



# Dietary adzuki bean paste dose-dependently reduces visceral fat accumulation in rats fed a normal diet

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journal or publication title	Food Research International
volume	130
page range	108890
year	2020-04
URL	<a href="http://id.nii.ac.jp/1588/00004616/">http://id.nii.ac.jp/1588/00004616/</a>

doi: [info:doi/http://doi.org/10.1016/j.foodres.2019.108890](https://doi.org/10.1016/j.foodres.2019.108890)



1 **Dietary adzuki bean paste dose-dependently reduces visceral fat accumulation in rats**  
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40 **Chemical compounds studied:** Acetic acid (PubChem CID: 176); Butyric acid (PubChem

41 CID: 264); Propionic acid (PubChem CID: 1032); Ammonia- nitrogen (PubChem CID:

42 6857397)

43

44 **Abstract**

45 The aim of this study was to evaluate the dose-dependent effect of adzuki bean (*Vigna*  
46 *angularis*) paste (ABP) on visceral fat accumulation in rats. ABP is a rich source of  
47 indigestible carbohydrates (18.5%) with fiber and resistant starch (RS) contents of 14.5% and  
48 4.0%, respectively. Animals were fed one of the following diets, control (CON), 30% ABP or  
49 58.9% ABP for 28 days. The daily dietary energy intake was lowered ( $p < 0.05$ ) and reduced  
50 visceral fat accumulation and lower serum lipid levels were observed in ABP fed groups.  
51 ABP consumption dose-dependently increased ( $p < 0.05$ ) the daily fecal lipid and fecal acidic  
52 sterol excretions. On the other hand, cecal content and fecal moisture content in the 58.9%  
53 ABP group were greater ( $p < 0.05$ ) than the CON group, while there was no significant  
54 difference between the two ABP fed groups. Both 30% and 58.9% ABP diets had  
55 significantly ( $p < 0.05$ ) higher contents of cecal acetic, propionic and *n*-butyric acids, and  
56 lowered cecal pH, independently of the ABP dose. Microbial community data of rats fed ABP  
57 diets exhibited higher alpha-diversities than the rats fed CON diet, based on the Shannon  
58 Index and the number of observed species index, where the two ABP groups exhibited a  
59 similar alpha diversity. The weighted UniFrac-based principal coordinate analysis plot of  
60 cecal microbial community data showed that the ABP had a substantial effect on the cecal  
61 microbial composition. Furthermore, cecal bacterial 16S rRNA gene sequencing revealed  
62 that the ABP supplemented diets decreased the ratio of Firmicutes to Bacteroidetes. These  
63 findings suggested that the cecal fermentation of fiber and RS in ABP, might have decreased  
64 the energy intake, altered the gut microbiota composition, increased fecal lipid output, and  
65 thereby reduced fat accumulation in rats.

66

67 **Keywords:** Adzuki bean paste, cecal bacteria, fecal lipid excretion, fermentation,  
68 indigestible carbohydrate, energy intakes, fat accumulation

69

## 70 **1. Introduction**

71 Rapidly escalating incidents of overweight and obesity have become global public health  
72 concerns linked to increased risks of chronic non-communicable diseases such as  
73 cardiovascular diseases and type II diabetes (Despres & Lemieux, 2000; World Health  
74 Organization, 2003; Animaw & Seyoum, 2017). The build-up of a calorie surplus due to the  
75 consumption of energy-dense foods, which is usually stored in the adipose tissue resulting in  
76 unnecessary fat accumulation, is one of the main risk factors of obesity development (Rolls  
77 *et al.*, 2005). The dietary interventions such as dietary adherence and caloric deficit play  
78 important roles in weight loss and weight-loss maintenance (World Health Organization,  
79 2003; MacLean *et al.*, 2015). Thus, restriction of energy intake together with sufficient  
80 physical activity are considered as healthy lifestyle habits in today's environment, in the  
81 context of preventing obesity (MacLean *et al.*, 2015; Heymsfield & Wadden, 2017).

82 Over the past decade, research suggests that the gut microbiota composition (including  
83 bacterial diversity) also influences obesity in animal models (Ley *et al.*, 2005, Turnbaugh *et al.*  
84 *et al.*, 2006; de La Serre CB *et al.*, 2010; Hamilton *et al.*, 2015; Sen *et al.*, 2017) and in humans  
85 (Ley *et al.*, 2006; Menni *et al.*, 2018). For example, a potential role of gut microbiota on the  
86 obesity development was shown with that the relative abundance of Firmicutes, one of the  
87 two most abundant gut microbial phyla, in obese mice was higher compared to that of the  
88 normal lean mice, while Bacteroidetes abundance was lower (Ley *et al.*, 2005). Mice  
89 gained more weight when their large intestines were colonized with the microbial members  
90 belonging to phylum Firmicutes (Turnbaugh *et al.*, 2006). Furthermore, a recent study of  
91 overweight or obesity shows a dysbiosis characterized by a lower gut microbiota diversity,  
92 and that *Bacteroides* species are associated with long-term weight gain (Menni *et al.*, 2018).  
93 However, the abundance of a specific genus, class or species of gut bacteria is not always  
94 positively or negatively associated with the incidence of obesity. Gut microbial dysbiosis in

95 obesity has been linked to an altered gut barrier, thereby promoting systemic endotoxemia,  
96 through the translocation of lipopolysaccharides (Cani, 2018). Similar to that, the excessive  
97 intake of specific food components such as fat (de La Serre CB *et al.*, 2010; Hamilton *et al.*,  
98 2015) or sugar (Sen *et al.*, 2017), also induces gut microbial dysbiosis, impairs the intestinal  
99 barrier function and/or alters vagal gut-brain communication in animals, which subsequently  
100 may lead to metabolic disorders (de La Serre CB *et al.*, 2010). On the other hand,  
101 supplementation of probiotics and fecal microbiota transplantation are practiced as strategies to  
102 modify the gut microbial composition. Another approach is to supplement diets with  
103 prebiotics that provide carbon and nitrogen sources for gut microbial metabolism, resulting in  
104 the production of short chain fatty acids (SCFA) and other fermentation products, which are  
105 known to modify the gut microbial composition. For example, dietary supplementation of  
106 inulin to obese rats led to a remarkable increase in the abundance of bifidobacteria (Han *et*  
107 *al.*, 2017). Other research also demonstrates that modification of dietary carbohydrates  
108 favorably changes the gut microbiota (Parnell & Reimer, 2012).

109         Studies in animal models and human subjects have reported an improved weight loss for  
110 fiber supplemented diets (Li *et al.*, 2016; Han *et al.*, 2017) and high-fiber foods (Han *et al.*,  
111 2016). According to a literature review by Howarth *et al.* (2001), changes in the energy  
112 intake and the body weight were observed after consuming dietary fiber supplements.  
113 Furthermore, dietary fiber is known to positively affect satiety and the subsequent  
114 food/energy intake (Slavin & Green, 2007). Additionally, a link between the consumption of  
115 dietary fiber and the reduced chronic diseases incidence in rats also has been suggested (Han  
116 *et al.*, 2017). Thus, owing to less energy-density ( $\sim 2 \text{ kcal g}^{-1}$ ) and gut microbiota  
117 modification effects (Parnell & Reimer, 2012), a fiber-rich meal is recommended as a  
118 potential candidate for controlling body weight (Rolls *et al.*, 2005).

