Calcium Bioavailability of Tempe and Boiled Soybean Flours and Its Effect on Osfemurs in Experimental Rats

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Summary Tempe is a nutritious food that contains both macronutrients and micronutrients in an adequate amount. Tempe contains a high amount of calcium, so it is an affordable food product that can be purchased by most people in Indonesia. Calcium deficiency can lead to several diseases and disorders such as osteoporosis, hypertension, heart disease, and nerve damage. This study was aimed to evaluate: (1) the calcium bioavailability (absorption and retention) of tempe and boiled soybean flours compared to casein (control), (2) the effect of tempe and boiled soybean flours on blood calcium and osfemurs in rats. This study was conducted using rats as a model. The rats were divided into four treatment groups based on the sources and protein concentrations in the diet; tempe flour 10% protein, tempe flour 20% protein, boiled soybean flour 10% protein, and casein 10% protein as a control. This experiment was conducted for over than 90 d. The results showed that the sources of protein (casein, tempe flour, and boiled soybean flour) and protein level in the diet (10% and 20%) had no significant effect ($p > 0.05$) to the calcium absorption, calcium retention, calcium content in serum, calcium content in osfemurs, and total calcium in osfemurs. Tempe flour and boiled soybean flour can be consumed as a calcium source to substitute the consumption of calcium from dairy products.

Key Words tempe flour, boiled soybean flour, calcium bioavailability, osfemurs

Tempe is an indigenous fermented food from Indonesia, prepared by the action of molds, *Rhizopus* spp., on cooked soybeans through a controlled fermentation process. Tempe is well known for its nutritional value and health benefit (1). Indonesia is acknowledged as the world’s biggest producer of tempe and the center of soybean market in Asia. In Indonesia, up to 70% of soybean stocks went to tempe industry. The average population consumption of tempe reached up to 10.1 kg per person per year (2).

Tempe is a nutritious food that contains both macronutrients and micronutrients in an adequate amount (1). Tempe has various macro and micro minerals such as calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, copper, and manganese (3). Tempe contains a high amount of calcium, which is 111 mg/100 g (3). In addition, the cost of tempe in Indonesia is very affordable, so it can be purchased by most people, thus making tempe suitable as an affordable calcium source. Tempe is also suitable for people with lactose intolerance, vegan, and ovo-vegetarian people who cannot obtain minerals from animal products specifically calcium from dairy products. Tempe consumption can prevent calcium deficiency which can lead to several diseases and disorders such as osteoporosis, hypertension, heart disease, and nerve damage (4).

Tempe has a short shelf life, which is only about one to two days at ambient temperature. Therefore, processing technology of tempe is needed to extend its shelf life, for example is the production of tempe flour (5). Drying and flouring processes can expand the shelf life of tempe. However, the effects of the processing to tempe calcium content and bioavailability have not been studied yet.

The objectives of this study were to evaluate: (1) the calcium bioavailability (absorption and retention) of tempe and boiled soybean flours toward casein as a control, (2) the effect of calcium from tempe and boiled soybean flours to calcium concentration in serum and osfemurs of rats.

MATERIALS AND METHODS

Materials. Ingredients for rats’ experimental diet were corn starch, casein, corn oil, carboxymethylcellulose, mineral premix, tempe flour, boiled soybean flour, and vitamin premix. Materials to anesthetize experiment rats before dissections were ketamine (Ilum, Australia) and xylazine (Interchemie, Netherlands) mixture. Materials for calcium analysis were aquadest, HCl 6M, weak acid, and calcium standard solvent.

Methods for making tempe and boiled soybean flours. Production of tempe was based on the procedures that have been implemented in Rumah Tempe Indonesia (Indonesia House of Tempe), Bogor-Indonesia which...
Tempe flour 20%**** 38.6 — 4.3 1.0 — 3.3 52.8
Tempe flour 10%*** 19.3 3.1 4.6 1.0 — 3.9 67.8
Boiled soybean flour 10%** 19.6 3.0 4.5 1.0 — 3.9 68.0
Casein 10%* 11.2 8.0 4.9 1.0 — 3.3 70.1

Table 1. Composition of experimental diets (percent of weight) for rat bioassay.

- * 10% protein in the diet came from casein.
- ** 10% protein in the diet came from boiled soybean flour.
- *** 10% protein in the diet came from tempe flour.
- **** 20% protein in the diet came from tempe flour.

