

# Chemical composition of tempeh from soybean cultivars specially developed for human consumption

*Composição química de tempeh de cultivares de soja especialmente desenvolvidas para o consumo humano*

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## Abstract

Tempeh is a food obtained by fermentation of soybean grains by the fungus *Rizophus oligosporus*. It is a traditional Indonesian food that presents benefits for human health protecting against diarrhea and chronic diseases. Tempeh processing includes dehulling, cooking, inoculation, and fermentation. In this study, chemical characteristics of tempeh prepared with soybean cultivars specially developed for human consumption (BRS 216, BRS 232, BRS 257, and BRS 267) were investigated. Soybean grains and tempeh obtained from these cultivars were analyzed for oil, protein, antinutritional factors, and isoflavone content. Cultivar BRS 216 presented the highest protein content in the grains (36.81%) and in tempeh (51.99%). On average, the protein content in tempeh increased 16% in relation to that of soybean grains. Isoflavone content was higher in the grains than in tempeh with significant differences among the cultivars. However, the aglycones content increased about 50% in tempeh (49.00 mg.100 g<sup>-1</sup> on average) compared to that of raw material (soybean grains - 21.49 mg.100 g<sup>-1</sup>, on average). The content of Kunitz trypsin inhibitor (KSTI) reduced 83% in tempeh, on average, as compared to the value found in the grains. Phytic acid content was similar in both tempeh and the grains.

**Keywords:** soybean; functional food; isoflavones; protein; antinutritional factors.

## Resumo

O tempeh é um alimento obtido por meio da fermentação dos grãos de soja pelo fungo *Rizophus oligosporus*. É um alimento tradicional da Indonésia que apresenta benefícios para a saúde humana, protegendo contra a diarreia e doenças crônicas. O processamento de tempeh inclui descasque, cozimento, inoculação e fermentação. Nesse estudo, as características químicas de tempeh produzido com cultivares de soja especialmente desenvolvidas para alimentação humana, BRS 216, BRS 232, BRS 257 e BRS 267 foram investigadas. Os grãos das cultivares e os tempehs foram analisados quanto aos teores de óleo e proteína, fatores antinutricionais e teor de isoflavonas. A cultivar BRS 216 apresentou o maior teor de proteína no tempeh (51,99%) e nos grãos (36,81%). Em média, no tempeh, o teor de proteína aumentou 16%. O teor total de isoflavonas foi maior nos grãos do que nos tempehs, com diferenças significativas entre as cultivares. O conteúdo de isoflavonas agliconas aumentou em média cerca de 50% nos tempehs (49,00 mg.100 g<sup>-1</sup> em média), quando comparados com a matéria-prima grãos de soja (21,49 mg.100 g<sup>-1</sup> em média). O conteúdo de inibidor de tripsina de Kunitz (KSTI) apresentou uma redução de 83% nos tempehs em relação ao conteúdo observado nos grãos. Os teores de ácido fítico nos tempehs e nos grãos de soja foram semelhantes.

**Palavras-chave:** soja; alimento funcional; isoflavonas; proteína; fatores antinutricionais.

## 1 Introduction

Innovations in the technology of food processing improve quality of soybean products. By genetic breeding, special quality characteristics of soybean are improved (LIU, 1997; CARRÃO-PANIZZI et al., 2009a). Soybean foods can be fermented, which in general are traditional oriental foods such as miso, shoyu, tempeh, and natto, or non-fermented, among which the most popular are soymilk and tofu (VILLARES et al., 2011).

Tempeh is a traditional fermented soybean food from Indonesia. It is consumed fried, boiled, steamed, or roasted,

and thanks to the Dutch in 1948, it became popular in Europe, where it has been consumed by vegetarians and non-vegetarians to reduce meat consumption (KARYADI, 2001). Its pleasant, mushroom-like aroma and delicious nutty flavor makes it an excellent alternative to meat, fish, and poultry dishes (PRIDE, 1984). It is a product that not only adds variety to the diet, but also enhances it nutritionally.