119 Legumes are excellent sources of non-digestible saccharides, and the relationship  
120 between the consumption of legumes and its effects on obesity has been previously well-  
121 reviewed (Rebello *et al.*, 2014). Several previous studies have reported a lower average body  
122 weight of the participants in communities where a higher average amount of legumes was  
123 consumed (Papanikolaou & Fulgoni, 2008; Jenkins *et al.*, 2012). Among various legumes,  
124 the small red bean, adzuki (*Vigna angularis*) has been mainly cultivated throughout East Asia  
125 (770,00 ha), where China is the world's biggest producer of adzuki beans (0.24 Mt in 2014,  
126 Li *et al.*, 2017). Adzuki bean has been consumed 0.14 Mt per year in Japan, where the most  
127 common use is the sweetened bean paste. A recent clinical trial suggested that the  
128 consumption of adzuki bean would attenuate inflammation and improve glycemic control in  
129 patients with type 2 diabetes (Liu *et al.*, 2018). In a previous study, we have found that the  
130 consumption of high fiber, less energy-dense red adzuki bean paste, improved serum lipid  
131 profile in rats, which was mostly attributed to its higher indigestible matter content,  
132 particularly dietary fiber and resistant starch (RS) (Han *et al.*, 2003a).

133 We hypothesized that higher fiber and RS contents in adzuki bean will positively affect  
134 the development of obesity by influencing energy intake and satiety. However, there is no  
135 information currently reported on the contribution of adzuki bean paste (ABP) to obesity  
136 development. Thus, in this study, we aimed to examine the effects of ABP consumption on  
137 the cecal fermentation, cecal microbiota and obesity development in rats.

138

## 139 **2. Materials and Methods**

### 140 *2.1. Preparation of adzuki bean paste*

141 Adzuki bean paste was purchased from a local company (Hosokawa Seian Co. Ltd,  
142 Obihiro, Japan) in 2017. The ABP was prepared according to a traditional method as  
143 described below. Raw beans (*Vigna angularis* cv. Erimoshouzu) were washed with water and

144 soaked at room temperature overnight. The soaked beans were boiled in water ( $\times 4$  folds,  $w/v$ )  
145 for 15 min and cooled to 15°C by adding water. Then beans were again boiled for 1 h and  
146 were later passed through a fine a strainer (60-mesh) to remove the bean husk after cooling  
147 for 1 h. The separated paste was put in a cloth bag, and was pressed to remove the moisture,  
148 and was freeze-dried (TFD-550-8, Takarass, Tokyo, Japan). Finally, the freeze-dried paste  
149 was ground to a particle size of 0.5 mm or less using an ultracentrifugal mill (ZM200,  
150 Retsch, Osaka, Japan).

151

## 152 *2.2. Composition of adzuki bean paste*

153 The nutrient composition of ABP was determined by the Official Methods of Analysis of  
154 AOAC International (1990) as follows: crude protein or total Kjeldhal nitrogen (AOAC  
155 method 920.87) with a conversion factor of 6.25, lipid (AOAC method 920.85), moisture  
156 (AOAC method 925.10) and ash (AOAC method 923.03). The dietary fiber content was  
157 determined by enzymatic-gravimetric method (AOAC method 991.43), and the RS content  
158 by a commercial test kit (Megazyme International Ireland, Ltd., Bray, Co. Wicklow, Ireland).

159

## 160 *2.3. Animal study*

161 Seven-week-old male Fischer 344 rats were purchased from Charles River Laboratories  
162 Japan (Yokohama, Japan). The F344 rats are inbred strains, which have been used for general  
163 multipurpose models such as aging, safety and efficacy testing, oncology or nutrition. All  
164 animals were individually housed in plastic cages with a steel mesh at the bottom (22°C,  
165 55% humidity, 12-h light: dark cycle). Rats were acclimatized for one week on a standard  
166 rodent diet (CE-2, CLEA Japan, Inc., Tokyo, Japan) and tap water. Then the rats were  
167 randomly separated into 3 similar body weight groups. The number of animals ( $n = 5$ ) were  
168 determined based on a previous report (Han *et al.*, 2003a). Each group was fed one of the



169 following experimental diets (formulated by Oriental Yeast Co., Ltd., Tokyo, Japan) to  
170 observe a dose-dependent effect of adzuki bean paste on obesity development for 4 weeks;  
171 AIN-93G semi-purified rodent diet (CON), 30% ABP (LAB), and 58.9% ABP (HAB), with a  
172 combined fiber and RS content of 5.0%, 10.6%, and 15.8%, respectively. Diet compositions  
173 are shown in Table 1 and the dose and the time of administration were determined partly  
174 based on a previous report (Han *et al.*, 2003b). All rats had access to *ad libitum* water and  
175 experimental diets throughout the experimental period. Immediately prior to the  
176 administration of the narcotic (Nembutal, 40 mg kg<sup>-1</sup> body weight; Abbott Laboratories,  
177 Illinois, USA), the rat anal area was gently massaged to collect fresh feces directly from the  
178 anus, which was later freeze-dried (Eyela PDU-2100, Tokyo Rikakikai Co., Ltd., Tokyo,  
179 Japan). Dry feces that had dropped on the bottom of the cage for three days before dissection  
180 were also collected. After euthanizing the rats, blood, liver, cecum and visceral fat tissues  
181 were quickly removed, and all tissues were weighed before freezing for storage. The animals  
182 were handled according to the guidelines of “Guide for the Care and Use of Laboratory  
183 Animals” (National Research Council, 1996). This experimental design was approved by the  
184 Animal Care and Experiment Committee of Obihiro University of Agriculture and Veterinary  
185 Medicine, Japan (animal protocol approval no. 29–94).

186

#### 187 2.4. Serum cholesterol and triglyceride

188 Blood (0.5 mL) was collected from the jugular vein of the fasted rats every week. Serum  
189 was prepared by centrifugation at 1500 × g for 20 min at 4°C after standing at room  
190 temperature for 2 h. Total cholesterol, HDL-cholesterol and triglyceride contents were  
191 analyzed using a clinical autoanalyzer (Model 7070, Hitachi, Tochigi, Japan). Non-HDL-  
192 cholesterol (VLDL + IDL + LDL) content was calculated as follows: [non-HDL-cholesterol]  
193 = [total cholesterol] – [HDL-cholesterol].

