mung bean seeds from 108.96 mg/g of dry weight in raw seeds without soaking to 159.81 mg/g of dry weight in seeds with 6 hours of soaking time (Widjajaseputra *et al.*, 2019b). The higher of soluble protein indicated the readiness of proteins to be digested. Based on protein digestibility shown in Table 2, the recommended soaking time for mung bean seed was 6 hours, because a germination process has taken place longer than 6 hours of

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soaking. During germination periods there were hydrolyzing processes that would effect on protein and starch degradation to produce energy for the new plant. This phenomena was revealed by Grewal and Jood (2009) that germination process decreased starch content, thereby increasing soluble sugars and improved starch digestibility to be 49% and 48% in two different of green gram (*Vigna radiata L.)* cultivars respectively.

### *3.3 Perspective of mung bean as healthy food source for people with diabetes mellitus*

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The high protein digestibility of mung beans as a result of soaking for 6 hours can support its usage as a good food source of protein. Combination of mung bean protein and rice protein in 3:4 ratio respectively can increase the chemical amino acid score to be 72 (Dahiya *et al*., 2015). Based on this recommendation, an increase in the protein bioavailability can be obtained. Consumption of legumes provides quality protein along with other micronutrients without adding extra energy or fat. According to Mak *et al.* (2018), in fact a diet high in protein-low starch was associated with a lower risk for gestational diabetes mellitus among women who were overweight at pre-pregnancy. Mung bean seeds are an affordable source of not only protein but also the starch, which has an advantage of consisting higher resistant starch compared to cereal, root, and tuber starch. Based on these characteristics, mung bean can be used as a good source of resistant starch with a high protein content in various food applications.

Mung beans as one kind of variety of pulses, are high in fiber and have a low glycemic index, making them particularly beneficial to people with diabetes by assisting in maintaining healthy blood glucose and insulin levels (Dipnaiki and Bathere, 2017). Previously, the same thing was also reported by Rebello *et al.* (2014), that mung bean like other legumes had a medium glycemic index (GI) and high content of dietary fibers, which makes benefit to be a healthy food source. High resistant starch content combined with medium GI is positive attributes that could promote the product as better food choice not only for diabetes mellitus patients but also for people which is suffered from celiac disease, obesity and other malnutrition symptoms. In particular for the nutrition management of gestational diabetes mellitus, it is important to focus on quality of carbohydrates and encourage consumption of vegetables, fruits, complex carbohydrates, and high-fibre foods (Kapur *et al*., 2020). A balanced diet consisting healthy carbohydrate sources with adequate proteins and fats based on individual and cultural food preferences as well as based on physical activity and physiological status will results in weight control as well as diabetes management (Devi *et al*., 2021).

## 4. Conclusion

Based on the rate of starch hydrolysis of soaked mung beans, the mung bean starch is a slow digesting starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking up to 6 hours of soaking time (in a range of 46.93%-51.29%). Based on the obtained data, the recommended soaking time was 6 hours which revealed the optimum digestibility of starch and protein for people with diabetes mellitus. The further investigation will be needed to provide to get better food choice not only for diabetes mellitus patients, but also for those suffering from celiac disease, obesity and other malnutrition symptoms.

## Conflict of interest

The authors declare no conflict of interest.


## Acknowledgments

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Table 1. Effects of soaking time on total starch, undigested starch and starch digestibility

| Soaking time (Hour) | Total starch (% dry basis ± SD)* | Undigested starch (% dry basis ± SD)* | Starch digestibility** (% ± SD)* |
|---|---|---|---|
| P0 | 41.13 ± 0.19 c | 19.16 ± 0.45 b | 53.42± 1.31 b |
| P1 | 39.14 ± 0.72 b | 16.65 ± 0.13 a | 57.46± 1.12 c |
| P2 | 38.85 ± 0.18 b | 18.70 ± 0.56 b | 51.87± 1.67 ab |
| P3 | 38.18 ± 0.44 b | 18.53± 0.34 b | 51.47± 0.31 a |
| P4 | 33.52 ± 1.29 a | 16.82± 0.21 a | 49.82 ± 1.30 a |

Values are means ± standard deviations (n=3).
P0 = control (without soaking), P1 = soaked 2 h , P2 = soaked 4 h, P3= soaked 6 h, P4= soaked 8 h.
* Different superscripts in the same column showed a significant difference based on the LSD Test ($p < 0.05$).
** Starch Digestibility (%) = (Total Starch – Undigested Starch)/(Total Starch) x 100%

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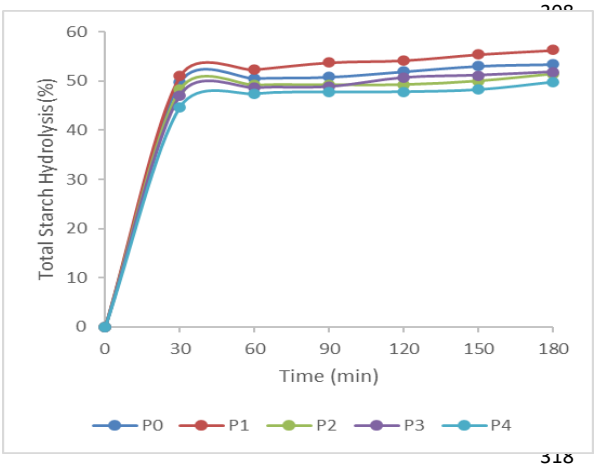


Figure 1. Mung bean starch hydrolysis rate. P0 = control (without soaking), P1 = soaked 2 h , P2 = soaked 4 h, P3= soaked 6 h, P4= soaked 8 h.

Table 2. Effects of soaking time on protein content and protein digestibility

| Soaking time (hour) | Protein content (% dry basis ± SD) | Protein digestibility* (% ± SD) |
|---|---|---|
| P0 | 26.29 ± 0.27 | 46.93 ± 1.53[a] |
| P1 | 25.88 ± 0.40 | 48.41 ± 0.38 [ab] |
| P2 | 25.90 ± 0.25 | 48.56 ± 0.54 [ab] |
| P3 | 25.68 ± 0.33 | 51.29 ± 1.20 [c] |
| P4 | 26.10 ± 0.82 | 49.83 ± 3.00 [bc] |

Values are means ± standard deviations (n=3)
P0 = control (without soaking), P1 = soaked 2 h , P2 = soaked 4 h, P3= soaked 6 h, P4= soaked 8 h.
* Different superscripts in the same column showed a significant difference based on the LSD Test ($p < 0.05$)

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# Controlling mung bean soaking time as a simple way to obtain alternative sources of healthy food for people with diabetes mellitus

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**Abstract**

Mung bean is one of the commodities with potency sources of starch and protein that is available and inexpensive. However, healthy foods ingredients for people with diabetes mellitus are high in protein but contain starch which is slow to digest. This research examines the effect of soaking time on the *in vitro* digestibility of starch and protein of mung bean, in order to obtain an alternative source of healthy food in a simple way. A single factor of randomized block design used in this study. Each treatment level was conducted in triplicates. The whole mung bean seeds were subjected to five different times of soaking, namely control (without soaking) and soaking for 2, 4, 6 and 8 hrs. Total starch, *in vitro* starch digestibility, protein content and *in vitro* protein digestibility of freeze dried and grounded whole mung beans were analyzed. The results showed a trend of the total starch content and starch digestibility decreasing during longer soaking time, in a range of 39.14-33.52 % dry basis and 57.46-49.82% respectively. Based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slow digesting starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking up to 6 hrs of soaking time (in a range of 46.93%-51.29%). Based on these results, the suggested soaking time was 6 hrs that gave optimum digestibility of starch and protein for human consumption. The recommendation in order to obtain the benefits as a healthy food source for people with diabetes mellitus.

**Keywords:** *In vitro* digestibility, Starch, Protein, Mung beans, Soaking time

## 1. Introduction

Mung bean (*Vigna radiata*) has been consumed in China for over 2,000 years, in the form of many kinds of food such as sprouts, noodles, cookies and others (Fayyaz *et al*., 2018). Mung bean, as one of the legumes, is being an important part of the dietaries of Indonesian people and contribute substantially to the nutrient intake of human beings, including children. Mung bean is less flatulent and is well tolerated by children (Dahiya *et al*., 2015).

Mung bean is one of the commodities with potency sources of starch and protein. Starch is the most component of carbohydrates in mung beans, about 30.74 ± 3.39% (Widjajaseputra *et al*., 2019a). Since starch is the most significant component in mung bean, the characteristics of starch will determine its suitability for its end use. Mung bean which its high amylose content can improve the swelling power and

gel texture of a starch noodle product. This is as reported by Li *et al*. (2008), that the high-quality starch noodle made from mung bean starch results from its high amylose content. Menon *et al.* (2016) studied gluten-free starch noodles from sweet potatoes and got that fortification with mung bean starch reduced the rate of release of glucose from cooked noodles *in vitro* condition. The high amylose content showed higher viscosities which could be used in food products that require this property, such as thickeners for creams, sauces, soups and puddings. However, Li *et al.* (2011) declared that starches from different mung bean varieties showed significant differences in the physicochemical, thermal, and pasting properties. So that they may suit diverse applications such as porridge, cakes, snacks, beverages etc.

Besides as a source of starch, mung bean is an excellent source of vitamins, minerals, and protein with ideal essential amino acid profile (Mubarak, 2005). The specific profile of mung bean amino acid allows its usage to supplement cereals and rice in particular. The mung bean flour could be used as a supplement for wheat flour, increasing the nutritional quality of bakery products (Marquezi *et al*., 2016). However, the original properties of the components in the mung bean will change depending on the processing applied.

Soaking is the beginning of legume processing which is usually done in preparation before use. During soaking, water entered the bean, its tissues hydrated, and some enzymes can be activated to break down complex structures such as starch and protein into simpler compounds. The treatment result in alteration of their nutritional quality which could either be reduction in nutrients and antinutrients or improvement in digestibility or availability of nutrients (Kaur *et al*., 2015). Pagar et al. (2021) who studied the horse gram, found that treatment having 6 hrs soaking, 72 hrs germination, and drying at 70˚C was the best where a maximum decrease in the anti-nutritional factors at the same time flour functional properties got enhanced due to the soaking and germination. It is essential to understand these changes to select appropriate techniques to obtain maximum nutritional and health benefits. In a previous study, it was reported that the digestible (total sugar) and indigestible carbohydrates (resistant starch) increased during four hours of soaking (Widjajaseputra *et al*., 2019a). Widjajaseputra *et al*. (2019b) also reported that the soaking treatment improved the protein quality. In relation to the provision of healthy food for people with diabetes, high-quality protein sources are needed, but the type and quality of carbohydrate digestibility need to be considered. The study of the potency of soaked mung bean as a healthy food source, especially for diabetic people, based on the digestibility perspective is needed.

## 2. Materials and methods

### *2.1 Materials*

71 The commercial mung bean was obtained from a local market in Surabaya, East Java, Indonesia.
72 All the chemicals, standards, and reagents were of analytical grade.
73
74 *2.2 Soaking procedure*
75 According to Widjajaseputra *et al*. (2019b), the mung bean was sorted. Only intact and sound
76 grains were washed and soaked (1:5 w/v) in distilled water at 30°C for 0 (control), 2, 4, 6 and 8 hrs,
77 afterward the grains were drained and freeze-dried (Bluewave B-10B Vacuum Freeze Drier; China) to
78 2% - 3% moisture content. The dried grains were ground (Miyako, Indonesia), wrapped in an airtight
79 plastic container and aluminum foil bag as secondary packaging, and then stored in a refrigerator
80 (LG, Indonesia) at 5°C ± 1 until analyzed.
81
82 *2.3 The experimental design and statistical analysis*
83 The experimental design used in this study was a single factor with a randomized block design.
84 The whole mung bean seeds were subjected to five different times of soaking, namely control
85 (without soaking/P0) and soaking for 2 (P1), 4 (P2), 6 (P3), and 8 hrs (P4). Each treatment level was
86 conducted in triplicates. Total starch, *in vitro* starch digestibility, protein content and *in vitro* protein
87 digestibility of freeze dried and grounded whole mung beans were analyzed. Water content analysis
88 was used to dry basis calculation. Data were expressed as mean ± standard deviation (SD) for the
89 three in each group (n=3). The data were subjected to one-way ANOVA ($p < 0.05$) with a least
90 significant difference (LSD) test at $p < 0.05$ using SPSS (version 19) for comparative analysis.
91
92 *2.4 Analysis Methods*
93 *2.4.1 Total starch analysis*
94 According to Goni *et al.* (1997), total starch was measured as glucose by incubating the
95 sample suspension in the optimum condition of amyloglucosidase for 45 mins measuring
96 activity. The factor conversion of glucose to starch was 0.9.
97 *2.4.2 In vitro starch digestibility analysis*
98 Starch digestibility was determined by measuring digestible starch *in vitro* (Goni *et al*., 1996
99 and Goni *et al.,* 1997). The principle of digestible starch determination was to analyze total
100 starch with enzymes and measured undigested starch during 180 mins within a 30 min interval,
101 then calculated the percentage of hydrolyzed starch (digestible starch) in equations (a) and (b)
102 as follows:

(a) Digestible starch = (total starch – undigested Starch)

(b) Starch digestibility (%) = (total starch – undigested starch)/total starch x 100%

*2.4.3 Protein content analysis*

The principle of determining protein content using the macro Kjeldahl method (AOAC, 2010). The protein in the sample was determined by measuring the amount of nitrogen (N). The measured nitrogen content was multiplied by the conversion factor resulting in protein content. The conversion factor used was 6.25.

*2.4.4 In vitro protein digestibility*

The principle of determining in vitro protein digestibility is to compare the total nitrogen content after the sample is treated with protein digestive enzymes (pepsin) with the total N of the initial sample. Nitrogen content was measured using the Kjeldahl micro method. Calculation of protein digestibility as stated in equation (c).

(c) $$\text{Protein digestibility} = \frac{\text{N total filtrate}}{\text{N total ingredients}} \times \text{diluting factor x } 100\%$$

## 3. Results and discussion

*3.1 Effects of soaking time on total starch, undigested starch and starch digestibility*

The total starch of mung bean seeds decreased from 41.13% dry to 33.52% dry basis during 8 hrs of soaking, because more intensive hydrolysis occurred during the longer soaking time (Table 1). The decrease of total starch was due to the leaching out of the soluble part of starch in soaking water and hydrolysis process to simpler compounds such as sugars and dextrin. The same thing was also found by Grewal and Jood (2009), that a significant decrease in starch content was followed by increased sugar content due to soaking and cooking of green gram.

Legume starch contains amylose higher than cereal or tuber starch. This starch has a lower bioavailability than most other starches when it is raw or retrograded (Guillon and Champ, 2002). Widjajaseputra *et al.* (2019a) stated about amylose level of mung beans (32.56 ± 0.31%) is higher than cereal (around 25%) and tuber starch (around 17%-19%). Singh *et al.* (2003) state that the factors which could influence starch retrogradation were the amylopectin content, intermediate materials, size and shape of the granules, the botanical source, and the amylose content. A higher proportion of amylose content being linked to a higher tendency to retrogradation would affect the level of digestibility.

The starch digestibility decreases (from 57.46% to 49.82%) with the longer soaking time, along with the decreasing of total starch during the soaking treatment up to 8 hrs of soaking from 39.14% to 33.52% (Table 1). If compared with the control, soaking for 2 hrs resulted in a significant increase in starch digestibility. This is because there were starch granules that were more readily hydrolyzed by enzymes that become active due to the imbibition of water in the soaked mung bean seeds. After 4 hrs of soaking time, the decrease in starch digestibility was not significantly different. Figure 1 shows that based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slow digesting starch.

The increasing of undigested starch up to 3 hrs of digestion (*in vitro*) can be affected by the number of resistant starch (RS). RS is defined as the portion of starch and starch products that resist to digestion, passing directly through the small intestine (Fabbri *et al*., 2016). Widjajaseputra *et al.* (2019a) stated that RS of mung bean was increased from 11.12% db to 18.49% db if soaked for 4 hrs, although it was decreased significantly (13.65% db) if soaking was continued for up to 8 hrs. This phenomenon is in line with the statement of Lang *et al.* (1999) that mung bean starch contains RS 11% db and has a long absorption period, which within 4.5 hrs after consumption has not been completely digested. It was due to the high level of amylose of mung bean starch (around 32 %– 34%). The difference in RS can be caused by differences in starch structure in various amylose-amylopectin ratio among of different starch granules which affects the RS level in processed food, included during seeds soaking and freeze-drying treatment in samples preparation. Retrogradation of amylose can be occurred during soaking and freeze drying treatment in sample preparation, and part of starch was being to exist as RS3. RS3 is starch that has been retrograded into more highly stabile crystalline structures. In addition, Fabbri *et al.* (2016) found that the cooling process of legumes can increase RS as a result of retrogradation. This phenomena caused a decrease in the level of starch digestibility. The different process following the soaking would give the different effect on starch digestibility. As an example, if soaking was followed by dehulling and cooking, the level of starch digestibility would increase as obtained by Grewal and Jood (2009).

