

Manuscript FR-2019-209 entitled " Mung bean as appropriate food for breastfeeding women with diabetes mellitus in Indonesia: Carbohydrate profiles at different soaking times"
Food Research <foodresearch.my@outlook.com>

To:Yahoo

Wed, Jun 19, 2019 at 2:52 PM

Dear Dr. Widjajaseputra,

Manuscript FR-2019-209 entitled " Mung bean as appropriate food for breastfeeding women with diabetes mellitus in Indonesia: Carbohydrate profiles at different soaking times" which you submitted to Food Research, has been reviewed. The comments of the reviewer(s) are included in the attached file.

The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript. Once the revised manuscript is prepared, please send it back to me for further processing.

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Once again, thank you for submitting your manuscript to Food Research and I look forward to receiving your revised manuscript.

Sincerely,

Professor Dr. Son Radu

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To:Food Research

Wed, Jun 19, 2019 at 6:44 PM

Dear Prof.Dr. Son Radu,

Thank you very much for the opportunity. I have received our manuscript's file included the reviewers comments. We will revise it as soon as possible.

Sincerely,

A. I. Widjajaseputra

On Wed, 6/19/19, Food Research <foodresearch.my@outlook.com> wrote:

Subject: FR-2019-209

To: "Yahoo" <ingani9456@yahoo.com>

Date: Wednesday, June 19, 2019, 2:52 PM

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Sincerely,

Professor Dr. Son Radu

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Editor-in-Chief, Food Research

Mung bean as food source for breastfeeding women with diabetes mellitus in Indonesia: carbohydrate profiles at different soaking times

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Abstract

The study reported carbohydrate profiles of mung bean that was soaked for different times, and how the bean can be a potential food in Indonesia for breastfeeding women with diabetes mellitus (DM). This study used a single factor with completely randomized design. The soaking time was a factor with five levels; 0 (control), 2, 4, 6, and 8 hrs. Each treatment level was replicated three times. Observed dependent variables of carbohydrate profiles were starch, amylose, total sugar, total dietary fiber (TDF), insoluble dietary fiber (IDF), soluble dietary fiber (SDF), and resistant starch (RS) contents. The results were subjected to one-way ANOVA and least significant difference test at $p < 0.05$ using SPSS (version 19). The soaking time resulted in a decrease of the starch content, relatively unchanged amylose content, and an increase of the reducing sugar content. The TDF tended to decrease, the RS was significantly ($p < 0.05$) higher than the unsoaked mung bean from 4 hrs of soaking and the SDF tended to increase with soaking. Generally, the digestible (total sugar) and indigestible (total dietary fiber and resistant starch) carbohydrates increased during the soaking. The overall results would indicate fewer calories and inhibition of blood sugar increases that are beneficial to breastfeeding women with DM. The findings provide a new scientific understanding of mung bean as an alternative food source for breastfeeding women with diabetes mellitus and can contribute to formulating national and global DM management food policy.

1. Introduction

Mung bean (*Vigna radiata* L), green gram or golden gram, is the third most cultivated legume in Indonesia after soybeans and peanuts (Purwono and Hartono, 2005). In 2015, mung bean production (dry seed) in Indonesia was about 271,000 MT compared to soybean production of about 963,000 MT (BPS-Statistics Indonesia, 2016). With its quantity of protein content being second to its carbohydrate content, mung bean can be used as a source of proteins, and it is also a good source of dietary fiber, essential minerals, and vitamins (Oghbaei and Prakash, 2017). As a legume, mung bean is important in human nutrition, but this can be limited because of its antinutrients such as protease inhibitors, phytic acid, and flatulence-causing oligosaccharides (Hussain and Burhanuddin, 2011). Mung bean carbohydrate content is 50-60% (Tang *et al.*, 2014) with starch being its most abundant carbohydrate at 37% with amylose fraction of about 32% and amylopectin fraction of about 68% (Sandhu and Lim, 2006). Mung bean starch granules have a round to oval shape and 7 μm - 28 μm in size (Hongsprabhas, 2006).

Prior to use, mung bean is usually soaked first to reduce its antinutrients (Kaur *et al.*, 2015). During soaking, water entered the bean, its tissues hydrated, and some enzymes can be activated to extents that are dependent on the soaking times culminating in minor and major changes in the bean structures and components. These changes will increase the digestibility of mung bean as a food source. In Indonesia, mung bean is a traditional food source for women that are breastfeeding, as it is believed that the consumption of mung bean porridge improved the quality of breast milk due to viscosity increases from the mung bean starch. However, starch is associated with lifestyle diseases, notably diabetes, which is projected globally to afflict 54% more adults in 2030, and individuals with diabetes in Indonesia are projected to increase from 7 million in 2010 to 12 million in 2030 (Whiting *et al.*, 2011; Sopade, 2017a). Hence, if breastfeeding women are diabetic, their consumption of starch-containing diets needs to be regulated with the overall aim of managing DM among Indonesians, for example. Therefore, this study examined the effects of soaking time on

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carbohydrate profiles of mung bean for understanding the potential of the bean as a good food source for breastfeeding women with DM.