During fermentation, there are nutritional and chemical changes that improve taste, flavor, and functional properties

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of the products (NOUT; KIERS, 2004). Enzymes produced during fermentation affect protein, fat, and carbohydrates. The complex sugars stachyose and raffinose, which cause flatulence, are broken down into digestible sugars. During fermentation, the fungus produces the enzyme phytase that mobilizes the phytic acid improving bioavailability of minerals. Fermentation also promotes the synthesis of B group vitamins (HERMANA; MAHMUD; KARYADI, 2001) improving flavor, nutritional, chemical and functional properties of the products (NOUT; KIERS, 2004).

Isoflavones are important biological active compounds that confer antioxidant activity to soybeans with health benefits for humans. Due to its antioxidant effects and other nutritional characteristics of tempeh, Nout and Kiers (2004) suggested that in addition to the traditional tempeh food, it could also be processed into powdered form to be used as a food ingredient of health foods, mainly to control diarrhea among children (SUDGIBIA, 2001)

During tempeh fermentation, the enzyme  $\beta$ -glycosidase hydrolyses the  $\beta$ -glycosides forming the aglycones, which is the readily available form. Carrão-Panizzi and Bordignon (2000) reported that soybean cultivars show a significant genetic variability for  $\beta$ -glucosidase activity. Genetic differences and environmental effects for isoflavone contents in Brazilian soybean cultivars were also investigated by Carrão-Panizzi et al. 2009b. Their results suggest that the choice of cultivars with better chemical composition will allow processing of high quality soy foods for human consumption. The present study had the objective to verify changes in the content of protein, lipids, ashes, isoflavones, and antinutritional factors in tempeh processed with different soybean cultivars specially developed for human consumption.

## 2 Materials e methods

Grains of soybean cultivars BRS 216, BRS 232, BRS 257, and BRS 267, produced at the experimental field of the National Soybean Research Center, in Londrina, Paraná State, Brazil were used to process tempeh. BRS 232 is a grain type cultivar (cv), BRS 216 is a small seed cv. for natto and sprout; BRS 257 is a null lipoxygenase cv.; and BRS 267 is a large seed cv. for tofu and edamame (CARRÃO-PANIZZI et al., 2009a). The freeze-dried inoculums of the fungus *Rhizopus microsporus* var. *Oligosporus* obtained from INTSOY (International Soybean Program, from the University of Illinois, USA) were used for preparation of tempeh.

### 2.1 Preparation of tempeh

Tempeh was prepared according to traditional method (WEI, 1991). Clean soybean grains were dehulled, and the cotyledons were boiled in water for 10 minutes. The hot water was drained, and the cotyledons were soaked in water for 17 hours at room temperature. The hydration water was discharged, and the hydrated grains were cooked in boiling water for 30 minutes. After cooking, the hot water was drained again, and the cooked soybean cotyledons were cooled down to room temperature. The cooked and cooled cotyledons were

inoculated with spores of the fungus *R. oligosporus* and were packed in polypropylene bags with small and equidistant perforations and were incubated at 32 °C for 26 hours to obtain the tempehs. Fresh tempehs were freeze-dried in a freeze drier (Liotop model L 110) and milled in a refrigerate mill (Tecnal model TE 631/2). Samples of freeze-dried milled tempeh were stored in glass containers for further chemical analysis.

### 2.2 Chemical composition

Samples of whole grains and freeze-dried tempehs were ground into fine particles and analyzed for moisture, protein, lipid and ash (in dry basis) according to the Food Analysis of Institute Adolfo Lutz (BRASIL, 2005).