194

## 195 2.5. Hepatic lipid and fecal lipid excretion

196 Total lipid fraction in the liver was extracted with chloroform-methanol (2:1, v/v)  
197 according to the method of Folch *et al.*, (1957). The organic solvents in the samples were  
198 removed using a Büchi rotavapor (R-114, Büchi, Tokyo, Japan). And the total lipid content  
199 was measured gravimetrically. The hepatic triglyceride and cholesterol levels were measured  
200 in the total lipid fraction dissolved in isopropyl alcohol using commercially available kits  
201 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) according to the manufacturer's  
202 instructions. The total lipid content in the feces was similarly extracted according to the  
203 Folch' method (1957). In brief, a portion of the pooled freeze-dried feces (Eyela PDU-2100)  
204 which were collected during the last three days of the experimental period was mixed with a  
205 3-fold volume of chloroform-methanol (2:1, v/v) and sonicated for 10 min. The resultant  
206 solution was mixed thoroughly with a little amount of water in a separate tube. After  
207 centrifugation ( $1500 \times g$  for 20 min at  $4^{\circ}\text{C}$ ), the upper layer was removed and the lower layer  
208 was dried. This procedure was repeated 3 times. Finally, the extracted lipid from feces was  
209 determined gravimetrically by the combined weight of the three dried lower layers. Fecal  
210 neutral and acidic sterol contents were measured by GLC (GC-2014, Shimadzu Co., Ltd.,  
211 Kyoto, Japan) following the method of Matsubara *et al.*, (1990) and Grundy *et al.*, (1965),  
212 respectively, as previously described (Han *et al.*, 2013). The daily excreted amounts of  
213 neutral and acidic sterol were obtained as follows: [concentration ( $\mu\text{mol g}^{-1}$ )  $\times$  dried feces  
214 weight ( $\text{g day}^{-1}$ )].

215

## 216 2.6. Adipocyte area

217 For measurement of adipose cell area, the mesenteric fat tissue was fixed with 10%  
218 neutral buffered-formalin solution and was embedded in paraffin. Standard, 4- $\mu\text{m}$  thick

219 sections were cut, stained with hematoxylin and eosin, and were visually examined under an  
220 optical microscope (DP-70, Olympus, Tokyo, Japan). Then, cell area ( $\mu\text{m}^2$ ) was measured  
221 using freely available software (Image J, version 1.46r, NIH, Bethesda, ML, USA) in  
222 comparison to a standard bar length (100  $\mu\text{m}$ ) within each image as the reference length.  
223 Finally, the adipocyte area was calculated as the average of 4 randomly obtained images (20 $\times$   
224 magnification, JPEG) of each sample.

225

## 226 2.7. Cecal bacterial DNA extraction, community analysis, and anaerobic bacteria count

227 Analytical conditions for cecal bacterial nucleic acid extraction and of 16S rRNA gene  
228 sequencing analysis were followed as previously described (Pelpolage *et al.*, 2019). In Brief,  
229 bacterial DNA was extracted from the lysates of cecal samples using QIAamp DNA Stool  
230 Mini Kit (Qiagen, Shanghai, China). The V3 and V4 variable regions of the 16S rRNA gene  
231 were amplified from the purified DNA using the following bacterial overhang adapters and  
232 the universal primers in the initial stage PCR; forward primer (5'-  
233 *TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3'*) and  
234 reverse primer (5'-  
235 *GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGATTACHVGGGTATCTAATCC-3'*). In  
236 the second stage PCR, Illumina sequencing adapters and dual index barcodes were added to  
237 the amplicons using a Nextera XT Index Kit (Illumina, San Diego, CA). PCR amplification  
238 conditions were as follows: an initial denaturation at 95°C for 30 s; 8 cycles of 95°C for  
239 30 s, 55°C for 30 s, and 72°C for 30 s; and an additional extension at 72°C for  
240 5 min. Taxonomic analysis of the 16S rRNA sequences was conducted using QIIME and the  
241 Calypso version 8.72.

242 For total anaerobes, an aliquot of the respective serial dilution samples (until  $10^{-7}$ ) of the  
243 cecal content was inoculated on Glucose Blood Liver agar plates (Eiken Chemical Co.,

244 Tokyo, Japan) containing 5% defibrinated horse blood (Benno *et al.*, 1986), and were  
245 incubated at 37°C for 2 days using an anaerobic gas generator (AnaeroPack-Anaero,  
246 Mitsubishi Gas Chemical, Tokyo, Japan) in an anaerobic jar. The number of colonies were  
247 counted, and the anaerobic bacteria population was expressed as log<sub>10</sub> colony-forming units  
248 (CFU) per gram of the cecal content.

249

### 250 2.8. Cecum SCFA and ammonia

251 Cecal content was diluted ( $\times 10$  folds, *w/w*) with desalting water and the pH was  
252 measured (Mettler Toledo Seven Easy S20 pH meter, Tokyo, Japan). The content was  
253 vigorously mixed and centrifuged at  $8,500 \times g$  for 10 min. A portion of the upper layer was  
254 deproteinized with 0.4 N HClO<sub>4</sub> and re-centrifuged under the same conditions. The  
255 supernatant was passed through a cellulose acetate membrane filter (0.45  $\mu$ m, Tosho, Tokyo,  
256 Japan). Each SCFA (acetic acid, propionic acid, and *n*-butyric acid) was measured by ST3-R  
257 (Shodex, Tokyo, Japan) dye post-column method using Shimadzu HPLC system (LC-10AD)  
258 as previously described (Pelopolage *et al.*, 2019).

259 An aliquot from the diluted cecal content (0.1 M PBS buffer, pH 5.5) was used to  
260 determine the ammonia nitrogen concentration using a commercially available kit (Wako  
261 Pure Chemical Industries, Ltd.) according to the manufacturer's instructions.

262

### 263 2.9. Statistical analysis

264 All data are presented as mean  $\pm$  standard error of the mean (SEM). The statistical  
265 significance of the differences among the 3 diet groups was analyzed by one-way analysis of  
266 variance (ANOVA) with Tukey's test at  $p < 0.05$  using SPSS software (IBM Corporation,  
267 Armonk, NY, USA). The relative abundance of the genera among the 3 groups were  
268 compared using Kruskal-Wallis H test. The statistical significance was determined using

269 Calypso (version 8.72) at  $p < 0.05$ .

270

### 271 **3. Results and Discussion**

#### 272 *3.1. Micronutrient in adzuki bean powder*

273 In this study, the nutrient composition of the ABP was as follows (g 100 g<sup>-1</sup>): protein,  
274 25.8; lipid, 1.3; moisture, 0.4; ash, 1.0; carbohydrate, 71.5 (including soluble dietary fiber,  
275 0.8; insoluble dietary fiber, 13.7; and RS, 4.0). Generally, the bean is characterized by a low-  
276 fat content and a higher carbohydrate content enriched in dietary fiber and starch (Rebello *et*  
277 *al.*, 2014; Kan *et al.*, 2018). Similarly, we have observed that the ABP contained a higher  
278 amount of indigestible carbohydrates comprising of dietary fiber and RS. For this reason,  
279 ABP, a traditional Japanese confectionery, is a source of less energy-dense food (3.54 kcal g<sup>-1</sup>  
280 dry). Interestingly, the RS contents of the raw whole bean and bean paste of adzuki-bean  
281 were similar (3.3 vs. 4.0 g 100 g<sup>-1</sup> dry, see supplementary Table 1). Native, type 2 RS is  
282 known to be gelatinized during thermal treatments and is dramatically hydrolyzed by  
283 digestive enzymes (Kawakami *et al.*, 2017). However, cooling of cooked starch is known to  
284 generate retrograded starch (Type 3 RS), which might have been the reason for the similar  
285 RS contents in bean paste. Besides, starch particles might have also been encapsulated by the  
286 denatured protein in the cooked adzuki bean, becoming resistant to hydrolysis.