2. Materials and methods

2.1 Materials

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.* (2019), the mung bean was sorted, and 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, after which 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±1°C until analyzed. The soaking experiment was replicated thrice.

2.3 Proximate analysis

Moisture and ash contents by thermogravimetry, protein analysis by micro Kjeldahl, fat analysis by Soxhlet method, and carbohydrate by difference were done following standard procedures (AOAC, 2010).

2.4 Carbohydrate profile

Starch and total sugar were measured using the Nelson Somogyi method (AOAC, 2010), in which the reducing sugar in the sample reduced cupric oxide to copper oxide, which was reacted with arsenomolybdate to form a blue complex that was spectrophotometrically (Shimadzu, UV-Vis 1800 Spectrophotometer, Japan) quantified at 540 nm wavelength from a standard absorbance curve. Amylose levels were measured following the procedure in Juliano (1971). Total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble

dietary fiber (IDF) contents were determined by a multi-enzymatic gravimetric method (Asp *et al.*, 1983), whereby starch and protein were digested with enzymes to small fragments to separate the fiber, before separating the IDF from the SDF by filtering and washing the residues with water. The IDF as the remaining residue was recovered by precipitation with 95% ethanol and washed with acetone. Resistant starch (RS) was analyzed using the method of Goni *et al.* (1996) that involves the removal of proteins and digestible starches, solubilization and enzymatic hydrolysis of RS and its quantification from the glucose released.

2.5 Statistical analysis

The experimental design used in this study was a single factor completely randomized design. Data are 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.

3. Results and discussion

3.1 Proximate analysis

As expected, the moisture content of the mung bean significantly (p<0.05) increased (12-53 g/100 g wet basis) with the soaking (Table 1) as the grains imbibed water and the cells were hydrated (Nonogaki *et al.*, 2010). The penetration of water into the grains would affect biochemical reactions in the grains as hydrolyzing enzymes became active, and α-amylase, invertase, lipase, and protease have been reported to be activated (Rahman *et al.*, 2007; Fayyaz *et al.*, 2018) thereby affecting the mung bean starch, protein and fat components. The protein content of the unsoaked mung bean (23.39 g/100 g wet basis or 26.64 g/100 g dry weight basis) is similar (27.5 g/100 g dry weight basis) reported by Mubarak (2005). Table 1 shows the significant (p<0.05) changes in fat and ash components of the mung bean with the fat content tended to increase, while the ash content decreased along with the longer soaking time. Various

Table 1. Effect of soaking time on the proximate composition of the mung bean

Soaking Time	Moisture content (g/100 g wet basis)	Protein (g/100 g wet basis)	Fat (g/100 g wet basis)	Ash (g/100 g wet basis)	Carbohydrate (g/100 g wet basis)
P0	12.18±0.46 ^a	23.39±2.16	2.56±0.36 ^b	4.35±0.06 ^b	57.52±2.92 ^c
P1	20.66±0.77 ^b	22.80±1.32	1.72±0.14 ^a	4.33±0.09 ^b	50.49±1.95 ^d
P2	32.60±0.38 ^c	23.56±0.89	4.02±0.18 ^c	4.20±0.11 ^b	35.62±0.98 ^c
P3	51.03±0.48 ^d	23.26±1.07	5.22±0.14 ^c	3.95±0.19 ^a	16.54±1.36 ^b
P4	53.00±0.58 ^e	24.55±0.59	4.43±0.18 ^d	3.98±0.09 ^a	14.04±0.58 ^a

Values are means ± standard deviations (n=3). Different alphabet superscripts in the same column showed a significant difference based on the LSD Test (p<0.05).