### 2.3 Isoflavone analysis

#### *Isoflavone extraction*

The extraction of isoflavones was performed according to the method described by Carrão-Panizzi, Favoni and Kikuchi (2002). Samples of 100 mg of freeze-dried tempeh and whole grains ground in fine particles that were defatted with N-hexane (HPLC grade) at room temperature under constant shake for 8 hours were added to test tubes with lid. Next, 4.0 mL of the solvent extraction (70:30 ethanol: water solution containing 0.1% V/V of glacial acetic acid) were added. The extraction was performed for one hour with shaking every 15 minutes in a "Vortex" tube shaker. The tubes containing the samples and the extraction solution were placed into an ultrasonic bath for 30 minutes at room temperature; 1.5 mL of the supernatant was transferred to 2.0 mL "Eppendorff" tubes that were centrifuged in an Eppendorff microcentrifuge (model 5417 R) at 21,000 g for 15 minutes at 4 °C. The supernatants were filtered through a 45  $\mu$ m Millipore membrane and transferred to a HPLC autosampler injection vials. 20  $\mu$ L of the extracts (injection volume) were used for profile analysis and quantification of the isoflavones in tempeh and in grains.

#### *Separation and quantification of isoflavones*

Separation and quantification of the isoflavones were conducted according to the method proposed by Berhow (2002) in a high pressure liquid chromatographer (HPLC – Waters), solvent pump model 600 equipped with an autosampler injector (Waters, model 717 plus). The isoflavones were eluted in a reverse phase ODS-AM C18 column (4.6 mm  $\times$  250 mm -YMC Pack) and 5  $\mu$ m particles. For separation, a linear binary gradient system with two mobile phases was used: 1) methanol with 0.025% trifluoroacetic acid (TFA) (solvent A); and 2) distilled deionized ultrapure water with 0,025% de TFA (solvent B). The initial gradient system condition consisted of 20% methanol acidified with 0.025% trifluoroacetic acid (pH 3.0) and 80% H<sub>2</sub>O acidified with 0.025% trifluoroacetic acid (pH 3.0). The proportion of 90% methanol and 10% H<sub>2</sub>O was reached in 35 minutes of elution. The gradient proportion of 100% methanol was reached at 40 minutes, remaining in this condition for 5 minutes for column cleaning, and then it returned to the initial condition of 20% methanol and 80% H<sub>2</sub>O in 20 minutes. The final elution time was 60 minutes. The solvent flow rate was

1 mL.min<sup>-1</sup>, and the temperature of the column was maintained constant at 25 °C.

The photodiode array detector (Waters, model 996) adjusted to 254 nm was used for the detection of the isoflavones. For the identification of the correspondents peaks of each isoflavone, it was used standards (Sigma) of the 12 forms of isoflavones (glycosides, glycitins, acetyls, malonyls e aglycons) solubilized in methanol (HPLC grade) at the following concentrations: 0.00625 mg.mL<sup>-1</sup>; 0.0125 mg.mL<sup>-1</sup>; 0.0250 mg.mL<sup>-1</sup>; 0.0500 mg.mL<sup>-1</sup>, and 0.1000 mg.mL<sup>-1</sup>, and standard curves were established for each form. These concentrations were used after the limit of detection (LD) and the limit of quantification (LQ) of the equipment were obtained with standards of daidzein and genistein. The LD was 0.0005 mg.mL<sup>-1</sup>, and the LQ was 0.0015 mg.mL<sup>-1</sup>. The peaks identified as the retention time standards of each isoflavone were: daidzin = 19.75 minutes, glycitin = 20.40 minutes, genistin = 22.75 minutes, malonyl-daidzin = 24.60 minutes, malonyl-glycitin = 25.20 minutes, malonyl-genistin = 27 minutes, acethyl-daizin = 26 minutes, acethyl-glycitin = 26.60 minutes, acethyl-genistin = 28.80 minutes, daizein = 29.00 minutes, glycitein = 29.50 minutes, and genistein = 31.60 minutes.

The content of the isoflavones was expressed in mg.100 g<sup>-1</sup> of samples in dry basis. The separated and quantified isoflavones were  $\beta$ -glycosides conjugates (daidzin, glycitin, and genistin), the derivatives malonyl- $\beta$ -glycosides (malonyldaidzin, malonylglycitin and malonylgenistin), and the aglycons (daidzein, glycitein, and genistein). The contents (mg.100 g<sup>-1</sup>) of malonylglycosides,  $\beta$ -glycosides, aglycons, and total isoflavones were expressed as aglycons in soybean grains and in tempeh after the conversion of each isoflavone form using a correcting factor calculated by the division of the molecular weight of each isoflavone for the corresponding aglycon form (TONGTONG et al. 1998; KLUMP et al. 2001).