287

#### 288 *3.2. Body weight, feed intake, tissue weight and feces weight*

289 Body weight, feed intake, and tissue and feces weights of rats are presented in Table 2.  
290 Although final body weight was not different among the groups, perirenal and epididymal  
291 adipose tissue weights (g 100<sup>-1</sup> body weight) of the rats fed ABP were lower ( $p < 0.05$ ) than  
292 that of the CON diet-fed rats (Table 2). Fat accumulation was dose-dependent in the two ABP  
293 fed groups. On the other hand, supplementation of ABP dose-dependently lowered the energy

294 intake also, where the feed intake of the HAB group was lower ( $p < 0.05$ ) than that of the  
295 CON group (Table 2). However, we did not observe a significant difference in the adipocyte  
296 area among the groups (CON, 2247  $\mu\text{m}^2$ ; LAB, 1912  $\mu\text{m}^2$ ; and HAB, 1892  $\mu\text{m}^2$ ).  
297 Furthermore, cecal content and fecal moisture content in the HAB group were greater ( $p <$   
298 0.05) than the CON group, while there was no difference between the two ABP fed groups  
299 (Table 2). Dry fecal weight was higher ( $p < 0.05$ ) in the two ABP groups (Table 2).

300 In this study, rats fed ABP exhibited a lower feed intake, gained less dietary energy, and  
301 were less obese than the CON diet-fed rats. During digestion, dietary fiber stimulates the  
302 salivary and gastric secretion that may cause stomach distension and which promotes satiety.  
303 Thus, we contend that the fiber and RS in ABP might have positively influenced satiety,  
304 regulated energy intake, and controlled fat accumulation. Besides, ABP increased the cecum  
305 volume and stool weight. Similarly, it also has been previously mentioned that the insoluble  
306 fiber increases fecal weight by adding water and bulk, which softens the stool (reviewed in  
307 Slavin & Green, 2007). Thus, a higher intake of fiber and RS partly explains the decrease in  
308 fat accumulation and increase in stool bulk observed in rats fed high amounts of ABP.

309

### 310 3.3. Serum cholesterol and triglyceride, and tissue lipid

311 Throughout the feeding period, fasting serum total cholesterol and non-HDL-cholesterol  
312 levels in the two ABP groups were lower ( $p < 0.05$ ) than those in the CON group (Table 3).  
313 Interestingly, total cholesterol and non-HDL-cholesterol levels in the two ABP groups were  
314 similar. The positive effect of the indigestible matter of adzuki bean on the serum cholesterol  
315 concentration was previously reported (Han *et al.*, 2003a; Han *et al.*, 2003b), and the  
316 hypocholesterolemic effect of dietary fiber can be attributed to the increased fecal steroid  
317 excretion (Gil-Ramírez *et al.*, 2018). Similarly, in this study also, a higher acidic sterol  
318 excretion was observed in the two ABP groups (Table 4). Further, supplementation of ABP

319 also lowered ( $p < 0.05$ ) the fasting serum triglyceride level (Table 3), where a higher ABP  
320 dose was more effective. The role of some dietary fibers in lowering the serum triglyceride  
321 content was attributed to the lower *de novo* lipogenesis (Daubioul *et al.*, 2002), due to a  
322 decrease in the activity and the expression of fatty acid synthase (Delzenne & Williams,  
323 2002). Similarly, our results also showed that the hepatic triglyceride concentration was  
324 reduced by ABP (Table 4). Lower energy absorption from lipids may also explain the lower  
325 serum triglyceride levels in rats fed ABP. Other research has shown that dietary fiber inhibits  
326 intestinal fatty acid absorption (reviewed in Sugano *et al.*, 1990) and subsequently reduces  
327 blood triglyceride levels (Han *et al.*, 2013; Han *et al.*, 2017). Further in this study, feeding  
328 ABP to rats significantly increased the daily total fecal lipid excretion also, and the higher  
329 dose of ABP had the highest total fecal lipid excretion (Table 4). Therefore, feeding ABP to  
330 rats resulted in lower energy intake, lower fat absorption from the small intestine, higher fat  
331 excretion via feces and lower fat storage in adipose tissue.

332 In this study, based on the nutritional composition of ABP, the fat content in the two ABP  
333 diets was also absolutely adjusted to be the same as that of the CON diet (7% in diet).  
334 Soybean oil had 61.0% polyunsaturated fatty acid, and the total lipid fraction of ABP also  
335 contained 58.3% polyunsaturated fatty acid (see supplementary Table 2). As a result, the  
336 concentration of omega-3 fatty acid ( $\alpha$ -linolenic acid) in the CON, LAB, and HAB diets was  
337 0.462%, 0.518%, and 0.572%, respectively. Furthermore, the concentration of omega-6 fatty  
338 acid (linoleic acid) in the CON, LAB, and HAB diets was 3.81%, 3.74%, and 3.68%,  
339 respectively. Therefore, the difference of polyunsaturated fatty acid content among the three  
340 diets is likely to be negligible to pose a significant impact on the adipose tissue weight and  
341 serum cholesterol level in rats.

342

343 *3.4. Cecal total anaerobic bacteria and relative composition of the microbiota*