*3.2 Effects of soaking time on protein content and protein digestibility*

The values of protein digestibility were significantly different on 6 hrs (P3) and 8 hrs (P4) of treated soaking time compared to P0, P1, and P2 as shown on Table 2. Protein digestibility increased during soaking treatment, up to 6 hrs of soaking time, and then the protein digestibility slightly decreased on 8 hrs of seed soaking.

This phenomenon was affected by increasing the soluble protein of mung bean seeds from 108.96 mg/g of dry weight in raw seeds without soaking to 159.81 mg/g of dry weight in seeds with 6 hrs of

soaking time (Widjajaseputra *et al*., 2019b). The higher of soluble protein indicated the readiness of proteins to be digested. Based on protein digestibility shown in Table 2, the recommended soaking time for mung bean seed was 6 hrs, because a germination process has taken place longer than 6 hrs of soaking. During germination periods there were hydrolyzing processes that would effect on protein and starch degradation to produce energy for the new plant. This phenomena was revealed by Grewal and Jood (2009) that germination process decreased starch content, thereby increasing soluble sugars and improved starch digestibility to be 49% and 48% in two different of green gram (*Vigna radiata L.)* cultivars respectively.

### *3.3 Perspective of mung bean as healthy food source for people with diabetes mellitus*

The high protein digestibility of mung beans as a result of soaking for 6 hrs can support its usage as a good food source of protein. Combination of mung bean protein and rice protein in 3:4 ratio respectively can increase the chemical amino acid score to be 72 (Dahiya *et al*., 2015). Based on this recommendation, an increase in the protein bioavailability can be obtained. Consumption of legumes provides quality protein along with other micronutrients without adding extra energy or fat. According to Mak *et al.* (2018), in fact a diet high in protein-low starch was associated with a lower risk for gestational diabetes mellitus among women who were overweight at pre-pregnancy. Mung bean seeds are an affordable source of not only protein but also the starch, which has an advantage of consisting higher resistant starch compared to cereal, root, and tuber starch. Based on these characteristics, mung bean can be used as a good source of resistant starch with a high protein content in various food applications.

Mung beans as one kind of variety of pulses, are high in fiber and have a low glycemic index, making them particularly beneficial to people with diabetes by assisting in maintaining healthy blood glucose and insulin levels (Dipnaiki and Bathere, 2017). Previously, the same thing was also reported by Rebello *et al.* (2014), that mung bean like other legumes had a medium glycemic index (GI) and high content of dietary fibers, which makes benefit to be a healthy food source. High resistant starch content combined with medium GI is positive attributes that could promote the product as better food choice not only for diabetes mellitus patients but also for people which is suffered from celiac disease, obesity and other malnutrition symptoms. In particular for the nutrition management of gestational diabetes mellitus, it is important to focus on quality of carbohydrates and encourage consumption of vegetables, fruits, complex carbohydrates, and high-fibre foods (Kapur *et al*., 2020). A balanced diet consisting healthy carbohydrate sources with adequate proteins and fats based on individual and cultural food

preferences as well as based on physical activity and physiological status will results in weight control as well as diabetes management (Devi *et al*., 2021).

## 4. Conclusion

Based on the rate of starch hydrolysis of soaked mung beans, the mung bean starch is a slow digesting starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking up to 6 hrs of soaking time (in a range of 46.93%-51.29%). Based on the obtained data, the recommended soaking time was 6 hrs which revealed the optimum digestibility of starch and protein for people with diabetes mellitus. The further investigation will be needed to provide to get better food choice not only for diabetes mellitus patients, but also for those suffering from celiac disease, obesity and other malnutrition symptoms.

## Conflict of interest

The authors declare no conflict of interest.


## Acknowledgments

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Widjajaseputra, A.I., Widyastuti, T.E.W. and Trisnawati, C.Y. (2019b). Potency of mung bean with different soaking times as protein source for breastfeeding women in Indonesia. *Food Research,* 3(5), 501-505. https://doi.org/10.26656/fr.2017.3(5).105.

290 Table 1. Effects of soaking time on total starch, undigested starch and starch digestibility

| Soaking time (hr) | Total starch (% dry basis ± SD)* | Undigested starch (% dry basis ± SD)* | Starch digestibility** (% ± SD)* |
|---|---|---|---|
| P0 | 41.13 ± 0.19 $^{c}$ | 19.16 ± 0.45 $^{b}$ | 53.42± 1.31 $^{b}$ |
| P1 | 39.14 ± 0.72 $^{b}$ | 16.65 ± 0.13 $^{a}$ | 57.46± 1.12 $^{c}$ |
| P2 | 38.85 ± 0.18 $^{b}$ | 18.70 ± 0.56 $^{b}$ | 51.87± 1.67 $^{ab}$ |
| P3 | 38.18 ± 0.44 $^{b}$ | 18.53± 0.34 $^{b}$ | 51.47± 0.31 $^{a}$ |
| P4 | 33.52 ± 1.29 $^{a}$ | 16.82± 0.21 $^{a}$ | 49.82 ± 1.30 $^{a}$ |

291 Values are means ± standard deviations (n=3).
292 P0 = control (without soaking), P1 = soaked 2 hrs , P2 = soaked 4 hrs, P3= soaked 6 hrs,
293 P4= soaked 8 hrs.
294 * Different superscripts in the same column showed a significant difference based
295 on the LSD Test ($p < 0.05$).
296 ** Starch Digestibility (%) = (Total Starch – Undigested Starch)/(Total Starch) x 100%

297

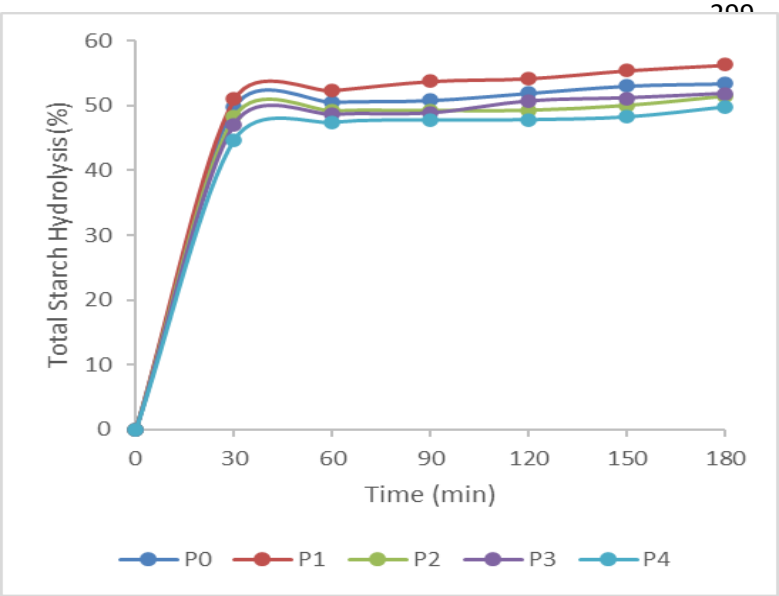


Figure 1. Mung bean starch hydrolysis rate. P0 = control (without soaking), P1 = soaked 2 hrs , P2 = soaked 4 hrs, P3= soaked 6 hrs, P4= soaked 8 hrs.

Table 2. Effects of soaking time on protein content and protein digestibility

| Soaking time (hr) | Protein content (% dry basis ± SD) | Protein digestibility* (% ± SD) |
|---|---|---|
| P0 | 26.29 ± 0.27 | 46.93 ± 1.53[a] |
| P1 | 25.88 ± 0.40 | 48.41 ± 0.38 [ab] |
| P2 | 25.90 ± 0.25 | 48.56 ± 0.54 [ab] |
| P3 | 25.68 ± 0.33 | 51.29 ± 1.20 [c] |
| P4 | 26.10 ± 0.82 | 49.83 ± 3.00 [bc] |

Values are means ± standard deviations (n=3)

P0 = control (without soaking), P1 = soaked 2 hrs , P2 = soaked 4 hr, P3= soaked 6 hr, P4= soaked 8 hr.

* Different superscripts in the same column showed a significant difference based on the LSD Test ($p < 0.05$)

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# Controlling mung bean soaking time as a simple way to obtain alternative sources of healthy food for people with diabetes mellitus


## Abstract

Mung bean is one of the commodities with potency sources of starch and protein that is available and inexpensive. However, healthy foods ingredients for people with diabetes mellitus are high in protein but contain starch which is slow to digest. This research examines the effect of soaking time on the *in vitro* digestibility of starch and protein of mung bean, in order to obtain an alternative source of healthy food in a simple way. A single factor of randomized block design used in this study. Each treatment level was conducted in triplicates. The whole mung bean seeds were subjected to five different times of soaking, namely control (without soaking) and soaking for 2, 4, 6 and 8 hrs. Total starch, *in vitro* starch digestibility, protein content and *in vitro* protein digestibility of freeze dried and grounded whole mung beans were analyzed. The results showed a trend of the total starch content and starch digestibility decreasing during longer soaking time, in a range of 39.14-33.52 % dry basis and 57.46-49.82% respectively. Based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slow digesting starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking up to 6 hrs of soaking time (in a range of 46.93%-51.29%). Based on these results, the suggested soaking time was 6 hrs that gave optimum digestibility of starch and protein for human consumption. The recommendation in order to obtain the benefits as a healthy food source for people with diabetes mellitus.



**Keywords:** *In vitro* digestibility, Starch, Protein, Mung beans, Soaking time


## 1. Introduction

Mung bean (*Vigna radiata*) has been consumed in China for over 2,000 years, in the form of many kinds of food such as sprouts, noodles, cookies and others (Fayyaz *et al*., 2018). Mung bean, as one of the legumes, is being an important part of the dietaries of Indonesian people and contribute substantially to the nutrient intake of human beings, including children. Mung bean is less flatulent and is well tolerated by children (Dahiya *et al*., 2015).

Mung bean is one of the commodities with potency sources of starch and protein. Starch is the most component of carbohydrates in mung beans, about 30.74 ± 3.39% (Widjajaseputra *et al*., 2019a). Since starch is the most significant component in mung bean, the characteristics of starch will determine its suitability for its end use. Mung bean which its high amylose content can improve the swelling power and gel texture of a starch noodle product. This is as reported by Li *et al*. (2008), that the high-quality starch noodle made from mung bean starch results from its high amylose content. Menon *et al.* (2016) studied gluten-free starch noodles from sweet potatoes and got that fortification with mung bean starch reduced the rate of release of glucose from cooked noodles *in vitro* condition. The high amylose content showed higher viscosities which could be used in food products that require this property, such as thickeners for creams, sauces, soups and puddings. However, Li *et al.* (2011) declared that starches from different mung

bean varieties showed significant differences in the physicochemical, thermal, and pasting properties. So that they may suit diverse applications such as porridge, cakes, snacks, beverages etc.

Besides as a source of starch, mung bean is an excellent source of vitamins, minerals, and protein with ideal essential amino acid profile (Mubarak, 2005). The specific profile of mung bean amino acid allows its usage to supplement cereals and rice in particular. The mung bean flour could be used as a supplement for wheat flour, increasing the nutritional quality of bakery products (Marquezi *et al*., 2016). However, the original properties of the components in the mung bean will change depending on the processing applied.

Soaking is the beginning of legume processing which is usually done in preparation before use. During soaking, water entered the bean, its tissues hydrated, and some enzymes can be activated to break down complex structures such as starch and protein into simpler compounds. The treatment result in alteration of their nutritional quality which could either be reduction in nutrients and antinutrients or improvement in digestibility or availability of nutrients (Kaur *et al*., 2015). Pagar et al. (2021) who studied the horse gram, found that treatment having 6 hrs soaking, 72 hrs germination, and drying at 70˚C was the best where a maximum decrease in the anti-nutritional factors at the same time flour functional properties got enhanced due to the soaking and germination. It is essential to understand these changes to select appropriate techniques to obtain maximum nutritional and health benefits. In a previous study, it was reported that the digestible (total sugar) and indigestible carbohydrates (resistant starch) increased during four hours of soaking (Widjajaseputra *et al*., 2019a). Widjajaseputra *et al*. (2019b) also reported that the soaking treatment improved the protein quality. In relation to the provision of healthy food for people with diabetes, high-quality protein sources are needed, but the type and quality of carbohydrate digestibility need to be considered. The study of the potency of soaked mung bean as a healthy food source, especially for diabetic people, based on the digestibility perspective is needed.

## 2. Materials and methods

### *2.1 Materials*

The commercial mung bean was obtained from a local market in Surabaya, East Java, Indonesia. All the chemicals, standards, and reagents were of analytical grade.

### *2.2 Soaking procedure*

According to Widjajaseputra *et al*. (2019b), the mung bean was sorted. Only intact and sound grains were washed and soaked (1:5 w/v) in distilled water at 30°C for 0 (control), 2, 4, 6 and 8 hrs,

afterward the grains were drained and freeze-dried (Bluewave B-10B Vacuum Freeze Drier; China) to 2% - 3% moisture content. The dried grains were ground (Miyako, Indonesia), wrapped in an airtight plastic container and aluminum foil bag as secondary packaging, and then stored in a refrigerator (LG, Indonesia) at 5°C ± 1 until analyzed.

*2.3 The experimental design and statistical analysis*

The experimental design used in this study was a single factor with a randomized block design. The whole mung bean seeds were subjected to five different times of soaking, namely control (without soaking/P0) and soaking for 2 (P1), 4 (P2), 6 (P3), and 8 hrs (P4). Each treatment level was conducted in triplicates. Total starch, *in vitro* starch digestibility, protein content and *in vitro* protein digestibility of freeze dried and grounded whole mung beans were analyzed. Water content analysis was used to dry basis calculation. Data were expressed as mean ± standard deviation (SD) for the three in each group (n=3). The data were subjected to one-way ANOVA ($p < 0.05$) with a least significant difference (LSD) test at $p < 0.05$ using SPSS (version 19) for comparative analysis.

*2.4 Analysis Methods*

**Dikomentari [A1]:** Add more details on each analysis method

*2.4.1 Total starch analysis*

According to Goni *et al.* (1997), total starch was measured as glucose by incubating the sample suspension in the optimum condition of amyloglucosidase for 45 mins measuring activity. The factor conversion of glucose to starch was 0.9.

**Dikomentari [A2]:** Then how to measure the glucose content?

*2.4.2 In vitro starch digestibility analysis*

Starch digestibility was determined by measuring digestible starch *in vitro* (Goni *et al*., 1996 and Goni *et al.,* 1997). The principle of digestible starch determination was to analyze total starch with enzymes and measured undigested starch during 180 mins within a 30 min interval, then calculated the percentage of hydrolyzed starch (digestible starch) in equations (a) and (b) as follows:

(a) Digestible starch = (total starch – undigested Starch)

(b) Starch digestibility (%) = (total starch – undigested starch)/total starch x 100%

*2.4.3 Protein content analysis*

The principle of determining protein content using the macro Kjeldahl method (AOAC, 2010). The protein in the sample was determined by measuring the amount of nitrogen (N). The

**Dikomentari [A3]:** ..through 3 steps....

measured nitrogen content was multiplied by the conversion factor resulting in protein content. The conversion factor used was 6.25.

*2.4.4 In vitro protein digestibility*

The principle of determining in vitro protein digestibility is to compare the total nitrogen content after the sample is treated with protein digestive enzymes (pepsin) with the total N of the initial sample. Nitrogen content was measured using the Kjeldahl micro method. Calculation of protein digestibility as stated in equation (c).

$$\text{(c) Protein digestibility} = \frac{\text{N total filtrate}}{\text{N total ingredients}} \times \text{diluting factor x } 100\% \quad (c)$$

## 3. Results and discussion

*3.1 Effects of soaking time on total starch, undigested starch and starch digestibility*

The total starch of mung bean seeds decreased from 41.13% dry to 33.52% dry basis during 8 hrs of soaking, because more intensive hydrolysis occurred during the longer soaking time (Table 1). The decrease of total starch was due to the leaching out of the soluble part of starch in soaking water and hydrolysis process to simpler compounds such as sugars and dextrin. The same thing was also found by Grewal and Jood (2009), that a significant decrease in starch content was followed by increased sugar content due to soaking and cooking of green gram.