#### 2.4 Kunitz trypsin inhibitor (KSTI)

Quantification of the Kunitz Trypsin Inhibitor (KSTI) content was made according to Kakade et al. (1974) with modifications of Hamerstrand, Black e Glover (1981).

##### Sample preparation

In Erlenmeyers of 250 mL, it was added five grams of tempeh and grain samples ground into fine particles in a refrigerated mill. Next, the samples were defatted with 50 mL of N-hexane under constant shaking on an orbital shaker table (Tecnal, model TE 140) for 16 hours at room temperature. After this procedure, the N-hexane was drained, and the samples were transferred to Petri dishes under air flushing to allow any residual solvent to evaporate. The defatted samples were passed through a 100 mesh sieve, and the fine flour obtained was used for the trypsin inhibitor extraction.

##### Extraction of the trypsin inhibitor

Trypsin inhibitor extraction was accomplished with three replicates for each sample. In 125 mL Erlenmeyers, 1.0 g of defatted milled samples was extracted with 50 mL of 0.01N

sodium hydroxide solution at room temperature under constant shaking for three hours. The solution pH was adjusted to 9.2 by using a pH meter, and 2.0 mL aliquots were taken. These aliquots were transferred to 100 mL volumetric flasks, and the volume was completed with ultrapure water. These diluted solutions were used for Kunitz trypsin inhibitor (KSTI) quantification.

##### Reagents preparation for KSTI determination

BAPA (benzoil-DL-arginine-p-nitroanilide) solution was prepared by weighting 0.080 g of BAPA dissolved in 2.0 mL of dimethyl sulfoxide and transferred to a 200 mL volumetric flask completed with a pre-heated (37 °C) Trizma buffer solution. This solution is stable for only 4 hours.

Trizma buffer was prepared by weighting 1.21 g of Trizma and 0.59 g of dihydrate calcium chloride (CaCl<sub>2</sub>.2H<sub>2</sub>O) and dissolved in 180 mL of ultrapure water, and the pH was adjusted to 8.2 with 1N hydrochloride acid. This solution was transferred to a 200 mL volumetric flask, and the volume was completed with ultrapure water. This solution is stable for only 8 hours.

Trypsin inhibitor solution was prepared by weighting 0.0020 g of trypsin and dissolved with 100 mL of 0.001N hydrochloride acid solution.

##### Kunitz trypsin inhibitor determination

Trypsin inhibitor quantification was accomplished in three replicates. After extraction, three aliquots of the diluted extract were taken for each replicate. Five test tubes were prepared for analysis: three for quantification of the trypsin inhibitor in the samples extracts (tubes 1, 2, and 3), one for the blank (4), and one for the trypsin standard (5).

Two mL of the diluted extract were added to tubes 1, 2, 3, and 4, and 2.0 mL of ultrapure water were added to tube 5. Next, 2.0 mL of trypsin inhibitor solution were added to tubes 1, 2, 3, and 5. The tubes were homogenized and placed in a water bath at 37 °C for exactly 10 minutes. Then, 5.0 mL of pre-heated (37 °C) BAPA solution were added to all tubes. Each tube was homogenized and placed back in the water bath immediately for another 10 minutes interval.

After that time, 1.0 mL of 30% acetic acid solution was added to all tubes and they were homogenized again. Then, 2.0 mL of trypsin solution were added to tube 4 (blank). The solutions were filtrated through a Whatman n° 3 paper filter, and their measurements of absorbance were performed in a spectrophotometer adjusted to 410 nm wavelength.

Observation: For complete homogenization, it is recommended 30 second intervals between each addition of solutions in the tubes.

The Kunitz trypsin inhibitor content (mgTI.g<sup>-1</sup> sample) was calculated according to the following formula 1:

$$mg\ TI.g^{-1} = \frac{\text{Standard tube absorption} - \text{sample tube absorption} \times 2,500}{38 \times \text{sample weight(g)}} \quad (1)$$

## 2.5 Phytic acid content

The phytic acid content in the soybean grains and in tempehs was determined according to the method proposed by Latta and Eskin (1980) modified by Bordignon, Silva and Carrão-Panizzi (2000).