344 The anaerobic bacteria count ( $\log_{10}$  CFU  $\text{g}^{-1}$  content) was similar among the three groups  
345 (CON,  $8.85 \pm 0.03$ ; LAB,  $8.90 \pm 0.20$ ; HAB,  $8.72 \pm 0.11$ ). Rats fed ABP diets exhibited  
346 higher alpha-diversity than the rats fed CON diet, based on the Shannon Index ( $p < 0.01$ ) and  
347 the number of observed species ( $p < 0.01$ ), where the LAB and HAB groups exhibited a  
348 similar alpha diversity (Fig. 1-A and -B). The weighted UniFrac-based principal coordinate  
349 analysis (PCoA) plot showed that the ABP had a substantial effect on the cecal microbial  
350 composition (Fig. 1-C). At the phylum level, pooled relative abundances of Bacteroidetes  
351 (5.8%), Firmicutes (89.2%) and Verrucomicrobia (3.5%) accounted for more than 98% of  
352 the total bacteria (Table 5). Many studies have shown that the increased ratio of Firmicutes  
353 to Bacteroidetes (F/B ratio) is associated with the obesity phenotype in humans (Ley *et al.*,  
354 2006) and animals (Ley *et al.*, 2005; Hamilton *et al.*, 2015; Sen *et al.*, 2017). On the other  
355 hand, a favorable effect of dietary fiber intake on the modifications in the intestinal microbial  
356 composition (or intestinal F/B ratio) and body weight loss has been reported (Parnell &  
357 Reimer, 2012; Li *et al.*, 2016). Moreover, the anti-obesity effect of unabsorbed polyphenol  
358 (proanthocyanidin) also seems to influence the changes in the intestinal F/B ratio  
359 (Masumoto *et al.*, 2016). In agreement with these findings, we have observed that the  
360 proportion of Firmicutes was significantly lowered in the HAB group compared to the  
361 CON group, whereas the proportion of Bacteroidetes in the LAB and HAB groups was  
362 increased (Fig.1-D). Thus, a lower F/B ratio in rats fed ABP may be a factor favoring  
363 visceral fat loss. We could not conclude that the abundance of a specific genus, class or a  
364 species of gut bacteria was positively associated with the fat loss. However, the ABP fed  
365 groups had a higher abundance of *Bacteroides* ( $p < 0.01$  for HAB) and *Parabacteroides* ( $p <$   
366  $0.01$  for HAB and  $p < 0.05$  for LAB) compared to the CON group (Table 5). These bacteria  
367 are associated with acetic acid (Sakamoto & Benno, 2006) or propionic acid production  
368 (Louis *et al.*, 2007).



369

### 370 3.5. Cecal fermentation

371 Cecal fermentation was enhanced by the consumption of ABP because of the 30%- and  
372 58.9%- ABP diets had significantly ( $p < 0.05$ ) higher concentrations of cecal acetic acid and  
373 *n*-butyric acid (Table 4). Cecal total SCFA concentrations were higher ( $p < 0.05$ ) and cecal  
374 pH was lower ( $p < 0.05$ ) in the ABP groups compared to the CON group (Table 4). The  
375 current findings were comparable with our previous results (Han *et al.*, 2003a).

376 The microbiota in the large intestine produces metabolites such as H<sub>2</sub>, CO<sub>2</sub>, and SCFA  
377 from fiber and RS. Recent research indicates that SCFA mediated release of gastrointestinal  
378 hormones modulates energy metabolism and control appetite (Samuel *et al.*, 2008; Monteiro  
379 & Batterham., 2017). Furthermore, propionic acid is known to inhibit lipolysis and *de novo*  
380 lipogenesis (Heimann *et al.*, 2015). Thus, it was suggested that SCFA production by cecal  
381 microbes reduces fat accumulation. In this study, cecal SCFA pools in the ABP groups were  
382 higher ( $p < 0.05$ ) than the CON group (Table 4). Furthermore, the propionic acid pool in the  
383 ABP groups was also higher than the CON group (Table 4). Interestingly, cecal SCFA  
384 production in the two ABP groups was not significantly different (Table 4). So, it was likely  
385 that the relationship between SCFA production and the incretin hormones was less related to  
386 the role of ABP on satiety because higher consumption of ABP only has influenced the  
387 decrease in food intake in this study. We do not clearly understand the reason for the similar  
388 cecal SCFA pool in the two ABP fed groups. It might be that the absorption of SCFA may  
389 have been higher in high ABP treatment. Moreover, adzuki beans contain proanthocyanidins  
390 and related polyphenols (see supplementary Table 1), that inhibit the production of SCFA in  
391 *in vitro* batch systems and in rats (Nagata *et al.*, 2018). Masumoto *et al.* (2016) reported that  
392 the anti-obesity effect of proanthocyanidin from apple is associated with gut microbial  
393 changes.

394 Cecal ammonia nitrogen concentration was lower ( $p < 0.05$ ) in two ABP diets when  
395 compared to CON diet (Table 4). Intestinal ammonia is generated when amino acids derived  
396 from food or host cells are decomposed by bacteria or broken down by ureases. Previously  
397 Rémésy & Demigné (1989) reported that some dietary fiber reduces cecal ammonia  
398 concentration. Bacteria that produce intestinal ammonia, include *Clostridium* species,  
399 *Peptostreptococcus*, *Fusobacterium*, *Actinomyces*, *Bacteroides*, *Megasphaera* and  
400 *Propionibacterium* (Richardson *et al.*, 2013), and the majority of the proteolytic bacteria  
401 reported in literature belongs to the genera *Bacteroides* and *Clostridium* (Shen *et al.*,  
402 2010). However, abundances of genera *Bacteroides* and *Clostridium* were higher in the LAB  
403 and HAB groups than the CON group (Table 5). Further, proanthocyanidins are known to  
404 inhibit proteolysis and deamination of amino acids during anaerobic fermentation (Tanner *et*  
405 *al.*, 1994), thus the proanthocyanidins found in ABP (supplementary Table 1) might have  
406 contributed to the reduced ammonia concentration in ABP fed groups, in a dose-dependent  
407 manner. The decrease in ammonia nitrogen concentration by ABP is also likely to be due to  
408 the osmotic pressure difference owing to higher cecal organic acid production, resulting in  
409 greater cecal wet content (dilution of cecal ammonia concentration). In fact, pooled ammonia  
410 nitrogen amount was similar among the groups (CON, 0.90 mg pool<sup>-1</sup>; LAB, 0.91 mg pool<sup>-1</sup>;  
411 and HAB, 0.91 mg pool<sup>-1</sup>).

412

#### 413 **4. Conclusion**

414 Our results demonstrated that the consumption of adzuki bean paste decreased visceral fat  
415 accumulation in rats, which was related to the content of fiber and RS. Increased satiety,  
416 lowered energy intake, increased lipid excretion, and altered the cecal microbiota (F/B ratio)  
417 upon ABP consumption, which led to a decrease in visceral fat accumulation. However, we  
418 acknowledge that a high intake of ABP (30% of the diet) was required to significantly reduce

419 body fat, which would not be practicable in a human diet. On the other hand, adzuki beans  
420 served with boiled rice along with the use of ABP in traditional confectioneries would  
421 increase the consumption of fiber and RS. Therefore, people who consume adzuki bean/  
422 paste incorporated diet are likely to be more satisfied, less likely to accumulate fat and gain  
423 weight, and have reduced blood lipid levels, suggesting a beneficial effect on the  
424 management of obesity development.

425

426 **Conflict of interest**

427 Authors declare no conflict of interests.

428

429 **Association.**

430 Appendix A. Supplementary material Supplementary data to this article can be found

431 online at [https:// xxx](https://xxx).

432

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605 2019.