Legume starch contains amylose higher than cereal or tuber starch. This starch has a lower bioavailability than most other starches when it is raw or retrograded (Guillon and Champ, 2002). Widjajaseputra *et al.* (2019a) stated about amylose level of mung beans (32.56 ± 0.31%) is higher than cereal (around 25%) and tuber starch (around 17%-19%). Singh *et al.* (2003) state that the factors which could influence starch retrogradation were the amylopectin content, intermediate materials, size and shape of the granules, the botanical source, and the amylose content. A higher proportion of amylose content being linked to a higher tendency to retrogradation would affect the level of digestibility.

The starch digestibility decreases (from 57.46% to 49.82%) with the longer soaking time, along with the decreasing of total starch during the soaking treatment up to 8 hrs of soaking from 39.14% to 33.52% (Table 1). If compared with the control, soaking for 2 hrs resulted in a significant increase in starch digestibility. This is because there were starch granules that were more readily hydrolyzed by enzymes that become active due to the imbibition of water in the soaked mung bean seeds. After 4 hrs of soaking time, the decrease in starch digestibility was not significantly different. Figure 1 shows that based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slow digesting starch.

The increasing of undigested starch up to 3 hrs of digestion (*in vitro*) can be affected by the number of resistant starch (RS). RS is defined as the portion of starch and starch products that resist to digestion, passing directly through the small intestine (Fabbri *et al*., 2016). Widjajaseputra *et al.* (2019a) stated that RS of mung bean was increased from 11.12% db to 18.49% db if soaked for 4 hrs, although it was decreased significantly (13.65% db) if soaking was continued for up to 8 hrs. This phenomenon is in line with the statement of Lang *et al.* (1999) that mung bean starch contains RS 11% db and has a long absorption period, which within 4.5 hrs after consumption has not been completely digested. It was due to the high level of amylose of mung bean starch (around 32 %– 34%). The difference in RS can be caused by differences in starch structure in various amylose-amylopectin ratio among of different starch granules which affects the RS level in processed food, included during seeds soaking and freeze-drying treatment in samples preparation. Retrogradation of amylose can be occurred during soaking and freeze drying treatment in sample preparation, and part of starch was being to exist as RS3. RS3 is starch that has been retrograded into more highly stabile crystalline structures. In addition, Fabbri *et al.* (2016) found that the cooling process of legumes can increase RS as a result of retrogradation. This phenomena caused a decrease in the level of starch digestibility. The different process following the soaking would give the different effect on starch digestibility. As an example, if soaking was followed by dehulling and cooking, the level of starch digestibility would increase as obtained by Grewal and Jood (2009).

*3.2 Effects of soaking time on protein content and protein digestibility*

The values of protein digestibility were significantly different on 6 hrs (P3) and 8 hrs (P4) of treated soaking time compared to P0, P1, and P2 as shown on Table 2. Protein digestibility increased during soaking treatment, up to 6 hrs of soaking time, and then the protein digestibility slightly decreased on 8 hrs of seed soaking.

This phenomenon was affected by increasing the soluble protein of mung bean seeds from 108.96 mg/g of dry weight in raw seeds without soaking to 159.81 mg/g of dry weight in seeds with 6 hrs of soaking time (Widjajaseputra *et al*., 2019b). The higher of soluble protein indicated the readiness of proteins to be digested. Based on protein digestibility shown in Table 2, the recommended soaking time for mung bean seed was 6 hrs, because a germination process has taken place longer than 6 hrs of soaking. During germination periods there were hydrolyzing processes that would effect on protein and starch degradation to produce energy for the new plant. This phenomena was revealed by Grewal and Jood (2009) that germination process decreased starch content, thereby increasing soluble sugars and

improved starch digestibility to be 49% and 48% in two different of green gram (*Vigna radiata L.)* cultivars respectively.

*3.3 Perspective of mung bean as healthy food source for people with diabetes mellitus*

The high protein digestibility of mung beans as a result of soaking for 6 hrs can support its usage as a good food source of protein. Combination of mung bean protein and rice protein in 3:4 ratio respectively can increase the chemical amino acid score to be 72 (Dahiya *et al*., 2015). Based on this recommendation, an increase in the protein bioavailability can be obtained. Consumption of legumes provides quality protein along with other micronutrients without adding extra energy or fat. According to Mak *et al.* (2018), in fact a diet high in protein-low starch was associated with a lower risk for gestational diabetes mellitus among women who were overweight at pre-pregnancy. Mung bean seeds are an affordable source of not only protein but also the starch, which has an advantage of consisting higher resistant starch compared to cereal, root, and tuber starch. Based on these characteristics, mung bean can be used as a good source of resistant starch with a high protein content in various food applications.

Mung beans as one kind of variety of pulses, are high in fiber and have a low glycemic index, making them particularly beneficial to people with diabetes by assisting in maintaining healthy blood glucose and insulin levels (Dipnaiki and Bathere, 2017). Previously, the same thing was also reported by Rebello *et al.* (2014), that mung bean like other legumes had a medium glycemic index (GI) and high content of dietary fibers, which makes benefit to be a healthy food source. High resistant starch content combined with medium GI is positive attributes that could promote the product as better food choice not only for diabetes mellitus patients but also for people which is suffered from celiac disease, obesity and other malnutrition symptoms. In particular for the nutrition management of gestational diabetes mellitus, it is important to focus on quality of carbohydrates and encourage consumption of vegetables, fruits, complex carbohydrates, and high-fibre foods (Kapur *et al*., 2020). A balanced diet consisting healthy carbohydrate sources with adequate proteins and fats based on individual and cultural food preferences as well as based on physical activity and physiological status will results in weight control as well as diabetes management (Devi *et al*., 2021).

## 4. Conclusion

Based on the rate of starch hydrolysis of soaked mung beans, the mung bean starch is a slow digesting starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking up to 6 hrs

of soaking time (in a range of 46.93%-51.29%). Based on the obtained data, the recommended soaking time was 6 hrs which revealed the optimum digestibility of starch and protein for people with diabetes mellitus. The further investigation will be needed to provide to get better food choice not only for diabetes mellitus patients, but also for those suffering from celiac disease, obesity and other malnutrition symptoms.

**Conflict of interest**

The authors declare no conflict of interest.

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Table 1. Effects of soaking time on total starch, undigested starch and starch digestibility

| Soaking time (hr) | Total starch (% dry basis ± SD)* | Undigested starch (% dry basis ± SD)* | Starch digestibility** (% ± SD)* |
|---|---|---|---|
| P0 | 41.13 ± 0.19 c | 19.16 ± 0.45 b | 53.42± 1.31 b |
| P1 | 39.14 ± 0.72 b | 16.65 ± 0.13 a | 57.46± 1.12 c |
| P2 | 38.85 ± 0.18 b | 18.70 ± 0.56 b | 51.87± 1.67 ab |
| P3 | 38.18 ± 0.44 b | 18.53± 0.34 b | 51.47± 0.31 a |
| P4 | 33.52 ± 1.29 a | 16.82± 0.21 a | 49.82 ± 1.30 a |

Values are means ± standard deviations (n=3).
P0 = control (without soaking), P1 = soaked 2 hrs , P2 = soaked 4 hrs, P3= soaked 6 hrs, P4= soaked 8 hrs.
* Different superscripts in the same column showed a significant difference based on the LSD Test ($p < 0.05$).
** Starch Digestibility (%) = (Total Starch – Undigested Starch)/(Total Starch) x 100%

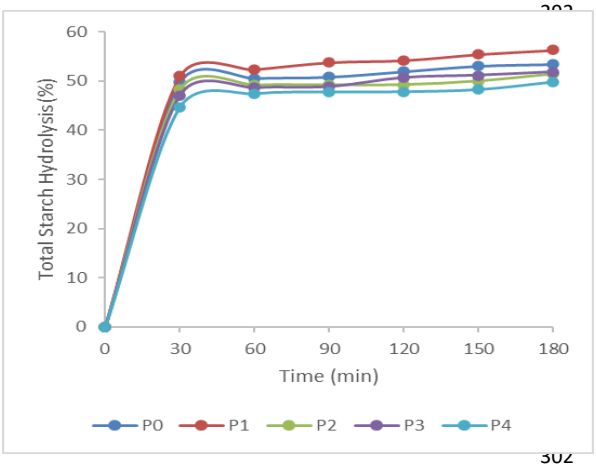


Figure 1. Mung bean starch hydrolysis rate. P0 = control (without soaking), P1 = soaked 2 hrs , P2 = soaked 4 hrs, P3= soaked 6 hrs, P4= soaked 8 hrs.

Table 2. Effects of soaking time on protein content and protein digestibility

| Soaking time (hr) | Protein content (% dry basis ± SD) | Protein digestibility* (% ± SD) |
|---|---|---|
| P0 | 26.29 ± 0.27 | 46.93 ± 1.53[a] |
| P1 | 25.88 ± 0.40 | 48.41 ± 0.38 [ab] |
| P2 | 25.90 ± 0.25 | 48.56 ± 0.54 [ab] |
| P3 | 25.68 ± 0.33 | 51.29 ± 1.20 [c] |
| P4 | 26.10 ± 0.82 | 49.83 ± 3.00 [bc] |

Values are means ± standard deviations (n=3)
P0 = control (without soaking), P1 = soaked 2 hrs , P2 = soaked 4 hr, P3= soaked 6 hr, P4= soaked 8 hr.
* Different superscripts in the same column showed a significant difference based on the LSD Test ($p < 0.05$)

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# Controlling mung bean soaking time as a simple way to obtain alternative sources of healthy food for people with diabetes mellitus

**Abstract**

Mung bean is one of the commodities with potency sources of starch and protein that is available and inexpensive. However, healthy foods ingredients for people with diabetes mellitus are high in protein but contain starch which is slow to digest. This research examines the effect of soaking time on the *in vitro* digestibility of starch and protein of mung bean, in order to obtain an alternative source of healthy food in a simple way. A single factor of randomized block design used in this study. Each treatment level was conducted in triplicates. The whole mung bean seeds were subjected to five different times of soaking, namely control (without soaking) and soaking for 2, 4, 6 and 8 hrs. Total starch, *in vitro* starch digestibility, protein content and *in vitro* protein digestibility of freeze dried and grounded whole mung beans were analyzed. The results showed a trend of the total starch content and starch digestibility decreasing during longer soaking time, in a range of 39.14-33.52 % dry basis and 57.46-49.82% respectively. Based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slow digesting starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking up to 6 hrs of soaking time (in a range of 46.93%-51.29%). Based on these results, the suggested soaking time was 6 hrs that gave optimum digestibility of starch and protein for human consumption. The recommendation in order to obtain the benefits as a healthy food source for people with diabetes mellitus.

**Keywords:** *In vitro* digestibility, Starch, Protein, Mung beans, Soaking time

## 1. Introduction

Mung bean (*Vigna radiata*) has been consumed in China for over 2,000 years, in the form of many kinds of food such as sprouts, noodles, cookies and others (Fayyaz *et al*., 2018). Mung bean, as one of the legumes, is being an important part of the dietaries of Indonesian people and contribute substantially to the nutrient intake of human beings, including children. Mung bean is less flatulent and is well tolerated by children (Dahiya *et al*., 2015).

Mung bean is one of the commodities with potency sources of starch and protein. Starch is the most component of carbohydrates in mung beans, about 30.74 ± 3.39% (Widjajaseputra *et al*., 2019a). Since starch is the most significant component in mung bean, the characteristics of starch will determine its suitability for its end use. Mung bean which its high amylose content can improve the swelling power and gel texture of a starch noodle product. This is as reported by Li *et al*. (2008), that the high-quality starch noodle made from mung bean starch results from its high amylose content. Menon *et al.* (2016) studied gluten-free starch noodles from sweet potatoes and got that fortification with mung bean starch reduced the rate of release of glucose from cooked noodles *in vitro* condition. The high amylose content showed higher viscosities which could be used in food products that require this property, such as thickeners for creams, sauces, soups and puddings. However, Li *et al.* (2011) declared that starches from different mung

bean varieties showed significant differences in the physicochemical, thermal, and pasting properties. So that they may suit diverse applications such as porridge, cakes, snacks, beverages etc.

Besides as a source of starch, mung bean is an excellent source of vitamins, minerals, and protein with ideal essential amino acid profile (Mubarak, 2005). The specific profile of mung bean amino acid allows its usage to supplement cereals and rice in particular. The mung bean flour could be used as a supplement for wheat flour, increasing the nutritional quality of bakery products (Marquezi *et al*., 2016). However, the original properties of the components in the mung bean will change depending on the processing applied.

Soaking is the beginning of legume processing which is usually done in preparation before use. During soaking, water entered the bean, its tissues hydrated, and some enzymes can be activated to break down complex structures such as starch and protein into simpler compounds. The treatment result in alteration of their nutritional quality which could either be reduction in nutrients and antinutrients or improvement in digestibility or availability of nutrients (Kaur *et al*., 2015). Pagar et al. (2021) who studied the horse gram, found that treatment having 6 hrs soaking, 72 hrs germination, and drying at 70˚C was the best where a maximum decrease in the anti-nutritional factors at the same time flour functional properties got enhanced due to the soaking and germination. It is essential to understand these changes to select appropriate techniques to obtain maximum nutritional and health benefits. In a previous study, it was reported that the digestible (total sugar) and indigestible carbohydrates (resistant starch) increased during four hours of soaking (Widjajaseputra *et al*., 2019a). Widjajaseputra *et al*. (2019b) also reported that the soaking treatment improved the protein quality. In relation to the provision of healthy food for people with diabetes, high-quality protein sources are needed, but the type and quality of carbohydrate digestibility need to be considered. The study of the potency of soaked mung bean as a healthy food source, especially for diabetic people, based on the digestibility perspective is needed.

## 2. Materials and methods

### *2.1 Materials*

The commercial mung bean was obtained from a local market in Surabaya, East Java, Indonesia. All the chemicals, standards, and reagents were of analytical grade.

### *2.2 Soaking procedure*

According to Widjajaseputra *et al*. (2019b), the mung bean was sorted. Only intact and sound grains were washed and soaked (1:5 w/v) in distilled water at 30°C for 0 (control), 2, 4, 6 and 8 hrs,

afterward the grains were drained and freeze-dried (Bluewave B-10B Vacuum Freeze Drier; China) to 2% - 3% moisture content. The dried grains were ground (Miyako, Indonesia), wrapped in an airtight plastic container and aluminum foil bag as secondary packaging, and then stored in a refrigerator (LG, Indonesia) at 5°C ± 1 until analyzed.

*2.3 The experimental design and statistical analysis*

The experimental design used in this study was a single factor with a randomized block design. The whole mung bean seeds were subjected to five different times of soaking, namely control (without soaking/P0) and soaking for 2 (P1), 4 (P2), 6 (P3), and 8 hrs (P4). Each treatment level was conducted in triplicates. Total starch, *in vitro* starch digestibility, protein content and *in vitro* protein digestibility of freeze dried and grounded whole mung beans were analyzed. Water content analysis was used to dry basis calculation. Data were expressed as mean ± standard deviation (SD) for the three in each group (n=3). The data were subjected to one-way ANOVA ($p < 0.05$) with a least significant difference (LSD) test at $p < 0.05$ using SPSS (version 19) for comparative analysis.

**Dikomentari [A1]:** Please check the suitability of experimental design with ANOVA method.

*2.4 Analysis Methods*

*2.4.1 Total starch analysis*

According to Goni *et al.* (1997), total starch was measured as glucose by incubating the sample suspension in the optimum condition of amyloglucosidase for 45 mins measuring activity. The factor conversion of glucose to starch was 0.9.

*2.4.2 In vitro starch digestibility analysis*

Starch digestibility was determined by measuring digestible starch *in vitro* (Goni *et al*., 1996 and Goni *et al.,* 1997). The principle of digestible starch determination was to analyze total starch with enzymes and measured undigested starch during 180 mins within a 30 min interval then calculated the percentage of hydrolyzed starch (digestible starch) in equations (a) and (b) as follows:

**Dikomentari [A2]:** How to measure it?

(a) Digestible starch = (total starch – undigested Starch)

(b) Starch digestibility (%) = (total starch – undigested starch)/total starch x 100%

*2.4.3 Protein content analysis*

The principle of determining protein content using the macro Kjeldahl method (AOAC, 2010). The protein in the sample was determined by measuring the amount of nitrogen (N). The

measured nitrogen content was multiplied by the conversion factor resulting in protein content. The conversion factor used was 6.25.

*2.4.4 In vitro protein digestibility*

The principle of determining in vitro protein digestibility is to compare the total nitrogen content after the sample is treated with protein digestive enzymes (pepsin) with the total N of the initial sample. Nitrogen content was measured using the Kjeldahl micro method. Calculation of protein digestibility as stated in equation (c).