The results were expressed as a mean ± SD. The statistical analysis was carried out using oneway ANOVA followed by DMRT post-hoc test.
RESULTS

Preparation of tempe and boiled soybean flour

Soybean variety used in this research was one of local soybean from Indonesia, which was Grobogan variety. The size of this Grobogan soybean was bigger (19.53 g/100 beans) than imported soybean, both in GMO and non-GMO varieties. In this experiment, production of tempe and boiled soybean flour were done in two batches. The average yield of boiled soybean flour was higher (65%) than that of tempe flour (57.5%).

Calcium bioavailability

Total consumption of diet and total calcium intake of the experimental rats during the last 7 d of 90 d experiment can be seen in Table 2. The total intake of diet containing boiled soybean flour was significantly lower compared to other groups, but there was no significant differences between the casein and tempe flour treatments. The total calcium intake of rats fed by diet containing soybean flour was higher (65%) than that of tempe flour (57.5%).

Table 2. Total diets and total calcium intake during the last 7 d of 90 d experiment.

| Treatments     | Total diet intake (g) | Total calcium intake (mg) |
|----------------|-----------------------|---------------------------|
| Casein 10%     | 133.1 ± 8.2 b         | 2,121.9 ± 131.1 bc        |
| Boiled soybean flour 10% | 116.1 ± 6.6 a         | 1,639.3 ± 93.3 a          |
| Tempe flour 10% | 123.6 ± 15.8 b        | 1,802.0 ± 232.2 b         |
| Tempe flour 20% | 139.7 ± 17.5 b        | 2,424.9 ± 276.3 a         |

Results were expressed as mean ± SD. Different letters following the values in a column showed that results were significantly different (p<0.05) using DMRT.

Calcium content in serum of rats

The calcium content in serum of rats can be seen in Table 3. It shows that experimental die gave no significant effect (p>0.05) to the calcium content in serum.

Table 3. Calcium contents in serum and osfemurs of rats by casein, boiled soybean and tempe flours treatments.

| Treatments     | Calcium in serum (mg/100 mL) | Calcium in osfemurs (g/100 g) |
|----------------|------------------------------|-------------------------------|
| Casein 10%     | 17.5 ± 1.9 a                 | 48.54 ± 13.72 a               |
| Boiled soybean flour 10% | 16.8 ± 0.5 a                 | 36.51 ± 3.54 a               |
| Tempe flour 10% | 17.0 ± 0.8 a                 | 41.96 ± 2.74 a               |
| Tempe flour 20% | 16.4 ± 0.4 a                 | 35.72 ± 3.11 a               |

Results were expressed as mean ± SD. Same letters following the values in a column showed that results were not significantly different (p>0.05) using DMRT.

Calcium content and total calcium in osfemurs of rats

The result of calcium content analysis in osfemurs is also shown in Table 3. It shows that experimental die gave no significant effect (p>0.05) to the calcium content in osfemurs.

Dry weight of rats’ osfemurs

The results of dry weight of rats’ osfemurs can be observed in Fig. 2. Figure 2 indicated that rats’ osfemurs with the biggest dry weight came from tempe flour 20% protein group, then followed by control (casein 10% protein), tempe flour 10% protein group, and the last is boiled soybean flour 10% protein group treatments. The figure indicates that experimental diets of rats had significantly influenced (p<0.05) the dry weight of rats’ osfemurs.

Fig. 1. Calcium absorption and retention by casein, boiled soybean, and tempe flours treatments. Same letters following the values in each treatment were not significantly different (p>0.05) using DMRT.

Fig. 2. Dry weight of osfemur of rats by casein, boiled soybean, and tempe flour treatments. Same letters following the values in each treatment were not significantly different (p>0.05) using DMRT.
DISCUSSION

Preparation of tempe and boiled soybean flour

The soybean variety used in this research was Grobogan variety which has several advantages, such as shorter cultivation time and larger bean size (14). Even though the size of Grobogan soybean is bigger than imported soybean, the yield of tempe was not significantly different ($p>0.05$) with the imported soybean (15). The tempe produced was compact and solid with normal and typical aroma, color, and taste, as required by Indonesian National Standard of Tempe—SNI 3144-2015 (16). The protein nutritional values of tempe from both local and imported soybean were also similar (17).