606

607 **Figure legend**

608

609 **Fig. 1.** Box plots of (A) Shannon Index and (B) observed species, and (C) principal co-  
610 ordinate analysis (PCoA) plot of cecal microbial community data, (D) the relative  
611 abundance of cecal microbiota at the Phylum level, and (E) the ratio of Firmicutes to  
612 Bacteroidetes in rats fed each diet for 4 weeks. Two-dimensional plot based on PCoA  
613 weighted UniFrac distance from the types and numbers of operational taxonomic units  
614 (OTUs) generated by the Calypso version of 8.84. \*  $p < 0.05$  vs. HAB group. \*\*  $p <$   
615  $0.01$  vs. LAB and HAB groups. CON, a control diet based on AIN-93G; LAB, 30% ABP  
616 diet; and HBP, 58.9% ABP diet.

617

618

**Table 1** Compositions of each experimental diet.

Ingredients (g/kg diet)	Dietary group		
	CON	LAB	HAB
Casein	200.0	122.6	48.0
L-Cystine	3.0	3.0	3.0
Sucrose	200.0	200.0	200.0
Soybean oil	70.00	66.10	62.34
<i>t</i> -Butylhydroquinone	0.014	0.014	0.014
Cellulose	50.0	50.0	50.0
Mineral Mix (AIN-93G)	35.0	35.0	35.0
Vitamin Mix (AIN-93G)	10.0	10.0	10.0
Choline bitartrate	2.5	2.5	2.5
Adzuki bean paste powder	—	300.000	589.144
$\alpha$ -Cornstarch	429.486	210.789	—
Dietary fiber content (%)	5.0	9.4	13.5
Indigestible carbohydrate content* (%)	5.0	10.6	15.8
Calorie (kcal/g)	3.96	3.86	3.76

619 CON, a control diet based on AIN-93G; LAB, a diet supplemented with 30% adzuki bean  
620 paste; HAB, a diet supplemented with 58.9% adzuki bean paste. \*Dietary fiber and  
621 resistant starch are included.

622

623 **Table 2** Body weight, feed intake, hepatic weight, cecal weight, fat weight, and fecal dry  
 624 weight and moisture content in rats fed a control diet or adzuki bean paste diets  
 625 for 28 days.

	Dietary group		
	CON	LAB	HAB
Initial body weight (g)	172±3 <sup>a</sup>	173±2 <sup>a</sup>	174±3 <sup>a</sup>
Final body weight (g)	241±3 <sup>a</sup>	237±2 <sup>a</sup>	240±4 <sup>a</sup>
Daily intake (g/day)	14.0±0.3 <sup>a</sup>	13.0±0.2 <sup>ab</sup>	12.7±0.4 <sup>b</sup>
Daily energy intake (kcal/day)	55.3±1.1 <sup>a</sup>	50.3±0.8 <sup>b</sup>	47.7±1.5 <sup>b</sup>
Liver weight (g/100 g body weight)	3.49±0.07 <sup>a</sup>	3.29±0.10 <sup>b</sup>	3.13±0.05 <sup>b</sup>
Cecal content (g)	2.18±0.08 <sup>b</sup>	3.14±0.33 <sup>ab</sup>	3.55±0.36 <sup>a</sup>
Cecal wall weight (g)	0.55±0.02 <sup>b</sup>	0.65±0.02 <sup>a</sup>	0.73±0.02 <sup>a</sup>
Perirenal fat weight (g/100 g body weight)	2.29±0.07 <sup>a</sup>	1.93±0.07 <sup>b</sup>	1.65±0.07 <sup>c</sup>
Epididymal fat weight (g/100 g body weight)	2.00±0.04 <sup>a</sup>	1.64±0.09 <sup>b</sup>	1.37±0.08 <sup>b</sup>
Fat accumulation* (g/100 g body weight)	4.30±0.10 <sup>a</sup>	3.57±0.16 <sup>b</sup>	3.02±0.14 <sup>c</sup>
Fecal weight (g/day, dry)	0.89±0.04 <sup>c</sup>	1.34±0.03 <sup>b</sup>	2.01±0.10 <sup>a</sup>
Fecal moisture content (%)	46.7±1.2 <sup>b</sup>	48.1±2.2 <sup>ab</sup>	54.7±2.2 <sup>a</sup>

626 Data are presented as means ± SEM, *n*=5. Values in the same row bearing dissimilar  
 627 superscript letters are significantly different (*p* < 0.05). CON, a control diet based on AIN-  
 628 93G; LAB, a diet supplemented with 30% adzuki bean paste; HAB, a diet supplemented with  
 629 58.9% adzuki bean paste. \*Fat accumulation is the sum of perirenal fat and epididymal fat.

630

631 **Table 3** Serum total cholesterol, high-density lipoprotein (HDL)-cholesterol, Non-HDL  
 632 cholesterol and triglyceride concentrations in rats fed a control diet or adzuki bean paste  
 633 diets for 28 days.

	Dietary group		
	CON	LAB	HAB
0 week (mmol/L)			
Total cholesterol	1.53±0.03	1.58±0.05	1.55±0.03
HDL cholesterol	0.49±0.01	0.50±0.02	0.47±0.00
Non-HDL cholesterol	1.04±0.03	1.08±0.03	1.07±0.03
Triglyceride	0.60±0.05	0.74±0.08	0.67±0.08
1 week (mmol/L)			
Total cholesterol	1.51±0.04 <sup>a</sup>	1.37±0.03 <sup>b</sup>	1.24±0.03 <sup>b</sup>
HDL cholesterol	0.44±0.01 <sup>a</sup>	0.44±0.01 <sup>a</sup>	0.40±0.01 <sup>a</sup>
Non-HDL cholesterol	1.07±0.04 <sup>a</sup>	0.93±0.02 <sup>b</sup>	0.83±0.02 <sup>b</sup>
Triglyceride	0.87±0.06 <sup>a</sup>	0.61±0.05 <sup>b</sup>	0.32±0.04 <sup>c</sup>
2 week (mmol/L)			
Total cholesterol	1.58±0.08 <sup>a</sup>	1.31±0.03 <sup>b</sup>	1.32±0.06 <sup>b</sup>
HDL cholesterol	0.47±0.02 <sup>a</sup>	0.42±0.01 <sup>a</sup>	0.43±0.02 <sup>a</sup>
Non-HDL cholesterol	1.11±0.06 <sup>a</sup>	0.89±0.03 <sup>b</sup>	0.89±0.04 <sup>b</sup>
Triglyceride	1.06±0.09 <sup>a</sup>	0.60±0.04 <sup>b</sup>	0.37±0.02 <sup>b</sup>
3 week (mmol/L)			
Total cholesterol	1.50±0.04 <sup>a</sup>	1.32±0.04 <sup>b</sup>	1.27±0.02 <sup>b</sup>
HDL cholesterol	0.45±0.01 <sup>a</sup>	0.43±0.01 <sup>a</sup>	0.43±0.00 <sup>a</sup>
Non-HDL cholesterol	1.05±0.04 <sup>a</sup>	0.89±0.04 <sup>b</sup>	0.84±0.01 <sup>b</sup>
Triglyceride	0.87±0.10 <sup>a</sup>	0.52±0.10 <sup>b</sup>	0.33±0.03 <sup>b</sup>
4 week (mmol/L)			
Total cholesterol	1.68±0.06 <sup>a</sup>	1.38±0.04 <sup>b</sup>	1.30±0.03 <sup>b</sup>
HDL cholesterol	0.46±0.01 <sup>a</sup>	0.43±0.01 <sup>ab</sup>	0.41±0.01 <sup>b</sup>