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$$\text{(c) Protein digestibility} = \frac{\text{N total filtrate}}{\text{N total ingredients}} \times \text{diluting factor x } 100\%$$

## 3. Results and discussion

*3.1 Effects of soaking time on total starch, undigested starch and starch digestibility*

The total starch of mung bean seeds decreased from 41.13% dry to 33.52% dry basis during 8 hrs of soaking, because more intensive hydrolysis occurred during the longer soaking time (Table 1). The decrease of total starch was due to the leaching out of the soluble part of starch in soaking water and hydrolysis process to simpler compounds such as sugars and dextrin. The same thing was also found by Grewal and Jood (2009), that a significant decrease in starch content was followed by increased sugar content due to soaking and cooking of green gram.

Legume starch contains amylose higher than cereal or tuber starch. This starch has a lower bioavailability than most other starches when it is raw or retrograded (Guillon and Champ, 2002). Widjajaseputra *et al.* (2019a) stated about amylose level of mung beans (32.56 ± 0.31%) is higher than cereal (around 25%) and tuber starch (around 17%-19%). Singh *et al.* (2003) state that the factors which could influence starch retrogradation were the amylopectin content, intermediate materials, size and shape of the granules, the botanical source, and the amylose content. A higher proportion of amylose content being linked to a higher tendency to retrogradation would affect the level of digestibility.

The starch digestibility decreases (from 57.46% to 49.82%) with the longer soaking time, along with the decreasing of total starch during the soaking treatment up to 8 hrs of soaking from 39.14% to 33.52% (Table 1). If compared with the control, soaking for 2 hrs resulted in a significant increase in starch digestibility. This is because there were starch granules that were more readily hydrolyzed by enzymes that become active due to the imbibition of water in the soaked mung bean seeds. After 4 hrs of soaking time, the decrease in starch digestibility was not significantly different. Figure 1 shows that based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slow digesting starch.

The increasing of undigested starch up to 3 hrs of digestion (*in vitro*) can be affected by the number of resistant starch (RS). RS is defined as the portion of starch and starch products that resist to digestion, passing directly through the small intestine (Fabbri *et al*., 2016). Widjajaseputra *et al.* (2019a) stated that RS of mung bean was increased from 11.12% db to 18.49% db if soaked for 4 hrs, although it was decreased significantly (13.65% db) if soaking was continued for up to 8 hrs. This phenomenon is in line with the statement of Lang *et al.* (1999) that mung bean starch contains RS 11% db and has a long absorption period, which within 4.5 hrs after consumption has not been completely digested. It was due to the high level of amylose of mung bean starch (around 32 %– 34%). The difference in RS can be caused by differences in starch structure in various amylose-amylopectin ratio among of different starch granules which affects the RS level in processed food, included during seeds soaking and freeze-drying treatment in samples preparation. Retrogradation of amylose can be occurred during soaking and freeze drying treatment in sample preparation, and part of starch was being to exist as RS3. RS3 is starch that has been retrograded into more highly stabile crystalline structures. In addition, Fabbri *et al.* (2016) found that the cooling process of legumes can increase RS as a result of retrogradation. This phenomena caused a decrease in the level of starch digestibility. The different process following the soaking would give the different effect on starch digestibility. As an example, if soaking was followed by dehulling and cooking, the level of starch digestibility would increase as obtained by Grewal and Jood (2009).

*3.2 Effects of soaking time on protein content and protein digestibility*

The values of protein digestibility were significantly different on 6 hrs (P3) and 8 hrs (P4) of treated soaking time compared to P0, P1, and P2 as shown on Table 2. Protein digestibility increased during soaking treatment, up to 6 hrs of soaking time, and then the protein digestibility slightly decreased on 8 hrs of seed soaking.

This phenomenon was affected by increasing the soluble protein of mung bean seeds from 108.96 mg/g of dry weight in raw seeds without soaking to 159.81 mg/g of dry weight in seeds with 6 hrs of soaking time (Widjajaseputra *et al*., 2019b). The higher of soluble protein indicated the readiness of proteins to be digested. Based on protein digestibility shown in Table 2, the recommended soaking time for mung bean seed was 6 hrs, because a germination process has taken place longer than 6 hrs of soaking. During germination periods there were hydrolyzing processes that would effect on protein and starch degradation to produce energy for the new plant. This phenomena was revealed by Grewal and Jood (2009) that germination process decreased starch content, thereby increasing soluble sugars and

improved starch digestibility to be 49% and 48% in two different of green gram (*Vigna radiata L.)* cultivars respectively.

*3.3 Perspective of mung bean as healthy food source for people with diabetes mellitus*

The high protein digestibility of mung beans as a result of soaking for 6 hrs can support its usage as a good food source of protein. Combination of mung bean protein and rice protein in 3:4 ratio respectively can increase the chemical amino acid score to be 72 (Dahiya *et al*., 2015). Based on this recommendation, an increase in the protein bioavailability can be obtained. Consumption of legumes provides quality protein along with other micronutrients without adding extra energy or fat. According to Mak *et al.* (2018), in fact a diet high in protein-low starch was associated with a lower risk for gestational diabetes mellitus among women who were overweight at pre-pregnancy. Mung bean seeds are an affordable source of not only protein but also the starch, which has an advantage of consisting higher resistant starch compared to cereal, root, and tuber starch. Based on these characteristics, mung bean can be used as a good source of resistant starch with a high protein content in various food applications.

Mung beans as one kind of variety of pulses, are high in fiber and have a low glycemic index, making them particularly beneficial to people with diabetes by assisting in maintaining healthy blood glucose and insulin levels (Dipnaiki and Bathere, 2017). Previously, the same thing was also reported by Rebello *et al.* (2014), that mung bean like other legumes had a medium glycemic index (GI) and high content of dietary fibers, which makes benefit to be a healthy food source. High resistant starch content combined with medium GI is positive attributes that could promote the product as better food choice not only for diabetes mellitus patients but also for people which is suffered from celiac disease, obesity and other malnutrition symptoms. In particular for the nutrition management of gestational diabetes mellitus, it is important to focus on quality of carbohydrates and encourage consumption of vegetables, fruits, complex carbohydrates, and high-fibre foods (Kapur *et al*., 2020). A balanced diet consisting healthy carbohydrate sources with adequate proteins and fats based on individual and cultural food preferences as well as based on physical activity and physiological status will results in weight control as well as diabetes management (Devi *et al*., 2021).

## 4. Conclusion

Based on the rate of starch hydrolysis of soaked mung beans, the mung bean starch is a slow digesting starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking up to 6 hrs

of soaking time (in a range of 46.93%-51.29%). Based on the obtained data, the recommended soaking time was 6 hrs which revealed the optimum digestibility of starch and protein for people with diabetes mellitus. The further investigation will be needed to provide to get better food choice not only for diabetes mellitus patients, but also for those suffering from celiac disease, obesity and other malnutrition symptoms.

**Conflict of interest**

The authors declare no conflict of interest.

**Acknowledgments**

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Table 1. Effects of soaking time on total starch, undigested starch and starch digestibility

| Soaking time (hr) | Total starch (% dry basis ± SD)* | Undigested starch (% dry basis ± SD)* | Starch digestibility** (% ± SD)* |
|---|---|---|---|
| P0 | 41.13 ± 0.19 c | 19.16 ± 0.45 b | 53.42± 1.31 b |
| P1 | 39.14 ± 0.72 b | 16.65 ± 0.13 a | 57.46± 1.12 c |
| P2 | 38.85 ± 0.18 b | 18.70 ± 0.56 b | 51.87± 1.67 ab |
| P3 | 38.18 ± 0.44 b | 18.53± 0.34 b | 51.47± 0.31 a |
| P4 | 33.52 ± 1.29 a | 16.82± 0.21 a | 49.82 ± 1.30 a |

Values are means ± standard deviations (n=3).
P0 = control (without soaking), P1 = soaked 2 hrs , P2 = soaked 4 hrs, P3= soaked 6 hrs, P4= soaked 8 hrs.
* Different superscripts in the same column showed a significant difference based on the LSD Test ($p < 0.05$).
** Starch Digestibility (%) = (Total Starch – Undigested Starch)/(Total Starch) x 100%

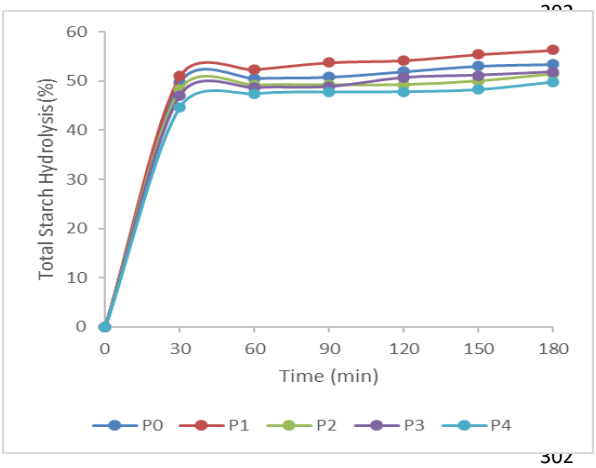


Figure 1. Mung bean starch hydrolysis rate. P0 = control (without soaking), P1 = soaked 2 hrs , P2 = soaked 4 hrs, P3= soaked 6 hrs, P4= soaked 8 hrs.

Table 2. Effects of soaking time on protein content and protein digestibility

| Soaking time (hr) | Protein content (% dry basis ± SD) | Protein digestibility* (% ± SD) |
|---|---|---|
| P0 | 26.29 ± 0.27 | 46.93 ± 1.53[a] |
| P1 | 25.88 ± 0.40 | 48.41 ± 0.38 [ab] |
| P2 | 25.90 ± 0.25 | 48.56 ± 0.54 [ab] |
| P3 | 25.68 ± 0.33 | 51.29 ± 1.20 [c] |
| P4 | 26.10 ± 0.82 | 49.83 ± 3.00 [bc] |

Values are means ± standard deviations (n=3)
P0 = control (without soaking), P1 = soaked 2 hrs , P2 = soaked 4 hr, P3= soaked 6 hr, P4= soaked 8 hr.
* Different superscripts in the same column showed a significant difference based on the LSD Test ($p < 0.05$)

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Bukti pengiriman Revisi *Manuscript* berdasar *review*
(terlampir Revisi *manuscript* dan Respon *Evaluation Form* untuk R*eviewer*)
(18 April 2023)

dan

Balasan Editor
(26 April 2023)

# Manuscript ID: FR-2023-081

**Ir. Theresia Endang Widoeri Widyastuti, MP.** <widoeri@ukwms.ac.id> Fri, Apr 14, 2023 at 5:23 AM
To: Food Research <foodresearch.my@outlook.com>

Dear Prof. Son Radu, PhD
Chief Editor, Food Research

Please kindly find the attached files of the revised manuscript FR-2023-081 and the response to the Reviewer's suggestion for further processing.

Thank you very much for your attention.

Sincerely,
Theresia Endang Widoeri Widyastuti

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# Controlling mung bean soaking time as a simple way to obtain alternative sources of healthy food for the diabetics

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**Abstract**

Mung bean is one of the commodities with potency sources of starch and protein that is available and inexpensive. However, healthy food ingredients which are needed by diabetics must be high in slow-digest starch and protein. This research examines the effect of soaking time on the *in vitro* digestibility of starch and protein of mung bean, to obtain an alternative source of healthy food simply. A single factor of randomized block design was used in this study. Each treatment level was conducted in triplicates. The whole mung bean seeds were subjected to five different times of soaking, namely control (without soaking) and soaking for 2, 4, 6, and 8 hrs. Total starch, *in vitro* starch digestibility, protein content, and *in vitro* protein digestibility of freeze-dried and grounded whole mung beans were analyzed. The results showed a trend of the total starch content and starch digestibility decreasing during the longer soaking time, in a range of 39.14-33.52% and 57.46-49.82% respectively. Based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slowly digestible starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking up to 6 hrs of soaking time (in a range of 46.93%-51.29%). Based on these results, the suggested soaking time was 6 hrs which gave optimum digestibility of starch and protein for human consumption. The recommendation is to obtain the benefits of a healthy food source for people with diabetes mellitus.

**Keywords:** *In vitro* digestibility, starch, protein, mung beans, soaking time

## 1. Introduction

Mung bean (*Vigna radiata*) has been consumed in China for over 2,000 years, in the form of many kinds of food such as sprouts, noodles, cookies, and others (Fayyaz *et al*., 2018). Mung bean, as one of the legumes, is an important part of the dietaries of Indonesian people and contributes substantially to the nutrient intake of human beings, including children. Mung bean is less flatulent and is well tolerated by children (Dahiya *et al*., 2015).

Mung bean is one of the commodities with potency sources of starch and protein. Starch is the most component of carbohydrates in mung beans, about 30.74 ± 3.39% (Widjajaseputra *et al*., 2019a). Since starch is the most significant component in mung bean, the characteristics of starch will determine its suitability for its end use. Mung bean which its high amylose content can improve the swelling power and

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gel texture of a starch noodle product. This is as reported by Li *et al*. (2008), that the high-quality starch noodle made from mung bean starch result from its high amylose content. Menon *et al.* (2016) studied gluten-free starch noodles from sweet potatoes and got that fortification with mung bean starch reduced the rate of release of glucose from cooked noodles *in vitro* condition. The high amylose content showed higher viscosities which could be used in certain food products such as thickeners for creams, sauces, soups, and puddings. Besides as a source of starch, mung bean is an excellent source of vitamins, minerals, and protein with an ideal essential amino acid profile (Mubarak, 2005). The specific profile of mung bean amino acid allows its use to supplement cereals and rice in particular. Mung bean flour could be used as a supplement for wheat flour, increasing the nutritional quality of bakery products (Marquezi *et al*., 2016). However, the original properties of the components in the mung bean will change depending on the applied processing.

Soaking is the beginning of legume processing which is usually done in preparation before use. During soaking, water entered the bean, its tissues hydrated, and some enzymes can be activated to break down complex structures such as starch and protein into simpler compounds. The treatment result in an alteration of their nutritional quality which could either be a reduction in nutrients and antinutrients or an improvement in the digestibility or availability of nutrients (Kaur *et al*., 2015). Pagar *et al*. (2021) who studied the horse gram, found that 6 hrs soaking, 72 hrs germination, and drying at 70˚C treatment was the best for a maximum decrease in the anti-nutritional factors and at the same time enhanced functional properties due to the soaking and germination.

In a previous study, it was reported that the digestible (total sugar) and indigestible carbohydrates (resistant starch) of the mung bean increased during four hours of soaking (Widjajaseputra *et al*., 2019a). There are five types of resistant starches (RS1 – RS5) and type RS2 is predominant in legumes as legume starches are physically enclosed within intact cell (protein) structures (Kaur *et al*., 2015). Such starches are indigestible by human digestive enzymes in the small intestine and pass to the large intestine or colon, thereby modifying postprandial glycemic responses. (Widjajaseputra *et al*. (2019b) also reported that the soaking treatment improved the protein quality. In relation to the provision of healthy food for people with diabetes, high-quality protein sources are needed, but the type and quality of carbohydrate digestibility need to be considered. The study of the potency of soaked mung bean as a healthy food source, especially for diabetic people, based on the digestibility perspective is needed.

## 2. Materials and methods

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## 2.1 Materials

The commercial mung bean was obtained from a local market in Surabaya, East Java, Indonesia. All the chemicals, standards, and reagents were of analytical grade.

## 2.2 Soaking procedure

According to Widjajaseputra *et al*. (2019b), the mung bean was sorted. Only intact and sound grains were washed and soaked (1:5 w/v) in distilled water at 30°C for 0 (control), 2, 4, 6, and 8 hrs, afterward the grains were drained and freeze-dried (Bluewave B-10B Vacuum Freeze Drier; China) to 2% - 3% moisture content. The dried grains were ground with a blender (Miyako, Indonesia), wrapped in an airtight plastic container and aluminum foil bag as secondary packaging, and then stored in a refrigerator (LG, Indonesia) at 5°C ± 1 until analyzed.

## 2.3 The experimental design and statistical analysis

The experimental design used in this study was a single factor with a randomized block design. The whole mung bean seeds were subjected to five different times of soaking, namely control (without soaking/P0) and soaking for 2 (P1), 4 (P2), 6 (P3), and 8 hrs (P4). Each treatment level was conducted in triplicates. Total starch, *in vitro* starch digestibility, protein content, and *in vitro* protein digestibility of freeze-dried and grounded whole mung beans were analyzed. Water content analysis was used to dry basis (db) calculation. Data were expressed as mean ± standard deviation (SD) for the three in each group (n=3). The data were subjected to ANOVA ($p < 0.05$) with a least significant difference (LSD) test at $p < 0.05$ using SPSS (version 19) for comparative analysis.