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

Table 2. Effect of soaking time on the carbohydrate profiles of the mung bean

Soaking Time	Starch (g/100 g dry weight basis)	Amylose (g/100 g dry weight basis)	Total sugar (g/100 g dry weight basis)	Total dietary fiber (g/100 g dry weight basis)	Insoluble dietary fiber (g/100 g dry weight basis)	Soluble dietary fiber (g/100 g dry weight basis)	Resistant starch (g/100 g dry weight basis)
P0	30.74±3.39 ^c	32.56±0.31	12.35±1.39 ^a	30.80±0.71 ^c	25.98±0.87 ^c	4.82±0.16 ^a	11.12±0.10 ^b
P1	24.38±0.27 ^d	33.09±1.18	27.36±0.22 ^b	38.30±0.08 ^d	28.99±0.03 ^d	9.30±0.11 ^c	8.33±0.09 ^a
P2	22.22±0.46 ^c	33.06±3.42	29.52±0.86 ^c	27.65±0.57 ^b	21.12±0.32 ^b	6.53±0.06 ^b	18.49±0.27 ^c
P3	18.81±0.44 ^b	34.55±0.43	34.91±0.58 ^d	24.80±0.35 ^a	17.95±0.29 ^a	6.86±0.06 ^c	14.49±0.06 ^d
P4	15.81±2.22 ^a	35.22±3.25	39.52±2.47 ^e	24.59±0.06 ^a	17.48±0.08 ^a	7.11±0.02 ^d	13.65±0.19 ^c

Values are means ± standard deviations (n=3). Different alphabet superscripts in the same column showed a significant difference based on the LSD Test (p<0.05).

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

studies (Desalegn, 2015; Fayyaz *et al.*, 2018) had measured the increase in the fat content of grains with soaking, which they related to the synthesis of the fat as a catabolic ingredient for growth.

Decreasing in ash content can be due to the use of certain minerals for the synthesis of several compounds that were needed to prepare germination such as Ca for the activation of amylase, Mg was needed for chlorophyll synthesis, PO₄⁻³ was needed for energy metabolism. The reduction in the ash content with the soaking could have resulted from the leaching of relevant soluble components from the mung bean with time (D'Souza, 2013).

3.2 Carbohydrate profile

Carbohydrates are the largest component of mung beans that need to be studied for their quantity and type or source is related to their potential as a source of calories for people with diabetes mellitus. Besides the amount of carbohydrate ingested, the type of carbohydrate also affects postprandial responses (American Diabetes Association, 2008). Foods with high resistant starches and amylose contents have been shown to exhibit slow rates of digestibility and reduced energy intakes by the intestinal cells, evident by low glycemic indices (Vatanasuchart *et al.*, 2009; Sopade, 2017b).

The starch content of the control sample (30.74 g/100 g dry weight basis), significantly (p<0.05) reduced with the soaking time (Table 2), which did not significantly affect (p>0.05) the amylose content (32.56 - 35.22 g/100 g dry starch). The increase in the total sugars with the soaking time (Table 2) could suggest that the mung bean starch changed into simpler compounds during the soaking due to the activity of α -amylase, being the dominant amylase in mung beans (Patong and Suarni, 2007). It is plausible that the solids lost during the soaking through leaching, though minimal, could contribute to the increases measured for these and other parameters.

The total dietary fiber (TDF) decreased with the

longer soaking time and ranged from 38.30-24.59 g/100 g dry weight basis (Table 2), with the seed coats or hulls contributing much of the soluble and insoluble fibers. Soluble dietary fiber (SDF) lowers the blood glucose level (Aguilera *et al.*, 1993). Chandalia *et al.* (2000) concluded that a high intake of dietary fiber, particularly of the soluble type, above the level of the recommendation by the American Diabetes Association, improved glycemic controls in patients with type 2 diabetes. Generally, with the mung bean, the insoluble dietary fiber (IDF) tend to decrease with the longer soaking (Table 2), which the SDF tend to increase. Possibly, hydrolytic enzymes degraded the TDF, solubilized a part of the IDF to yield more SDF as the soaking progressed beyond two hours. The resistant starch (RS) measured for the control mung bean (11.12±0.10 g/100 g dry weight basis) is similar to reported data (about 11%) by Guillon and Champ (2002), and upon soaking, this initially reduced after two hours before increasing. There are five types (RS1 = RS5) of resistant starches (Sopade, 2017b), and type RS2 is predominant in legumes as legume starches are physically enclosed within intact cell (protein) structures (Kaur *et al.*, 2015; Sopade, 2017b). 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.

4. Conclusion

The carbohydrate profiles showed that the soaking time decreased the starch level with relatively fixed amylose content and an increase in the total sugar levels. While the TDF tended to decrease, the RS was significantly (p<0.05) higher than the unsoaked mung bean from 4 hrs of soaking and the SDF increased with the soaking. In general, the digestible carbohydrates increased during the soaking along with an increase in several types of indigestible carbohydrates. These changes in the carbohydrate profiles of the mung bean with the soaking are expected to lead to reduced calories and a significant reduction in the blood glucose upon

consumption of the soaked mung bean. This will benefit for breastfeeding women with DM. Further studies will investigate soaked mung bean digestibility to confirm the beneficial deductions from the present study.

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