Non-HDL cholesterol	1.22±0.05 <sup>a</sup>	0.96±0.03 <sup>b</sup>	0.89±0.02 <sup>b</sup>
Triglyceride	1.27±0.09 <sup>a</sup>	0.83±0.07 <sup>b</sup>	0.54±0.06 <sup>c</sup>

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634 Data are presented as means ± SEM, *n*=5. Values in the same row bearing dissimilar  
635 superscript letters are significantly different (*p* < 0.05). CON, a control diet based on AIN-  
636 93G; LAB, a diet supplemented with 30% adzuki bean paste; HAB, a diet supplemented with  
637 58.9% adzuki bean paste. Non-HDL cholesterol (very low-density lipoprotein cholesterol +  
638 intermediate density lipoprotein cholesterol + low density lipoprotein cholesterol) = Total  
639 cholesterol – high density lipoprotein cholesterol.

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641

642 **Table 4** Hepatic total lipid, triglyceride and cholesterol concentrations, fecal lipid excretions,  
 643 and cecal short-chain fatty acid (SCFA), pooled SCFA content, ammonia nitrogen  
 644 concentrations, and cecal pH in rats fed a control diet or adzuki bean paste diets for 28  
 645 days.

	Dietary group		
	CON	LAB	HAB
<b>Liver</b>			
Total lipids (mg/g)	44.3±1.9 <sup>a</sup>	44.3±1.2 <sup>a</sup>	41.6±1.8 <sup>a</sup>
Triglyceride (mg/g)	12.7±0.5 <sup>a</sup>	9.9±0.4 <sup>b</sup>	8.6±0.7 <sup>b</sup>
Cholesterol (mg/g)	3.39±0.17 <sup>a</sup>	3.39±0.20 <sup>a</sup>	3.24±0.17 <sup>a</sup>
<b>Feces</b>			
Total lipids excretion (mg/day)	26.3±2.6 <sup>b</sup>	51.3±6.2 <sup>a</sup>	67.1±5.8 <sup>a</sup>
Neutral sterol excretion (µmol/day)	2.66±0.46 <sup>a</sup>	2.36±0.48 <sup>a</sup>	2.63±0.19 <sup>a</sup>
Bile acid excretion (µmol/day)	0.78±0.12 <sup>b</sup>	1.95±0.62 <sup>ab,*</sup>	2.60±0.44 <sup>a</sup>
<b>Cecum</b>			
pH	7.89±0.05 <sup>a</sup>	7.22±0.04 <sup>b</sup>	7.15±0.03 <sup>b</sup>
Total SCFA (µmol/g content)	80±6 <sup>b</sup>	122±6 <sup>a</sup>	125±6 <sup>a</sup>
Acetic acid	67±5 <sup>b</sup>	100±5 <sup>a</sup>	104±4 <sup>a</sup>
Propionic acid	10.5±1.9 <sup>a</sup>	12.8±1.0 <sup>a</sup>	12.8±1.4 <sup>a</sup>
Butyric acid	2.1±0.2 <sup>b</sup>	9.0±0.8 <sup>a</sup>	8.4±1.3 <sup>a</sup>
Total SCFA (µM/pool)	173±12 <sup>b</sup>	397±58 <sup>a</sup>	414±69 <sup>a</sup>
Acetic acid	146±8 <sup>b</sup>	327±49 <sup>a</sup>	343±55 <sup>a</sup>
Propionic acid	22.6±3.9 <sup>b</sup>	40.6±5.3 <sup>a</sup>	42.6±8.2 <sup>a</sup>
Butyric acid	4.5±0.4 <sup>b</sup>	29.2±4.9 <sup>a</sup>	28.8±6.8 <sup>a</sup>
Ammonia nitrogen (mg/g content)	0.41±0.03 <sup>a</sup>	0.29±0.03 <sup>b</sup>	0.26±0.03 <sup>b</sup>

646 Data are presented as means ± SEM, *n*=5. Values in the same row bearing dissimilar  
 647 superscript letters are significantly different (*p* < 0.05). CON, a control diet based on AIN-

648 93G; LAB, a diet supplemented with 30% adzuki bean paste; HAB, a diet supplemented with  
649 58.9% adzuki bean paste.  
650

651 **Table 5** Relative abundance of cecal microbial taxa at the levels of phylum and genus in rats  
 652 fed a control diet or adzuki bean paste diets for 28 days.

		Dietary group		
		CON	LAB	HAB
Phylum	Genus			
Actinobacteria (%)		0.10±0.03 <sup>b</sup>	0.23±0.06 <sup>ab</sup>	1.66±1.34 <sup>a</sup>
Bacteroidetes (%)		1.48±0.32 <sup>b</sup>	6.71±1.93 <sup>a</sup>	9.06±1.40 <sup>a</sup>
	<i>Bacteroides</i>	0.50±0.10 <sup>b</sup>	1.19±0.33 <sup>ab</sup>	1.89±0.47 <sup>a</sup>
	<i>Parabacteroides</i>	0.96±0.34 <sup>b</sup>	5.52±1.62 <sup>a</sup>	7.17±1.36 <sup>a</sup>
Firmicutes (%)		93.0±2.6 <sup>a</sup>	90.1±2.3 <sup>ab</sup>	84.7±2.0 <sup>b</sup>
	<i>Blautia</i>	7.83±2.01 <sup>a</sup>	1.09±0.40 <sup>b</sup>	1.68±0.32 <sup>b</sup>
	<i>Dorea</i>	11.8±2.1 <sup>a</sup>	1.03±0.24 <sup>b</sup>	1.68±0.32 <sup>b</sup>
	<i>Lactococcus</i>	3.89±1.08 <sup>a</sup>	0.34±0.04 <sup>b</sup>	0.15±0.02 <sup>c</sup>
	<i>Oscillospira</i>	0.80±0.12 <sup>c</sup>	1.87±0.23 <sup>b</sup>	2.71±0.47 <sup>a</sup>
	<i>Clostridium</i>	0.24±0.22 <sup>b</sup>	1.86±0.53 <sup>a</sup>	0.15±0.12 <sup>b</sup>
	<i>Eubacterium</i>	0.25±0.08 <sup>a</sup>	0.04±0.04 <sup>b</sup>	0.01±0.01 <sup>b</sup>
Proteobacteria (%)		0.35±0.23 <sup>a</sup>	0.78±0.24 <sup>a</sup>	1.29±0.36 <sup>a</sup>
Tenericutes (%)		0.02±0.01 <sup>a</sup>	0.04±0.03 <sup>a</sup>	0.07±0.03 <sup>a</sup>
Verrucomicrobia (%)		5.10±2.07 <sup>a</sup>	2.14±0.38 <sup>b</sup>	3.21±0.22 <sup>ab</sup>

653 Data are presented as means ± SEM, *n*=5. Values in the same row bearing dissimilar  
 654 superscript letters are significantly different (*p* < 0.05). The relative abundance of genera  
 655 among the treatment groups were compared using Kruskal-Wallis H test. CON, a control diet  
 656 based on AIN-93G; LAB, a diet supplemented with 30% adzuki bean paste; HAB, a diet  
 657 supplemented with 58.9% adzuki bean paste.