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## 2.4 Analysis Methods

### 2.4.1 Total starch analysis

According to Goni *et al.* (1997), total starch was measured by incubating the sample suspension in the optimum condition of amyloglucosidase, at 60°C in a controlled shaking water bath, for 45 mins measuring activity. Starch was measured as glucose with Peridochrom Glucose GOD-PAP (Ref 676 543, Boehringer). The glucose content was measured by the enzymatic photometric test. A red quinone imine as the reaction product was measured at a wavelength of 500 nm. The absorbance of the colored complex was proportional to the concentration of glucose. The factor conversion of glucose to starch was 0.9.

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### 2.4.2 In vitro starch digestibility analysis

Starch digestibility was determined by measuring digestible starch *in vitro* (Goni *et al*., 1996 and Goni *et al.,* 1997). The principle of digestible starch determination was to analyze total starch with enzymes and measured undigested starch for 180 mins within a 30 min interval. Then calculated the percentage of hydrolyzed starch (digestible starch) in equations (a) and (b) as follows:

(a) Digestible starch = (total starch – undigested Starch)

(b) Starch digestibility (%) = (total starch – undigested starch)/total starch x 100%

*2.4.3 Protein content analysis*

The protein content analysis used the macro Kjeldahl method (AOAC, 2010). The protein in the sample was determined by measuring the amount of nitrogen (N) through three-step, namely digestion, distillation, and titration. The measured nitrogen content was multiplied by the conversion factor resulting in protein content. The conversion factor used was 6.25.

*2.4.4 In vitro protein digestibility*

The principle of determining in vitro protein digestibility is to compare the total nitrogen content after the sample is treated with protein digestive enzymes (pepsin) with the total N of the initial sample (Mertz *et al*., 1984). Nitrogen content was measured using the micro Kjeldahl method. Calculation of protein digestibility as stated in equation (c).

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$$\text{(c) Protein digestibility} = \frac{\text{N total filtrate}}{\text{N total ingredients}} \times \text{diluting factor x } 100\%$$

## 3. Results and discussion

*3.1 Effects of soaking time on total starch, undigested starch, and starch digestibility*

The total starch of mung bean seeds decreased significantly from 41.13% db to 33.52% db during 8 hrs of soaking, because more intensive hydrolysis occurred during the longer soaking time (Table 1). The decrease in total starch was due to the leaching out of the soluble part of starch in soaking water and the hydrolysis process to simpler compounds such as sugars and dextrin. The same thing was also found by Grewal and Jood (2009), that a significant decrease in starch content was followed by increased sugar content due to soaking and cooking of green gram.

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Legume starch contains amylose higher than cereal or tuber starch. This starch has a lower bioavailability than most other starches when it is raw or retrograded (Guillon and Champ, 2002).

Widjajaseputra *et al.* (2019a) stated about the amylose level of mung beans (32.56 ± 0.31%) is higher than cereal (around 25%) and tuber starch (around 17%-19%). Singh *et al.* (2003) state that the factors which could influence starch retrogradation were the amylopectin content, intermediate materials, size and shape of the granules, the botanical source, and the amylose content. A higher proportion of amylose content being linked to a higher tendency to retrograde would affect the level of digestibility.

The starch digestibility decreases (from 57.46% to 49.82%) with the longer soaking time and the decrease of total starch during the soaking treatment up to 8 hrs of soaking from 39.14% to 33.52% (Table 1). If compared with the control, soaking for 2 hrs resulted in a significant increase in starch digestibility. This is due to part of the starch granules that were more readily hydrolyzed by enzymes that were affected by the imbibition of water in the soaked mung bean seeds. After 4 hrs of soaking time, the decrease in starch digestibility was not significantly different. Figure 1 shows that based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slow-digesting starch.

The increasing of undigested starch up to 3 hrs of digestion (*in vitro*) can be affected by the number of resistant starch (RS). RS is defined as the portion of starch and starch products that resist digestion, passing directly through the small intestine (Fabbri *et al.*, 2016). Widjajaseputra *et al.* (2019a) found that the RS of mung bean was increased from 11.12% db to 18.49% db if soaked for 4 hrs, although it was decreased significantly (13.65% db) if soaking was continued for up to 8 hrs. This phenomenon is in line with the statement of Lang *et al.* (1999) that mung bean starch contains RS 11% db and has a long absorption period, which within 4.5 hrs after consumption has not been completely digested. It was due to the high level of amylose in mung bean starch (around 32 %– 34%). The difference in RS can be influenced by differences in starch structure in various amylose-amylopectin ratios among different starch granules which affect the RS level in processed food, including during seeds soaking and freeze-drying treatment in sample preparation. Retrogradation of amylose can occur during soaking and freeze-drying treatment in sample preparation, and part of starch was being to exist as RS3. RS3 is starch that has been retrograded into more highly stabile crystalline structures. In addition, Fabbri *et al.* (2016) found that the cooling process of legumes can increase RS as a result of retrogradation. These phenomena caused a decrease in the level of starch digestibility. RS is one kind of dietary fiber. Dietary fiber is bound together in such a way that it can not be ready in the small intestine. Besides it, dietary fiber may make humans feel full longer. This causes mung bean to help keep blood sugar levels low. Nevertheless, the different processes following the soaking would give different effects on starch digestibility, as example, if soaking was followed by dehulling and cooking, the level of starch digestibility would increase as obtained by Grewal and Jood (2009).

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### *3.2 Effects of soaking time on protein content and protein digestibility*

The values of protein digestibility were significantly different on 6 hrs (P3) and 8 hrs (P4) of treated soaking time compared to P0, P1, and P2 as shown in Table 2. Protein digestibility increased during soaking treatment, up to 6 hrs of soaking time, and then the protein digestibility slightly decreased on 8 hrs of seed soaking.

This phenomenon was affected by increasing the soluble protein of mung bean seeds from 108.96 mg/g of dry weight in raw seeds without soaking to 159.81 mg/g of dry weight in seeds with 6 hrs of soaking time (Widjajaseputra *et al*., 2019b). The higher soluble protein indicated the readiness of proteins to be digested. Based on protein digestibility shown in Table 2, the recommended soaking time for mung bean seed was 6 hrs, because a germination process has taken place longer than 6 hrs of soaking. During germination periods there were hydrolyzing processes that would affect protein and starch degradation to produce energy for the new plant. These phenomena were revealed by Grewal and Jood (2009) that the germination process decreased starch content, thereby increasing soluble sugars and improved starch digestibility to 49% and 48% in two different green gram (*Vigna radiata L.)* cultivars respectively.

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### *3.3 Perspective of mung bean as a healthy food source for the diabetics*

The high protein digestibility of mung beans as a result of soaking for 6 hrs can support its usage as a good food source of protein. A combination of mung bean protein and rice protein in a 3:4 ratio respectively can increase the chemical amino acid score to 72 (Dahiya *et al*., 2015). Based on this recommendation, an increase in protein bioavailability can be obtained. Consumption of legumes provides qualified protein along with other micronutrients without adding extra energy or fat. According to Mak *et al.* (2018), a diet high in protein-low starch was associated with a lower risk for gestational diabetes mellitus among women who were overweight at pre-pregnancy. Mung bean seeds are an affordable source of not only protein but also starch, which has the advantage of consisting of higher resistant starch compared to cereal, root, and tuber starch. Based on these characteristics, mung bean can be used as a good source of resistant starch with a high protein content in various food applications.

Mung beans as one kind of variety of pulses, are high in fiber and have a low glycemic index, making them particularly beneficial to people with diabetes by assisting in maintaining healthy blood glucose and insulin levels (Dipnaiki and Bathere, 2017). Previously, the same thing was also reported by Rebello *et al.*

(2014), that mung bean like other legumes had a medium glycemic index (GI) and high content of dietary fibers, which makes benefit to be a healthy food source. High resistant starch content combined with medium GI is a positive attribute that could promote the product as a better food choice not only for diabetes mellitus patients but also for people which is suffered from celiac disease, obesity, and other malnutrition symptoms (Rebello *et al.*, 2014). In particular, for the nutrition management of gestational diabetes mellitus, it is important to focus on the quality of carbohydrates and encourage the consumption of vegetables, fruits, complex carbohydrates, and high-fiber foods (Kapur *et al*., 2020). A balanced diet consisting of healthy carbohydrate sources with adequate proteins and fats based on individual and cultural food preferences as well as based on physical activity and physiological status will result in weight control as well as diabetes management (Devi *et al*., 2021).

## 4. Conclusion

Based on the rate of starch hydrolysis of soaked mung beans, mung bean starch is a slow-digesting starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking up to 6 hrs of soaking time (in a range of 46.93%-51.29%). Based on the obtained data, the recommended soaking time was 6 hrs which revealed the optimum digestibility of starch and protein for diabetics. Further investigation in the processing field will be needed to get better food choices for diabetics.

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## Conflict of interest

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The authors declare no conflict of interest.


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Table 1. Effects of soaking time on total starch, undigested starch and starch digestibility

| Soaking time (hr) | Total starch (% db ± SD)* | Undigested starch (% db ± SD)* | Starch digestibility** (% ± SD)* |
|---|---|---|---|
| P0 | 41.13 ± 0.19 c | 19.16 ± 0.45 b | 53.42± 1.31 b |
| P1 | 39.14 ± 0.72 b | 16.65 ± 0.13 a | 57.46± 1.12 c |
| P2 | 38.85 ± 0.18 b | 18.70 ± 0.56 b | 51.87± 1.67 ab |
| P3 | 38.18 ± 0.44 b | 18.53± 0.34 b | 51.47± 0.31 a |
| P4 | 33.52 ± 1.29 a | 16.82± 0.21 a | 49.82 ± 1.30 a |

Values are means ± standard deviations (n=3).
P0 = control (without soaking), P1 = soaked 2 hrs , P2 = soaked 4 hrs, P3= soaked 6 hrs, P4= soaked 8 hrs.
* Different superscripts in the same column showed a significant difference based on the LSD Test ($p < 0.05$).
** Starch Digestibility (%) = (Total Starch – Undigested Starch)/(Total Starch) x 100%

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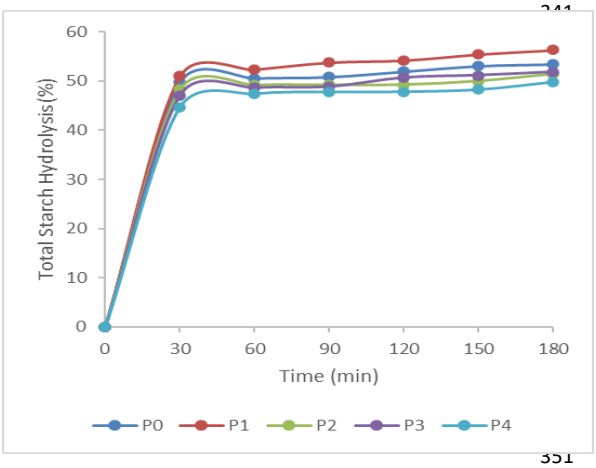


Figure 1. Mung bean starch hydrolysis rate. P0 = control (without soaking), P1 = soaked 2 hrs, P2 = soaked 4 hrs, P3= soaked 6 hrs, P4= soaked 8 hrs.

Table 2. Effects of soaking time on protein content and protein digestibility

| Soaking time (hr) | Protein content (% db ± SD) | Protein digestibility* (% ± SD) |
|---|---|---|
| P0 | 26.29 ± 0.27 | 46.93 ± 1.53[a] |
| P1 | 25.88 ± 0.40 | 48.41 ± 0.38 [ab] |
| P2 | 25.90 ± 0.25 | 48.56 ± 0.54 [ab] |
| P3 | 25.68 ± 0.33 | 51.29 ± 1.20 [c] |
| P4 | 26.10 ± 0.82 | 49.83 ± 3.00 [bc] |

Values are means ± standard deviations (n=3)
P0 = control (without soaking), P1 = soaked 2 hrs , P2 = soaked 4 hr, P3= soaked 6 hr, P4= soaked 8 hr.
* Different superscripts in the same column showed a significant difference based on the LSD Test ($p < 0.05$)

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| **Please return by** | : | 23rd March 2023 |
| **Title of Manuscript** | : | Controlling mung bean soaking time as a simple way to obtain alternative sources of healthy food for people with diabetes mellitus |

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| | | |
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| Manuscript Title | : | Controlling mung bean soaking time as a simple way to obtain alternative sources of healthy food for the diabetics |
| Authors | : | Widyastuti, T.E.W. and Widjajaseputra, A.I. |

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RESEARCH PAPER

# Controlling mung bean soaking time as a simple way to obtain alternative sources of healthy food for the diabetics

*Widyastuti, T.E.W. and Widjajaseputra, A.I.

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**Abstract**

Mung bean is one of the commodities with potency sources of starch and protein that is available and inexpensive. However, healthy food ingredients which are needed by diabetics must be high in slow-digest starch and protein. This research examined the effect of soaking time on the *in vitro* digestibility of starch and protein of mung bean, to obtain an alternative source of healthy food simply. A single factor of randomized block design was used in this study. Each treatment level was conducted in triplicates. The whole mung bean seeds were subjected to five different times of soaking, namely control (without soaking) and soaking for 2, 4, 6, and 8 hrs. Total starch, *in vitro* starch digestibility, protein content, and *in vitro* protein digestibility of freeze-dried and grounded whole mung beans were analyzed. The results showed a trend of the total starch content and starch digestibility decreasing during the longer soaking time, in a range of 39.14-33.52% and 57.46-49.82%, respectively. Based on the rate of starch hydrolysis, the digestibility of mung bean starch classified as slowly digestible starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking up to 6 hrs of soaking time (in a range of 46.93-51.29%). Based on these results, the suggested soaking time was 6 hrs which gave optimum digestibility of starch and protein for human consumption. The recommendation is to obtain the benefits of a healthy food source for people with diabetes

## 1. Introduction

Mung bean (*Vigna radiata*) has been consumed in China for over 2,000 years, in the form of many kinds of food such as sprouts, noodles, cookies, and others (Fayyaz *et al*., 2018). Mung bean, as one of the legumes, is an important part of the dietaries of Indonesian people and contributes substantially to the nutrient intake of human beings, including children. Mung bean is less flatulent and is well tolerated by children (Dahiya *et al*., 2015).

Mung bean is one of the commodities with potency sources of starch and protein. Starch is the most important component of carbohydrates in mung beans, about 30.74±3.39% (Widjajaseputra *et al*., 2019a). Since starch is the most significant component in mung bean, the characteristics of starch will determine its suitability for its end use. Mung bean with its high amylose content can improve the swelling power and gel texture of a starch noodle product. This is as reported by Li *et al*. (2008), that the high-quality starch noodle made from mung bean starch result from its high amylose content. Menon *et al.* (2016) studied gluten-free starch noodles from sweet potatoes and found that fortification with mung bean starch reduced the rate of release of glucose from cooked noodles *in vitro* conditions. The high amylose content showed higher viscosities which could be used in certain food products such as thickeners for creams, sauces, soups, and puddings. Besides as a source of starch, mung bean is an excellent source of vitamins, minerals, and protein with an ideal essential amino acid profile (Mubarak, 2005). The specific profile of mung bean amino acid allows its use to supplement cereals and rice in particular. Mung bean flour could be used as a supplement for wheat flour, increasing the nutritional quality of bakery products (Marquezi *et al*., 2016). However, the original properties of the components in the mung bean will change depending on the applied processing.

Soaking is the beginning of legume processing which is usually done in preparation before use. During soaking, water enters the bean, its tissues hydrated, and some enzymes can be activated to break down complex structures such as starch and protein into simpler compounds. The treatment results in an alteration of their

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nutritional quality which could either be a reduction in nutrients and antinutrients or an improvement in the digestibility or availability of nutrients (Kaur *et al*., 2015). Pagar *et al*. (2021) who studied the horse gram, found that 6 hrs soaking, 72 hrs germination, and drying at 70°C treatment was the best for a maximum decrease in the anti-nutritional factors and at the same time enhanced functional properties due to the soaking and germination.