The boiled soybean flour in this research was different from raw (no-boiling) soybean flour. Raw soybean flour consists of antinutrient compounds that can prevent mineral absorption in the body, one of which is calcium. On the other hand, boiled soybean flour has fewer antinutrient compounds because boiling process can reduce antinutrient factors in raw soybean. Phytic acid, one of the antinutrients in soybean may dissolve in water and decreased during the boiling process. Nevertheless, boiling cannot reduce phytic acid or phytate in high content because it has heat stable properties. In tempe making, high content phytic acid can be reduced. Research by Almasyhuri et al. (18) showed that phytic acid content in tempe had been decreased from 77.6–86.9% of its raw soybean form.

The average yield of boiled soybean flour was higher (65%) than tempe flour (57.5%), which was caused by the fermentation process in tempe production. During the fermentation, various nutrients such as water, carbohydrate, protein, and lipid, were used by tempe mold (Rhizopus spp.) to support its growth. This condition will reduce the yield of tempe flour compared to boiled soybean flour.

Calcium bioavailability

As mentioned before, the total consumption of diet and total calcium intake of the experimental rats is shown in Table 2 above. Calcium balance will be reached when the consumption amount of calcium replace the loss of calcium through fecal and urine. A positive balance is even needed in some special phase, such as growth phase, pregnancy, and lactation. Calcium bioavailability can be mentioned as calcium absorption and calcium retention (12, 19). Measurement of calcium retention was done to examine how much calcium that can be retained by the body after absorption process.

Calcium bioavailability in food products, including tempe and boiled soybean flours, is very important to be determined, because the high amount of calcium content in food products does not always mean that it can be absorbed well by the body. Calcium bioavailability value can describe how much calcium from food products that can be absorbed and utilized in the body.

The result showed that experimental diet resulted in no significant difference ($p>0.05$) to calcium absorption of experimental rats. In other words, tempe flour and boiled soybean flour can be used as an alternative food calcium source aside from animal products, such as dairy products.

Calcium retention is needed to evaluate how much calcium which can be retained in the body, so that it can be metabolized. Figure 1 also showed that experimental diets had no significant effect ($p>0.05$) on calcium retention in the body of rats. These results shows that tempe and boiled soybean flour can be consumed as a good source of calcium to substitute the consumption of dairy products.

The statistical analysis of calcium bioavailability describes that both calcium absorption and retention from all samples were not significantly different ($p>0.05$) compared to casein 10% protein as control. Calcium absorption and retention of boiled soybean flour 10% protein and casein 10% protein in this research were supported by previous research of Kamao et al. (20). Their results indicated that calcium absorption and retention of experimental rats with soy protein isolate diet were not significantly different compared to casein diet. Another research by Haron et al. (21) in human model also described the same pattern with this research, where calcium absorption of tempe was almost similar with milk. Some bioactive compounds in fermented milk (synbiotic yoghurt) were also found in tempe, such as anti-diarrheal and antioxidative agents (22, 23). Soybean and other legumes, especially in raw form contain some antinutrients (phytic acid, oxalate, and tannin) which can reduce calcium bioavailability (24, 25). During fermentation process, the phytic acid content of soybean was reduced by about one-third. The reduction of phytic acid was due to phytase elaborated by Rhizopus spp. (26). Therefore, mineral like calcium which is chelated by phytate will be released, so that the mineral absorption will be easier in human body (2).

Tempe and boiled soybean flours in this research had high calcium bioavailability and even similar to casein because the antinutrient content had decreased significantly, especially in tempe. It was because tempe making consists of more steps aside from boiling process, especially soaking and fermentation process. Those three steps can decrease oxalate content significantly in raw soybean (27). While boiling only, in fact, is not sufficient to reduce phytate and tannin significantly because both phytate and tannin are heat-stable enough compounds (28).

In soybean, tannin mostly accumulated at the hull. Dehulling step in tempe making could remove most of the tannin in soybean. In other words, lower antinutrients content in tempe flour resulted in higher calcium bioavailability than boiled soybean flour as described in Fig. 1. However, single boiling process was effective in reducing oxalate in raw soybean (27), so that calcium bioavailability of boiled soybean flour in this research was still sufficiently high.