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660 **Supplementary data**

661 **Table 1** Chemical composition in adzuki bean paste and whole adzuki bean.

g/100 g	Dried materials	
	Bean paste	Whole bean
Water	0.4	1.6
Protein	25.8	23.0
Lipid	1.3	1.8
Carbohydrate	71.5	70.1
Total starch	49.5	40.3
Digestible starch	45.5	37.0
Resistant starch	4.0	3.3
Dietary fiber	14.5	15.5
Soluble dietary fiber	0.8	1.3
Insoluble dietary fiber	13.7	14.2
Reducing sugars (g glucose equivalents/100 g, dry)	1.3	5.7
Unknown	6.2	8.6
Ash	1.0	3.5
Total polyphenols (mg gallic acid equivalents/g, dry)	2.53	10.9
Proanthocyanidin (mg Batch13 equivalents/g, dry)	14.0	25.1
Soluble proanthocyanidin	6.80	8.23
Insoluble proanthocyanidin	7.16	16.9

662 Polyphenol in dried adzuki materials was repeatedly extracted with 70% acetone and the  
663 extract was fractionated through a LH-20 column. Total polyphenol was determined by the  
664 Folin-Ciocalteu method using gallic acid as standard. Soluble and insoluble  
665 proanthocyanidins were determined respectively by DMAC assay and Butanol-HCl assay  
666 using Batch13, which is a specific standard from cranberry proanthocyanidin. Reducing  
667 sugar was measured by DNS method. Starch particles were encapsulated by the denatured  
668 protein in the cooked adzuki bean. At that time, the ABP became reddish, actually white,

669 because that pigment-containing polyphenols was incorporated into starch and combined  
670 with the protein. Thus, the bean paste exhibited a higher level of proanthocyanidin as  
671 remains.

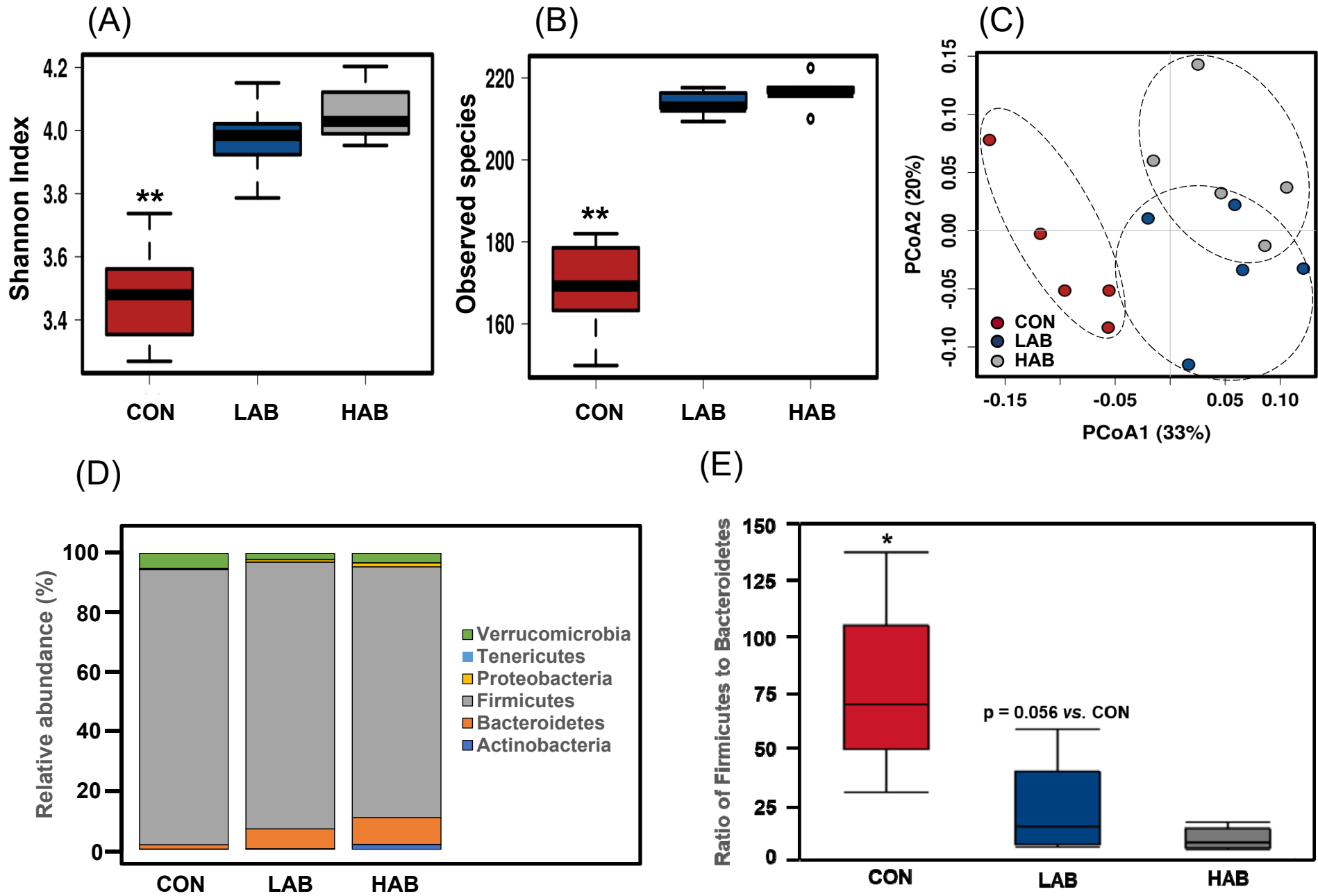
672

673 **Table 2** Fatty acid composition in soybean and adzuki bean paste oils.

Fatty acid	Soybean	Adzuki bean paste
		Mole % <sup>1</sup>
Lauric acid (12:0)	0.0	0.4
Myristic acid (14:0)	0.1	0.1
Palmitic acid (16:0)	11.1	24.9
Palmitoleic acid (16:1)	0.1	0.1
Stearic acid (18:0)	4.2	0.2
Oleic acid (18:1)	23.4	12.5
Linoleic acid (18:2)	54.4	37.3
Linolenic acid (18:3)	6.6	21.0
Behenic acid (22:0)	0.0	3.4
Sum	100	100

674 Each fatty acid was converted to fatty acid methyl ester (FAME), and then was analyzed by  
675 gas chromatography.

676 <sup>1</sup>The relative proportion of each fatty acid component in the total moles contained in the  
677 FAME mixture.



**Fig. 1. Han *et al.***