In a previous study, it was reported that the digestible (total sugar) and indigestible carbohydrates (resistant starch) of the mung bean increased during four hours of soaking (Widjajaseputra *et al*., 2019a). There are five types of resistant starches (RS1-RS5) and type RS2 is predominant in legumes as legume starches are physically enclosed within intact cell (protein) structures (Kaur *et al*., 2015). Such starches are indigestible by human digestive enzymes in the small intestine and pass to the large intestine or colon, thereby modifying postprandial glycemic responses. Widjajaseputra *et al*. (2019b) also reported that the soaking treatment improved the protein quality. In relation to the provision of healthy food for people with diabetes, high-quality protein sources are needed, but the type and quality of carbohydrate digestibility need to be considered. The study of the potency of soaked mung bean as a healthy food source, especially for diabetic people, based on the digestibility perspective is needed.

## 2. Materials and methods

### *2.1 Materials*

The commercial mung bean was obtained from a local market in Surabaya, East Java, Indonesia. All the chemicals, standards, and reagents were of analytical grade.

### *2.2 Soaking procedure*

According to Widjajaseputra *et al*. (2019b), the mung bean was sorted. Only intact and sound grains were washed and soaked (1:5 w/v) in distilled water at 30°C for 0 (control), 2, 4, 6, and 8 hrs, afterward the grains were drained and freeze-dried (Bluewave B-10B Vacuum Freeze Drier; China) to 2-3% moisture content. The dried grains were ground with a blender (Miyako, Indonesia), wrapped in an airtight plastic container and aluminum foil bag as secondary packaging, and then stored in a refrigerator (LG, Indonesia) at 5±1°C until analyzed.

### *2.3 Experimental design and statistical analysis*

The experimental design used in this study was a single factor with a randomized block design. The whole mung bean seeds were subjected to five different times of soaking, control (without soaking/P0) and soaking for 2 (P1), 4 (P2), 6 (P3), and 8 hrs (P4). Each treatment level was conducted in triplicates. Total starch, *in vitro* starch digestibility, protein content, and *in vitro* protein digestibility of freeze-dried and grounded whole mung beans were analyzed. Water content analysis was used for dry basis (db) calculation. Data were expressed as mean ± standard deviation (SD) for the three in each group (n = 3). The data were subjected to ANOVA ($p<0.05$) with a least significant difference (LSD) test at $p < 0.05$ using SPSS (version 19) for comparative analysis.

### *2.4 Analysis methods*

#### *2.4.1 Total starch analysis*

According to Goni *et al.* (1997), total starch was measured by incubating the sample suspension in the optimum condition of amyloglucosidase, at 60°C in a controlled shaking water bath, for 45 mins measuring activity. Starch was measured as glucose with Peridochrom Glucose GOD-PAP (Ref 676 543, Boehringer). The glucose content was measured by the enzymatic photometric test. A red quinone imine as the reaction product was measured at a wavelength of 500 nm. The absorbance of the colored complex was proportional to the concentration of glucose. The factor conversion of glucose to starch was 0.9.

#### *2.4.2 In vitro starch digestibility analysis*

Starch digestibility was determined by measuring digestible starch *in vitro* (Goni *et al*., 1996; Goni *et al.,* 1997). The principle of digestible starch determination was to analyze total starch with enzymes and measured undigested starch for 180 mins within a 30 mins interval. Then calculated the percentage of hydrolyzed starch (digestible starch) in equations (1) and (2) as follows:

$$\text{Digestible starch} = (\text{total starch} - \text{undigested starch}) \quad (1)$$

$$\text{Starch digestibility (\%)} = (\text{total starch} - \text{undigested starch})/\text{total starch} \times 100\% \quad (2)$$

#### *2.4.3 Protein content analysis*

The protein content analysis used the macro Kjeldahl method (Association of the Official Analytical Collaboration (AOAC) International, 2010). The protein in the sample was determined by measuring the amount of nitrogen (N) through three steps, namely digestion, distillation, and titration. The measured nitrogen content was multiplied by the conversion factor resulting in protein content. The conversion factor used was 6.25.

*2.4.4 In vitro protein digestibility*

The principle of determining in vitro protein digestibility is to compare the total nitrogen content after the sample is treated with protein digestive enzymes (pepsin) with the total N of the initial sample (Mertz *et al*., 1984). Nitrogen content was measured using the micro Kjeldahl method.

## 3. Results and discussion

*3.1 Effects of soaking time on total starch, undigested starch, and starch digestibility*

The total starch of mung bean seeds decreased significantly from 41.13% db to 33.52% db during 8 hrs of soaking, because more intensive hydrolysis occurred during the longer soaking time (Table 1). The decrease in total starch was due to the leaching out of the soluble part of starch in soaking water and the hydrolysis process to simpler compounds such as sugars and dextrin. The same thing was also found by Grewal and Jood (2009), that a significant decrease in starch content was followed by increased sugar content due to soaking and cooking of green gram.

Table 1. Effects of soaking time on total starch, undigested starch and starch digestibility.

| Soaking time (hr) | Total starch (% db) | Undigested starch (% db) | Starch digestibility (%) |
|---|---|---|---|
| P0 | 41.13±0.19$^{c}$ | 19.16±0.45$^{b}$ | 53.42±1.31$^{b}$ |
| P1 | 39.14±0.72$^{b}$ | 16.65±0.13$^{a}$ | 57.46±1.12$^{c}$ |
| P2 | 38.85±0.18$^{b}$ | 18.70±0.56$^{b}$ | 51.87±1.67$^{ab}$ |
| P3 | 38.18±0.44$^{b}$ | 18.53±0.34$^{b}$ | 51.47±0.31$^{a}$ |
| P4 | 33.52±1.29$^{a}$ | 16.82±0.21$^{a}$ | 49.82±1.30$^{a}$ |

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are statistically significantly different based on the LSD Test ($p<0.05$). P0: control (without soaking), P1: soaked for 2 hrs, P2: soaked for 4 hrs, P3: soaked for 6 hrs, P4: soaked for 8 hrs.

Legume starch contains amylose higher than cereal or tuber starch. This starch has a lower bioavailability than most other starches when it is raw or retrograded (Guillon and Champ, 2002). Widjajaseputra *et al.* (2019a) stated that the amylose level of mung beans (32.56±0.31%) is higher than cereal (around 25%) and tuber starch (around 17-19%). Singh *et al.* (2003) state that the factors which could influence starch retrogradation were the amylopectin content, intermediate materials, size and shape of the granules, the botanical source, and the amylose content. A higher proportion of amylose content being linked to a higher tendency to retrograde would affect the level of digestibility.

The starch digestibility decreases (from 57.46% to 49.82%) with the longer soaking time and the decrease of total starch during the soaking treatment up to 8 hrs of soaking from 39.14% to 33.52% (Table 1). If compared with the control, soaking for 2 hrs resulted in a significant increase in starch digestibility. This is due to part of the starch granules that were more readily hydrolyzed by enzymes that were affected by the imbibition of water in the soaked mung bean seeds. After 4 hrs of soaking time, the decrease in starch digestibility was not significantly different. Figure 1 shows that based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slow-digesting starch.

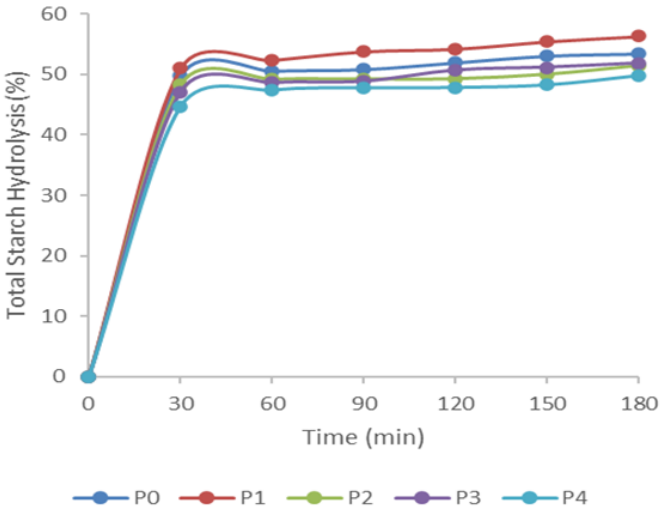


Figure 1. Mung bean starch hydrolysis rate. P0: control (without soaking), P1: soaked for 2 hrs, P2: soaked for 4 hrs, P3: soaked for 6 hrs, P4: soaked for 8 hrs.

The increasing of undigested starch up to 3 hrs of digestion (*in vitro*) can be affected by the number of resistant starch (RS). RS is defined as the portion of starch and starch products that resist digestion, passing directly through the small intestine (Fabbri *et al*., 2016). Widjajaseputra *et al.* (2019a) found that the RS of mung bean was increased from 11.12% db to 18.49% db if soaked for 4 hrs, although it was decreased significantly (13.65% db) if soaking was continued for up to 8 hrs. This phenomenon is in line with the statement of Lang *et al.* (1999) that mung bean starch contains RS 11% db and has a long absorption period, which within 4.5 hrs after consumption has not been completely digested. It was due to the high level of amylose in mung bean starch (around 32%-34%). The difference in RS can be influenced by differences in starch structure in various amylose-amylopectin ratios among different starch granules which affect the RS level in processed food, including during seeds soaking and freeze-drying treatment in sample preparation. Retrogradation of amylose can occur during soaking and freeze-drying treatment in sample preparation, and part of starch was to exist as RS3. RS3 is starch that has been retrograded into

more highly stabile crystalline structures. In addition, Fabbri *et al.* (2016) found that the cooling process of legumes can increase RS as a result of retrogradation. These phenomena caused a decrease in the level of starch digestibility. RS is one kind of dietary fiber. Dietary fiber is bound together in such a way that it can not be ready in the small intestine. Besides it, dietary fiber may make humans feel full longer. This causes mung bean to help keep blood sugar levels low. Nevertheless, the different processes following the soaking would give different effects on starch digestibility, as example, if soaking was followed by dehulling and cooking, the level of starch digestibility would increase as obtained by Grewal and Jood (2009).

### *3.2 Effects of soaking time on protein content and protein digestibility*

The values of protein digestibility were significantly different on 6 hrs (P3) and 8 hrs (P4) of treated soaking time compared to P0, P1, and P2 as shown in Table 2. Protein digestibility increased during soaking treatment, up to 6 hrs of soaking time, and then the protein digestibility slightly decreased on 8 hrs of seed soaking.

Table 2. Effects of soaking time on protein content and protein digestibility.

| Soaking time (hr) | Protein content (% db) | Protein digestibility (%) |
|---|---|---|
| P0 | 26.29±0.27 | 46.93±1.53$^{a}$ |
| P1 | 25.88±0.40 | 48.41±0.38$^{ab}$ |
| P2 | 25.90±0.25 | 48.56±0.54$^{ab}$ |
| P3 | 25.68±0.33 | 51.29±1.20$^{c}$ |
| P4 | 26.10±0.82 | 49.83±3.00$^{bc}$ |

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are statistically significantly different based on the LSD Test ($p<0.05$). P0: control (without soaking), P1: soaked for 2 hrs, P2: soaked for 4 hrs, P3: soaked for 6 hrs, P4: soaked for 8 hrs.

This phenomenon was affected by increasing the soluble protein of mung bean seeds from 108.96 mg/g of dry weight in raw seeds without soaking to 159.81 mg/g of dry weight in seeds with 6 hrs of soaking time (Widjajaseputra *et al*., 2019b). The higher soluble protein indicated the readiness of proteins to be digested. Based on protein digestibility shown in Table 2, the recommended soaking time for mung bean seed was 6 hrs, because a germination process has taken place longer than 6 hrs of soaking. During germination periods there were hydrolyzing processes that would affect protein and starch degradation to produce energy for the new plant. These phenomena were revealed by Grewal and Jood (2009) that the germination process decreased starch content, thereby increasing soluble sugars and improved starch digestibility to 49% and 48% in two different green gram (*Vigna radiata* L.) cultivars respectively.

### *3.3 Perspective of mung bean as a healthy food source for the diabetics*

The high protein digestibility of mung beans as a result of soaking for 6 hrs can support its usage as a good food source of protein. A combination of mung bean protein and rice protein in a 3:4 ratio respectively can increase the chemical amino acid score to 72 (Dahiya *et al*., 2015). Based on this recommendation, an increase in protein bioavailability can be obtained. Consumption of legumes provides qualified protein along with other micronutrients without adding extra energy or fat. According to Mak *et al.* (2018), a diet high in protein-low starch was associated with a lower risk for gestational diabetes mellitus among women who were overweight at pre-pregnancy. Mung bean seeds are an affordable source of not only protein but also starch, which has the advantage of consisting of higher resistant starch compared to cereal, root, and tuber starch. Based on these characteristics, mung bean can be used as a good source of resistant starch with a high protein content in various food applications.

Mung beans as one kind of variety of pulses, are high in fiber and have a low glycemic index, making them particularly beneficial to people with diabetes by assisting in maintaining healthy blood glucose and insulin levels (Dipnaiki and Bathere, 2017). Previously, the same thing was also reported by Rebello *et al.* (2014), that mung bean like other legumes had a medium glycemic index (GI) and high content of dietary fibers, which makes benefit to be a healthy food source. High resistant starch content combined with medium GI is a positive attribute that could promote the product as a better food choice not only for diabetes mellitus patients but also for people which is suffered from celiac disease, obesity, and other malnutrition symptoms (Rebello *et al.*, 2014). In particular, for the nutrition management of gestational diabetes mellitus, it is important to focus on the quality of carbohydrates and encourage the consumption of vegetables, fruits, complex carbohydrates, and high-fiber foods (Kapur *et al*., 2020). A balanced diet consisting of healthy carbohydrate sources with adequate proteins and fats based on individual and cultural food preferences as well as based on physical activity and physiological status will result in weight control as well as diabetes management (Devi *et al*., 2021).

## 4. Conclusion

Based on the rate of starch hydrolysis of soaked mung beans, mung bean starch is a slow-digesting starch. There was a trend of increasing protein digestibility of

mung bean seeds during soaking up to 6 hrs of soaking time (in a range of 46.93-51.29%). Based on the obtained data, the recommended soaking time was 6 hrs which revealed the optimum digestibility of starch and protein for diabetics. Further investigation in the processing field will be needed to get better food choices for diabetics.

## Conflict of interest

The authors declare no conflict of interest.

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# Controlling mung bean soaking time as a simple way to obtain alternative sources of healthy food for the diabetics

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**Abstract**

Mung bean is one of the commodities with potency sources of starch and protein that is available and inexpensive. However, healthy food ingredients which are needed by diabetics must be high in slow-digest starch and protein. This research examined the effect of soaking time on the *in vitro* digestibility of starch and protein of mung bean, to obtain an alternative source of healthy food simply. A single factor of randomized block design was used in this study. Each treatment level was conducted in triplicates. The whole mung bean seeds were subjected to five different times of soaking, namely control (without soaking) and soaking for 2, 4, 6, and 8 hrs. Total starch, *in vitro* starch digestibility, protein content, and *in vitro* protein digestibility of freeze-dried and grounded whole mung beans were analyzed. The results showed a trend of the total starch content and starch digestibility decreasing during the longer soaking time, in a range of 39.14-33.52% and 57.46-49.82%, respectively. Based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slowly digestible starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking up to 6 hrs of soaking time (in a range of 46.93-51.29%). Based on these results, the suggested soaking time was 6 hrs which gave optimum digestibility of starch and protein for human consumption. The recommendation is to obtain the benefits of a healthy food source for people with diabetes mellitus.

## 1. Introduction

Mung bean (*Vigna radiata*) has been consumed in China for over 2,000 years, in the form of many kinds of food such as sprouts, noodles, cookies, and others (Fayyaz *et al*., 2018). Mung bean, as one of the legumes, is an important part of the dietaries of Indonesian people and contributes substantially to the nutrient intake of human beings, including children. Mung bean is less flatulent and is well tolerated by children (Dahiya *et al*., 2015).

Mung bean is one of the commodities with potency sources of starch and protein. Starch is the most important component of carbohydrates in mung beans, about 30.74±3.39% (Widjajaseputra *et al*., 2019a). Since starch is the most significant component in mung bean, the characteristics of starch will determine its suitability for its end use. Mung bean with its high amylose content can improve the swelling power and gel texture of a starch noodle product. This is as reported by Li *et al*. (2008), that the high-quality starch noodle made from mung bean starch result from its high amylose content. Menon *et al.* (2016) studied gluten-free starch noodles from sweet potatoes and found that fortification with mung bean starch reduced the rate of release of glucose from cooked noodles *in vitro* conditions. The high amylose content showed higher viscosities which could be used in certain food products such as thickeners for creams, sauces, soups, and puddings. Besides as a source of starch, mung bean is an excellent source of vitamins, minerals, and protein with an ideal essential amino acid profile (Mubarak, 2005). The specific profile of mung bean amino acid allows its use to supplement cereals and rice in particular. Mung bean flour could be used as a supplement for wheat flour, increasing the nutritional quality of bakery products (Marquezi *et al*., 2016). However, the original properties of the components in the mung bean will change depending on the applied processing.