Calcium content in serum of rats

Calcium content in serum is calcium which is used for physiological function. Calcium in serum can be
sourced from the digestion tract which absorbs calcium from food or calcium resorption from bone if calcium intake from food is not enough. About 99% of calcium in the body is located in bone and teeth, and only 1% found in extracellular plasma such as serum and blood plasma. Calcium has broad functions aside from bone and teeth formation, there are also physiological roles in blood clotting mechanism, and muscle contraction-relaxation (29).

Calcium balance in serum is regulated through calcium homeostatic process. If the body detects low calcium level, it will cause in the increase of calcium absorption from food or calcium preservation in the bone if calcium intake from food is not sufficient. Oppositely, when calcium level in serum is high, the body will inhibit or reduce calcium absorption. Excessive calcium then will be kept in bone but if it cannot be either absorbed or kept, it will be removed through fecal and urine. Several factors which influence calcium absorption are daily calcium intake, hormones’ performance such as parathormone (PTH), 1,25 dihydroxy itamin D3, and also estrogen (30).

**Calcium content and total calcium in osfemurs of rats**

Calcium content in osfemurs of rats indicates how much calcium accumulation in the osfemurs. The calcium content measurement was done when the experimental rats reached its end phase of growth, which in humans is the same as the last of skeletal (bones) growth. On average, a human will reach that phase at about 30 y old. At the same time, bone mass density has reached its peak. Consumption of food rich in calcium resulted in calcium storage, so it will contribute to the growth and bone formation that manifests in both density and bone size (31).

The results show that experimental diets gave no significant influence ($p>0.05$) to calcium content in osfemurs of rats. This indicated that calcium consumption from boiled soybean flour 10% protein, tempe flour 10% protein and 20% protein, were similar with calcium content from milk consumption. This data indicates that tempe and boiled soybean flour have the potential to substitute milk as a source of calcium. Calcium content in osfemurs of rats can be determined directly from AAS measurement, while total calcium in osfemurs was calculated by multiplying calcium content with the dry weight of osfemurs. Total calcium means the real calcium content in the osfemurs.

**Dry weight of rats’ osfemurs**

Adequate consumption and absorption of minerals which construct bone mass in the adolescence period will maximize the density and bone size to peak bone mass (31). The peak bone mass occurred at the end of the growth phase. The higher density of bone will result in the higher mass and weight of bone. In this research, it was examined by using rats as experimental model. The experimental diets were given every day from the beginning of the growth phase (around 21-28 d old) until the end of the growth phase (around 100 d old).

The weighing of dried osfemurs was done to evaluate the effect of experimental diet to bone condition. The higher calcium bioavailability will result in higher bone weight. It is supported by Haron et al. (21) study which explains that tempe consumption could fulfill the calcium absorption significantly of postmenopausal women in Malaysia because the bone weight was affected by calcium absorption. Figure 2 indicated that the dry weight of rats’ osfemurs with casein 10% protein treatment had no significant difference ($p>0.05$) with other treatments, but tempe flour 20% protein treatment gave a significantly higher effect ($p<0.05$) than tempe flour 10% and boiled soybean flour 10% protein treatments. It happened because protein content of the experimental diet from tempe flour 20% protein treatment was higher than tempe flour 10% and boiled soybean flour 10% protein treatments.

According to NIH (32), bone is not only composed of minerals but in fact, the major composer of bone is collagen. Collagen is a protein that composes the soft skeleton and by the presence of mineral, the bone or skeleton will be strengthened. Collagen and calcium combinations cause in a strong bone that could withstand pressure. Besides protein and calcium, phytate was also influencing the weight and density of bone. That is proven in the research by Kamao et al. (20) in osfemurs of rats fed by phytate-free soybean protein, soy protein isolate, and casein. The research indicated that mineral density, ash weight, and mechanical strength of bone were not different in those three sample treatments, except in the mineral density of osfemurs. Treatment of phytate-free soybean protein resulted in a significantly higher value of osfemurs mineral density than soy protein isolate. Tempe has lower phytate content than raw and boiled soybean (27). This condition contributed to higher weight and density of osfemurs of rats which were fed with tempe.

In conclusion, protein sources (casein, tempe flour, and boiled soybean flour) and protein level in the diet (10% and 20%) had no significant effect ($p>0.05$) to calcium absorption, calcium retention, calcium content in serum, calcium content in osfemurs, and total calcium in osfemurs. Tempe flour and boiled soybean flour can be consumed as calcium source to substitute the consumption of calcium from dairy product.

**Disclosure of state of COI**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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