Soaking is the beginning of legume processing which is usually done in preparation before use. During soaking, water enters the bean, its tissues hydrated, and some enzymes can be activated to break down complex structures such as starch and protein into simpler compounds. The treatment results in an alteration of their

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nutritional quality which could either be a reduction in nutrients and antinutrients or an improvement in the digestibility or availability of nutrients (Kaur *et al*., 2015). Pagar *et al*. (2021) who studied the horse gram, found that 6 hrs soaking, 72 hrs germination, and drying at 70°C treatment was the best for a maximum decrease in the anti-nutritional factors and at the same time enhanced functional properties due to the soaking and germination.

In a previous study, it was reported that the digestible (total sugar) and indigestible carbohydrates (resistant starch) of the mung bean increased during four hours of soaking (Widjajaseputra *et al*., 2019a). There are five types of resistant starches (RS1-RS5) and type RS2 is predominant in legumes as legume starches are physically enclosed within intact cell (protein) structures (Kaur *et al*., 2015). Such starches are indigestible by human digestive enzymes in the small intestine and pass to the large intestine or colon, thereby modifying postprandial glycemic responses. Widjajaseputra *et al*. (2019b) also reported that the soaking treatment improved the protein quality. In relation to the provision of healthy food for people with diabetes, high-quality protein sources are needed, but the type and quality of carbohydrate digestibility need to be considered. The study of the potency of soaked mung bean as a healthy food source, especially for diabetic people, based on the digestibility perspective is needed.

## 2. Materials and methods

### *2.1 Materials*

The commercial mung bean was obtained from a local market in Surabaya, East Java, Indonesia. All the chemicals, standards, and reagents were of analytical grade.

### *2.2 Soaking procedure*

According to Widjajaseputra *et al*. (2019b), the mung bean was sorted. Only intact and sound grains were washed and soaked (1:5 w/v) in distilled water at 30°C for 0 (control), 2, 4, 6, and 8 hrs, afterward the grains were drained and freeze-dried (Bluewave B-10B Vacuum Freeze Drier; China) to 2-3% moisture content. The dried grains were ground with a blender (Miyako, Indonesia), wrapped in an airtight plastic container and aluminum foil bag as secondary packaging, and then stored in a refrigerator (LG, Indonesia) at 5±1°C until analyzed.

### *2.3 Experimental design and statistical analysis*

The experimental design used in this study was a single factor with a randomized block design. The whole mung bean seeds were subjected to five different times of soaking, control (without soaking/P0) and soaking for 2 (P1), 4 (P2), 6 (P3), and 8 hrs (P4). Each treatment level was conducted in triplicates. Total starch, *in vitro* starch digestibility, protein content, and *in vitro* protein digestibility of freeze-dried and grounded whole mung beans were analyzed. Water content analysis was used for dry basis (db) calculation. Data were expressed as mean ± standard deviation (SD) for the three in each group (n = 3). The data were subjected to ANOVA ($p<0.05$) with a least significant difference (LSD) test at $p < 0.05$ using SPSS (version 19) for comparative analysis.

### *2.4 Analysis methods*

#### *2.4.1 Total starch analysis*

According to Goni *et al.* (1997), total starch was measured by incubating the sample suspension in the optimum condition of amyloglucosidase, at 60°C in a controlled shaking water bath, for 45 mins measuring activity. Starch was measured as glucose with Peridochrom Glucose GOD-PAP (Ref 676 543, Boehringer). The glucose content was measured by the enzymatic photometric test. A red quinone imine as the reaction product was measured at a wavelength of 500 nm. The absorbance of the colored complex was proportional to the concentration of glucose. The factor conversion of glucose to starch was 0.9.

#### *2.4.2 In vitro starch digestibility analysis*

Starch digestibility was determined by measuring digestible starch *in vitro* (Goni *et al*., 1996; Goni *et al.,* 1997). The principle of digestible starch determination was to analyze total starch with enzymes and measured undigested starch for 180 mins within a 30 mins interval. Then calculated the percentage of hydrolyzed starch (digestible starch) in equations (1) and (2) as follows:

$$\text{Digestible starch} = (\text{total starch} - \text{undigested starch}) \quad (1)$$

$$\text{Starch digestibility (\%)} = (\text{total starch} - \text{undigested starch})/\text{total starch} \times 100\% \quad (2)$$

#### *2.4.3 Protein content analysis*

The protein content analysis used the macro Kjeldahl method (Association of the Official Analytical Collaboration (AOAC) International, 2010). The protein in the sample was determined by measuring the amount of nitrogen (N) through three steps, namely digestion, distillation, and titration. The measured nitrogen content was multiplied by the conversion factor resulting in protein content. The conversion factor used was 6.25.

#### *2.4.4 In vitro protein digestibility*

The principle of determining in vitro protein digestibility is to compare the total nitrogen content after the sample is treated with protein digestive enzymes (pepsin) with the total N of the initial sample (Mertz *et al*., 1984). Nitrogen content was measured using the micro Kjeldahl method. Calculation of protein digestibility as stated in equation (3).

$$\text{Protein digestibility} = \frac{\text{N total filtrate}}{\text{N total ingredients}} \times \text{diluting factor} \times 100\% \quad (3)$$

## 3. Results and discussion

### *3.1 Effects of soaking time on total starch, undigested starch, and starch digestibility*

The total starch of mung bean seeds decreased significantly from 41.13% db to 33.52% db during 8 hrs of soaking, because more intensive hydrolysis occurred during the longer soaking time (Table 1). The decrease in total starch was due to the leaching out of the soluble part of starch in soaking water and the hydrolysis process to simpler compounds such as sugars and dextrin. The same thing was also found by Grewal and Jood (2009), that a significant decrease in starch content was followed by increased sugar content due to soaking and cooking of green gram.

Table 1. Effects of soaking time on total starch, undigested starch and starch digestibility.

| Soaking time (hr) | Total starch (% db) | Undigested starch (% db) | Starch digestibility (%) |
|---|---|---|---|
| P0 | 41.13±0.19$^{c}$ | 19.16±0.45$^{b}$ | 53.42±1.31$^{b}$ |
| P1 | 39.14±0.72$^{b}$ | 16.65±0.13$^{a}$ | 57.46±1.12$^{c}$ |
| P2 | 38.85±0.18$^{b}$ | 18.70±0.56$^{b}$ | 51.87±1.67$^{ab}$ |
| P3 | 38.18±0.44$^{b}$ | 18.53±0.34$^{b}$ | 51.47±0.31$^{a}$ |
| P4 | 33.52±1.29$^{a}$ | 16.82±0.21$^{a}$ | 49.82±1.30$^{a}$ |

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are statistically significantly different based on the LSD Test ($p<0.05$). P0: control (without soaking), P1: soaked for 2 hrs, P2: soaked for 4 hrs, P3: soaked for 6 hrs, P4: soaked for 8 hrs.

Legume starch contains amylose higher than cereal or tuber starch. This starch has a lower bioavailability than most other starches when it is raw or retrograded (Guillon and Champ, 2002). Widjajaseputra *et al.* (2019a) stated that the amylose level of mung beans (32.56±0.31%) is higher than cereal (around 25%) and tuber starch (around 17-19%). Singh *et al.* (2003) state that the factors which could influence starch retrogradation were the amylopectin content, intermediate materials, size and shape of the granules, the botanical source, and the amylose content. A higher proportion of amylose content being linked to a higher tendency to retrograde would affect the level of digestibility.

The starch digestibility decreases (from 57.46% to 49.82%) with the longer soaking time and the decrease of total starch during the soaking treatment up to 8 hrs of soaking from 39.14% to 33.52% (Table 1). If compared with the control, soaking for 2 hrs resulted in a significant increase in starch digestibility. This is due to part of the starch granules that were more readily hydrolyzed by enzymes that were affected by the imbibition of water in the soaked mung bean seeds. After 4 hrs of soaking time, the decrease in starch digestibility was not significantly different. Figure 1 shows that based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slow-digesting starch.

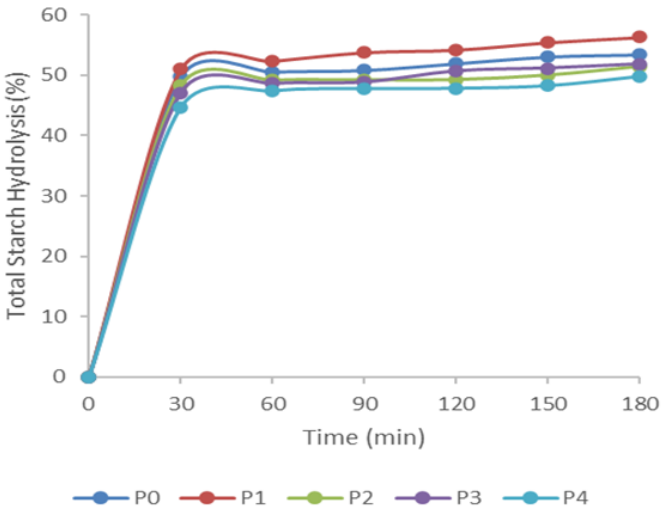


Figure 1. Mung bean starch hydrolysis rate. P0: control (without soaking), P1: soaked for 2 hrs, P2: soaked for 4 hrs, P3: soaked for 6 hrs, P4: soaked for 8 hrs.

The increasing of undigested starch up to 3 hrs of digestion (*in vitro*) can be affected by the number of resistant starch (RS). RS is defined as the portion of starch and starch products that resist digestion, passing directly through the small intestine (Fabbri *et al*., 2016). Widjajaseputra *et al.* (2019a) found that the RS of mung bean was increased from 11.12% db to 18.49% db if soaked for 4 hrs, although it was decreased significantly (13.65% db) if soaking was continued for up to 8 hrs. This phenomenon is in line with the statement of Lang *et al.* (1999) that mung bean starch contains RS 11% db and has a long absorption period, which within 4.5 hrs after consumption has not been completely digested. It was due to the high level of amylose in mung bean starch (around 32%-34%). The difference in RS can be influenced by differences in starch structure in various amylose-amylopectin ratios among different starch granules which affect the RS level in processed food, including during seeds soaking and freeze-drying treatment in sample preparation. Retrogradation of amylose can occur during soaking and freeze-drying treatment in sample preparation, and part of starch was to exist as RS3. RS3 is starch that has been retrograded into

more highly stabile crystalline structures. In addition, Fabbri *et al.* (2016) found that the cooling process of legumes can increase RS as a result of retrogradation. These phenomena caused a decrease in the level of starch digestibility. RS is one kind of dietary fiber. Dietary fiber is bound together in such a way that it can not be ready in the small intestine. Besides it, dietary fiber may make humans feel full longer. This causes mung bean to help keep blood sugar levels low. Nevertheless, the different processes following the soaking would give different effects on starch digestibility, as example, if soaking was followed by dehulling and cooking, the level of starch digestibility would increase as obtained by Grewal and Jood (2009).

### *3.2 Effects of soaking time on protein content and protein digestibility*

The values of protein digestibility were significantly different on 6 hrs (P3) and 8 hrs (P4) of treated soaking time compared to P0, P1, and P2 as shown in Table 2. Protein digestibility increased during soaking treatment, up to 6 hrs of soaking time, and then the protein digestibility slightly decreased on 8 hrs of seed soaking.

Table 2. Effects of soaking time on protein content and protein digestibility.

| Soaking time (hr) | Protein content (% db) | Protein digestibility (%) |
|---|---|---|
| P0 | 26.29±0.27 | 46.93±1.53$^{a}$ |
| P1 | 25.88±0.40 | 48.41±0.38$^{ab}$ |
| P2 | 25.90±0.25 | 48.56±0.54$^{ab}$ |
| P3 | 25.68±0.33 | 51.29±1.20$^{c}$ |
| P4 | 26.10±0.82 | 49.83±3.00$^{bc}$ |

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are statistically significantly different based on the LSD Test ($p<0.05$). P0: control (without soaking), P1: soaked for 2 hrs, P2: soaked for 4 hrs, P3: soaked for 6 hrs, P4: soaked for 8 hrs.

This phenomenon was affected by increasing the soluble protein of mung bean seeds from 108.96 mg/g of dry weight in raw seeds without soaking to 159.81 mg/g of dry weight in seeds with 6 hrs of soaking time (Widjajaseputra *et al*., 2019b). The higher soluble protein indicated the readiness of proteins to be digested. Based on protein digestibility shown in Table 2, the recommended soaking time for mung bean seed was 6 hrs, because a germination process has taken place longer than 6 hrs of soaking. During germination periods there were hydrolyzing processes that would affect protein and starch degradation to produce energy for the new plant. These phenomena were revealed by Grewal and Jood (2009) that the germination process decreased starch content, thereby increasing soluble sugars and improved starch digestibility to 49% and 48% in two different green gram (*Vigna radiata* L.) cultivars respectively.

### *3.3 Perspective of mung bean as a healthy food source for the diabetics*

The high protein digestibility of mung beans as a result of soaking for 6 hrs can support its usage as a good food source of protein. A combination of mung bean protein and rice protein in a 3:4 ratio respectively can increase the chemical amino acid score to 72 (Dahiya *et al*., 2015). Based on this recommendation, an increase in protein bioavailability can be obtained. Consumption of legumes provides qualified protein along with other micronutrients without adding extra energy or fat. According to Mak *et al.* (2018), a diet high in protein-low starch was associated with a lower risk for gestational diabetes mellitus among women who were overweight at pre-pregnancy. Mung bean seeds are an affordable source of not only protein but also starch, which has the advantage of consisting of higher resistant starch compared to cereal, root, and tuber starch. Based on these characteristics, mung bean can be used as a good source of resistant starch with a high protein content in various food applications.

Mung beans as one kind of variety of pulses, are high in fiber and have a low glycemic index, making them particularly beneficial to people with diabetes by assisting in maintaining healthy blood glucose and insulin levels (Dipnaiki and Bathere, 2017). Previously, the same thing was also reported by Rebello *et al.* (2014), that mung bean like other legumes had a medium glycemic index (GI) and high content of dietary fibers, which makes benefit to be a healthy food source. High resistant starch content combined with medium GI is a positive attribute that could promote the product as a better food choice not only for diabetes mellitus patients but also for people which is suffered from celiac disease, obesity, and other malnutrition symptoms (Rebello *et al.*, 2014). In particular, for the nutrition management of gestational diabetes mellitus, it is important to focus on the quality of carbohydrates and encourage the consumption of vegetables, fruits, complex carbohydrates, and high-fiber foods (Kapur *et al*., 2020). A balanced diet consisting of healthy carbohydrate sources with adequate proteins and fats based on individual and cultural food preferences as well as based on physical activity and physiological status will result in weight control as well as diabetes management (Devi *et al*., 2021).

## 4. Conclusion

Based on the rate of starch hydrolysis of soaked mung beans, mung bean starch is a slow-digesting starch.

There was a trend of increasing protein digestibility of mung bean seeds during soaking up to 6 hrs of soaking time (in a range of 46.93-51.29%). Based on the obtained data, the recommended soaking time was 6 hrs which revealed the optimum digestibility of starch and protein for diabetics. Further investigation in the processing field will be needed to get better food choices for diabetics.

## Conflict of interest

The authors declare no conflict of interest.

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RESEARCH PAPER

# Controlling mung bean soaking time as a simple way to obtain alternative sources of healthy food for the diabetics

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**Abstract**

Mung bean is one of the commodities with potency sources of starch and protein that is available and inexpensive. However, healthy food ingredients which are needed by diabetics must be high in slow-digest starch and protein. This research examined the effect of soaking time on the *in vitro* digestibility of starch and protein of mung bean, to obtain an alternative source of healthy food simply. A single factor of randomized block design was used in this study. Each treatment level was conducted in triplicates. The whole mung bean seeds were subjected to five different times of soaking, namely control (without soaking) and soaking for 2, 4, 6, and 8 hrs. Total starch, *in vitro* starch digestibility, protein content, and *in vitro* protein digestibility of freeze-dried and grounded whole mung beans were analyzed. The results showed a trend of the total starch content and starch digestibility decreasing during the longer soaking time, in a range of 39.14-33.52% and 57.46-49.82%, respectively. Based on the rate of starch hydrolysis, the digestibility of mung bean starch classified as slowly digestible starch. There was a trend of increasing protein digestibility of mung bean seeds during soaking up to 6 hrs of soaking time (in a range of 46.93-51.29%). Based on these results, the suggested soaking time was 6 hrs which gave optimum digestibility of starch and protein for human consumption. The recommendation is to obtain the benefits of a healthy food source for people with diabetes

## 1. Introduction

Mung bean (*Vigna radiata*) has been consumed in China for over 2,000 years, in the form of many kinds of food such as sprouts, noodles, cookies, and others (Fayyaz *et al*., 2018). Mung bean, as one of the legumes, is an important part of the dietaries of Indonesian people and contributes substantially to the nutrient intake of human beings, including children. Mung bean is less flatulent and is well tolerated by children (Dahiya *et al*., 2015).

Mung bean is one of the commodities with potency sources of starch and protein. Starch is the most important component of carbohydrates in mung beans, about 30.74±3.39% (Widjajaseputra *et al*., 2019a). Since starch is the most significant component in mung bean, the characteristics of starch will determine its suitability for its end use. Mung bean with its high amylose content can improve the swelling power and gel texture of a starch noodle product. This is as reported by Li *et al*. (2008), that the high-quality starch noodle made from mung bean starch result from its high amylose content. Menon *et al.* (2016) studied gluten-free starch noodles from sweet potatoes and found that fortification with mung bean starch reduced the rate of release of glucose from cooked noodles *in vitro* conditions. The high amylose content showed higher viscosities which could be used in certain food products such as thickeners for creams, sauces, soups, and puddings. Besides as a source of starch, mung bean is an excellent source of vitamins, minerals, and protein with an ideal essential amino acid profile (Mubarak, 2005). The specific profile of mung bean amino acid allows its use to supplement cereals and rice in particular. Mung bean flour could be used as a supplement for wheat flour, increasing the nutritional quality of bakery products (Marquezi *et al*., 2016). However, the original properties of the components in the mung bean will change depending on the applied processing.

Soaking is the beginning of legume processing which is usually done in preparation before use. During soaking, water enters the bean, its tissues hydrated, and some enzymes can be activated to break down complex structures such as starch and protein into simpler compounds. The treatment results in an alteration of their

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nutritional quality which could either be a reduction in nutrients and antinutrients or an improvement in the digestibility or availability of nutrients (Kaur *et al*., 2015). Pagar *et al*. (2021) who studied the horse gram, found that 6 hrs soaking, 72 hrs germination, and drying at 70°C treatment was the best for a maximum decrease in the anti-nutritional factors and at the same time enhanced functional properties due to the soaking and germination.

In a previous study, it was reported that the digestible (total sugar) and indigestible carbohydrates (resistant starch) of the mung bean increased during four hours of soaking (Widjajaseputra *et al*., 2019a). There are five types of resistant starches (RS1-RS5) and type RS2 is predominant in legumes as legume starches are physically enclosed within intact cell (protein) structures (Kaur *et al*., 2015). Such starches are indigestible by human digestive enzymes in the small intestine and pass to the large intestine or colon, thereby modifying postprandial glycemic responses. Widjajaseputra *et al*. (2019b) also reported that the soaking treatment improved the protein quality. In relation to the provision of healthy food for people with diabetes, high-quality protein sources are needed, but the type and quality of carbohydrate digestibility need to be considered. The study of the potency of soaked mung bean as a healthy food source, especially for diabetic people, based on the digestibility perspective is needed.

## 2. Materials and methods

### *2.1 Materials*

The commercial mung bean was obtained from a local market in Surabaya, East Java, Indonesia. All the chemicals, standards, and reagents were of analytical grade.

### *2.2 Soaking procedure*

According to Widjajaseputra *et al*. (2019b), the mung bean was sorted. Only intact and sound grains were washed and soaked (1:5 w/v) in distilled water at 30°C for 0 (control), 2, 4, 6, and 8 hrs, afterward the grains were drained and freeze-dried (Bluewave B-10B Vacuum Freeze Drier; China) to 2-3% moisture content. The dried grains were ground with a blender (Miyako, Indonesia), wrapped in an airtight plastic container and aluminum foil bag as secondary packaging, and then stored in a refrigerator (LG, Indonesia) at 5±1°C until analyzed.

### *2.3 Experimental design and statistical analysis*

The experimental design used in this study was a single factor with a randomized block design. The whole mung bean seeds were subjected to five different times of soaking, control (without soaking/P0) and soaking for 2 (P1), 4 (P2), 6 (P3), and 8 hrs (P4). Each treatment level was conducted in triplicates. Total starch, *in vitro* starch digestibility, protein content, and *in vitro* protein digestibility of freeze-dried and grounded whole mung beans were analyzed. Water content analysis was used for dry basis (db) calculation. Data were expressed as mean ± standard deviation (SD) for the three in each group (n = 3). The data were subjected to ANOVA ($p<0.05$) with a least significant difference (LSD) test at $p < 0.05$ using SPSS (version 19) for comparative analysis.

### *2.4 Analysis methods*

#### *2.4.1 Total starch analysis*

According to Goni *et al.* (1997), total starch was measured by incubating the sample suspension in the optimum condition of amyloglucosidase, at 60°C in a controlled shaking water bath, for 45 mins measuring activity. Starch was measured as glucose with Peridochrom Glucose GOD-PAP (Ref 676 543, Boehringer). The glucose content was measured by the enzymatic photometric test. A red quinone imine as the reaction product was measured at a wavelength of 500 nm. The absorbance of the colored complex was proportional to the concentration of glucose. The factor conversion of glucose to starch was 0.9.

#### *2.4.2 In vitro starch digestibility analysis*

Starch digestibility was determined by measuring digestible starch *in vitro* (Goni *et al*., 1996; Goni *et al.,* 1997). The principle of digestible starch determination was to analyze total starch with enzymes and measured undigested starch for 180 mins within a 30 mins interval. Then calculated the percentage of hydrolyzed starch (digestible starch) in equations (1) and (2) as follows:

$$\text{Digestible starch} = (\text{total starch} - \text{undigested starch}) \quad (1)$$

$$\text{Starch digestibility (\%)} = (\text{total starch} - \text{undigested starch})/\text{total starch} \times 100\% \quad (2)$$

#### *2.4.3 Protein content analysis*

The protein content analysis used the macro Kjeldahl method (Association of the Official Analytical Collaboration (AOAC) International, 2010). The protein in the sample was determined by measuring the amount of nitrogen (N) through three steps, namely digestion, distillation, and titration. The measured nitrogen content was multiplied by the conversion factor resulting in protein content. The conversion factor used was 6.25.

*2.4.4 In vitro protein digestibility*

The principle of determining in vitro protein digestibility is to compare the total nitrogen content after the sample is treated with protein digestive enzymes (pepsin) with the total N of the initial sample (Mertz *et al*., 1984). Nitrogen content was measured using the micro Kjeldahl method.

## 3. Results and discussion

*3.1 Effects of soaking time on total starch, undigested starch, and starch digestibility*

The total starch of mung bean seeds decreased significantly from 41.13% db to 33.52% db during 8 hrs of soaking, because more intensive hydrolysis occurred during the longer soaking time (Table 1). The decrease in total starch was due to the leaching out of the soluble part of starch in soaking water and the hydrolysis process to simpler compounds such as sugars and dextrin. The same thing was also found by Grewal and Jood (2009), that a significant decrease in starch content was followed by increased sugar content due to soaking and cooking of green gram.

Table 1. Effects of soaking time on total starch, undigested starch and starch digestibility.

| Soaking time (hr) | Total starch (% db) | Undigested starch (% db) | Starch digestibility (%) |
|---|---|---|---|
| P0 | 41.13±0.19$^{c}$ | 19.16±0.45$^{b}$ | 53.42±1.31$^{b}$ |
| P1 | 39.14±0.72$^{b}$ | 16.65±0.13$^{a}$ | 57.46±1.12$^{c}$ |
| P2 | 38.85±0.18$^{b}$ | 18.70±0.56$^{b}$ | 51.87±1.67$^{ab}$ |
| P3 | 38.18±0.44$^{b}$ | 18.53±0.34$^{b}$ | 51.47±0.31$^{a}$ |
| P4 | 33.52±1.29$^{a}$ | 16.82±0.21$^{a}$ | 49.82±1.30$^{a}$ |

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are statistically significantly different based on the LSD Test ($p<0.05$). P0: control (without soaking), P1: soaked for 2 hrs, P2: soaked for 4 hrs, P3: soaked for 6 hrs, P4: soaked for 8 hrs.

Legume starch contains amylose higher than cereal or tuber starch. This starch has a lower bioavailability than most other starches when it is raw or retrograded (Guillon and Champ, 2002). Widjajaseputra *et al.* (2019a) stated that the amylose level of mung beans (32.56±0.31%) is higher than cereal (around 25%) and tuber starch (around 17-19%). Singh *et al.* (2003) state that the factors which could influence starch retrogradation were the amylopectin content, intermediate materials, size and shape of the granules, the botanical source, and the amylose content. A higher proportion of amylose content being linked to a higher tendency to retrograde would affect the level of digestibility.

The starch digestibility decreases (from 57.46% to 49.82%) with the longer soaking time and the decrease of total starch during the soaking treatment up to 8 hrs of soaking from 39.14% to 33.52% (Table 1). If compared with the control, soaking for 2 hrs resulted in a significant increase in starch digestibility. This is due to part of the starch granules that were more readily hydrolyzed by enzymes that were affected by the imbibition of water in the soaked mung bean seeds. After 4 hrs of soaking time, the decrease in starch digestibility was not significantly different. Figure 1 shows that based on the rate of starch hydrolysis, the digestibility of mung bean starch is a slow-digesting starch.

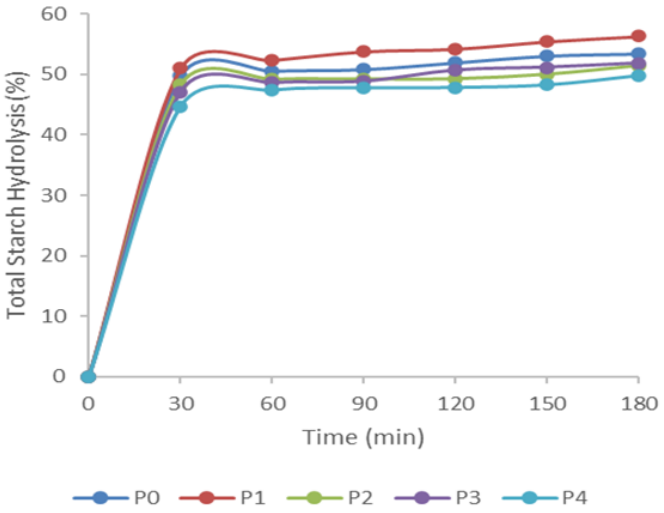


Figure 1. Mung bean starch hydrolysis rate. P0: control (without soaking), P1: soaked for 2 hrs, P2: soaked for 4 hrs, P3: soaked for 6 hrs, P4: soaked for 8 hrs.

The increasing of undigested starch up to 3 hrs of digestion (*in vitro*) can be affected by the number of resistant starch (RS). RS is defined as the portion of starch and starch products that resist digestion, passing directly through the small intestine (Fabbri *et al*., 2016). Widjajaseputra *et al.* (2019a) found that the RS of mung bean was increased from 11.12% db to 18.49% db if soaked for 4 hrs, although it was decreased significantly (13.65% db) if soaking was continued for up to 8 hrs. This phenomenon is in line with the statement of Lang *et al.* (1999) that mung bean starch contains RS 11% db and has a long absorption period, which within 4.5 hrs after consumption has not been completely digested. It was due to the high level of amylose in mung bean starch (around 32%-34%). The difference in RS can be influenced by differences in starch structure in various amylose-amylopectin ratios among different starch granules which affect the RS level in processed food, including during seeds soaking and freeze-drying treatment in sample preparation. Retrogradation of amylose can occur during soaking and freeze-drying treatment in sample preparation, and part of starch was to exist as RS3. RS3 is starch that has been retrograded into

more highly stabile crystalline structures. In addition, Fabbri *et al.* (2016) found that the cooling process of legumes can increase RS as a result of retrogradation. These phenomena caused a decrease in the level of starch digestibility. RS is one kind of dietary fiber. Dietary fiber is bound together in such a way that it can not be ready in the small intestine. Besides it, dietary fiber may make humans feel full longer. This causes mung bean to help keep blood sugar levels low. Nevertheless, the different processes following the soaking would give different effects on starch digestibility, as example, if soaking was followed by dehulling and cooking, the level of starch digestibility would increase as obtained by Grewal and Jood (2009).

### *3.2 Effects of soaking time on protein content and protein digestibility*

The values of protein digestibility were significantly different on 6 hrs (P3) and 8 hrs (P4) of treated soaking time compared to P0, P1, and P2 as shown in Table 2. Protein digestibility increased during soaking treatment, up to 6 hrs of soaking time, and then the protein digestibility slightly decreased on 8 hrs of seed soaking.

Table 2. Effects of soaking time on protein content and protein digestibility.

| Soaking time (hr) | Protein content (% db) | Protein digestibility (%) |
|---|---|---|
| P0 | 26.29±0.27 | 46.93±1.53$^{a}$ |
| P1 | 25.88±0.40 | 48.41±0.38$^{ab}$ |
| P2 | 25.90±0.25 | 48.56±0.54$^{ab}$ |
| P3 | 25.68±0.33 | 51.29±1.20$^{c}$ |
| P4 | 26.10±0.82 | 49.83±3.00$^{bc}$ |

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are statistically significantly different based on the LSD Test ($p<0.05$). P0: control (without soaking), P1: soaked for 2 hrs, P2: soaked for 4 hrs, P3: soaked for 6 hrs, P4: soaked for 8 hrs.

This phenomenon was affected by increasing the soluble protein of mung bean seeds from 108.96 mg/g of dry weight in raw seeds without soaking to 159.81 mg/g of dry weight in seeds with 6 hrs of soaking time (Widjajaseputra *et al*., 2019b). The higher soluble protein indicated the readiness of proteins to be digested. Based on protein digestibility shown in Table 2, the recommended soaking time for mung bean seed was 6 hrs, because a germination process has taken place longer than 6 hrs of soaking. During germination periods there were hydrolyzing processes that would affect protein and starch degradation to produce energy for the new plant. These phenomena were revealed by Grewal and Jood (2009) that the germination process decreased starch content, thereby increasing soluble sugars and improved starch digestibility to 49% and 48% in two different green gram (*Vigna radiata* L.) cultivars respectively.

### *3.3 Perspective of mung bean as a healthy food source for the diabetics*

The high protein digestibility of mung beans as a result of soaking for 6 hrs can support its usage as a good food source of protein. A combination of mung bean protein and rice protein in a 3:4 ratio respectively can increase the chemical amino acid score to 72 (Dahiya *et al*., 2015). Based on this recommendation, an increase in protein bioavailability can be obtained. Consumption of legumes provides qualified protein along with other micronutrients without adding extra energy or fat. According to Mak *et al.* (2018), a diet high in protein-low starch was associated with a lower risk for gestational diabetes mellitus among women who were overweight at pre-pregnancy. Mung bean seeds are an affordable source of not only protein but also starch, which has the advantage of consisting of higher resistant starch compared to cereal, root, and tuber starch. Based on these characteristics, mung bean can be used as a good source of resistant starch with a high protein content in various food applications.

Mung beans as one kind of variety of pulses, are high in fiber and have a low glycemic index, making them particularly beneficial to people with diabetes by assisting in maintaining healthy blood glucose and insulin levels (Dipnaiki and Bathere, 2017). Previously, the same thing was also reported by Rebello *et al.* (2014), that mung bean like other legumes had a medium glycemic index (GI) and high content of dietary fibers, which makes benefit to be a healthy food source. High resistant starch content combined with medium GI is a positive attribute that could promote the product as a better food choice not only for diabetes mellitus patients but also for people which is suffered from celiac disease, obesity, and other malnutrition symptoms (Rebello *et al.*, 2014). In particular, for the nutrition management of gestational diabetes mellitus, it is important to focus on the quality of carbohydrates and encourage the consumption of vegetables, fruits, complex carbohydrates, and high-fiber foods (Kapur *et al*., 2020). A balanced diet consisting of healthy carbohydrate sources with adequate proteins and fats based on individual and cultural food preferences as well as based on physical activity and physiological status will result in weight control as well as diabetes management (Devi *et al*., 2021).

## 4. Conclusion

Based on the rate of starch hydrolysis of soaked mung beans, mung bean starch is a slow-digesting starch. There was a trend of increasing protein digestibility of

mung bean seeds during soaking up to 6 hrs of soaking time (in a range of 46.93-51.29%). Based on the obtained data, the recommended soaking time was 6 hrs which revealed the optimum digestibility of starch and protein for diabetics. Further investigation in the processing field will be needed to get better food choices for diabetics.

## Conflict of interest

The authors declare no conflict of interest.

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