### Phytic acid extraction

For phytic acid extraction, one gram of tempehs and grain samples ground to fine particles in a mill were weighed and transferred to 125 mL Erlenmeyers, and 10 mL of 0.8M hydrochloric acid were added. The samples were constantly shaken in an orbital shaker table (Tecnal, model TE 140) for 2 hours at 250 rpm, followed by centrifugation at 10,000 g for 10 minutes. The supernatant was used for phytic acid separation through an ion exchange column.

### Phytic acid separation

For phytic acid separation, an ion exchange column prepared with 0.5 g of Dowex resin (type 1 X 4-50) and built over a 1 cm glass wool layer was used. The columns were washed with ultrapure water and equilibrate with 10 mL of 0.7M sodium chloride (NaCl) solution. Two mL of the supernatant solution previously obtained in the extraction step was slowly applied to the top of the column. Undesirable cations were eluted from the column by adding 10 mL of 0.1M sodium chloride (NaCl) solution. Phytic acid was then eluted from the column with 10 mL of 0.7M sodium chloride (NaCl) solution and collected for spectrophotometric determination.

### Spectrophotometric determination of the phytic acid

For phytic acid spectrophotometric determination, a calibration curve prepared with dodecassodium phytate solution (35.29 mg of phytic acid, 85% purity, in 100 mL ultrapure water) was used. Fifteen mL of this primary solution were diluted in volumetric flask to a 100 mL final volume with ultrapure water. From this secondary solution, different volume aliquots between 0.1 and 0.5 mL were taken. Aliquots were transferred to test tubes and diluted to a 3 mL final volume with ultrapure water. Next, 1 mL of Wade Reagent (0.03 g monohydrate ferric chloride (FeCl<sub>3</sub>.H<sub>2</sub>O) and 0.30 g of sulfosalicylic acid, diluted in 100 mL ultrapure water were added to each tube. Phytic acid concentration was determined in a spectrophotometer adjusted to 500 nm wavelength. In order to verify the Wade Reagent

stability, its pure solution absorbance should be between 0.490 and 0.530 AU.

## 2.6 Statistical analysis

Exploratory data analyses were performed to verify homogeneity of variance, which were arranged in a complete randomized design with 2 treatments (soybean grains and tempeh) and four cultivars (BRS 216, BRS 232, BRS 257, and BRS 267), with three replicates. The data were submitted to analysis of variance (ANOVA), and the means were compared by the Tukey test at 5% probability. The data was analyzed by SAS - Statistical Analysis System (STATISTICAL..., 2001) and Sanest (ZONTA; MACHADO; SILVEIRA JÚNIOR, 1982).

## 3 Results and discussion

The protein contents in both the samples of soybean grains and samples of tempeh were significantly different ( $P \leq 0.05$ ) between the cultivars. Cultivar BRS 216 had the highest protein content in grains (36.81%), and in tempeh (51.99%) (Table 1). The high protein content of this cultivar was corroborated by Carrão-Panizzi et al. (2009a). Wang (1986) and Vaidehi and Rathnamani (1990) reported protein content for tempeh of 48%.

Protein content increased in tempeh (Table 1) due to the cooking and fermentation steps during tempeh processing, which favor losses of soluble substances (mineral and sugars) from soybean grains. Similar result was described by Vaidehi and Rathnamani (1990), who observed an increase in protein content of 14.35% higher in tempeh than in whole grains. Ferreira et al. (2011) working with Brazilian soybean cultivars (BR-36 e IAS 5) observed, on average, an increase of 21% in tempeh as compared to cotyledons. Since tempeh is prepared with dehulled soybean, increases in concentrations of the nutrients are expected.

The oil content observed in the soybean grains and in tempeh samples (Table 1) were also different ( $P \leq 0.05$ ). The grain samples presented an average oil content similar to that normally observed for soybean (LIU, 1997), while the oil content in the tempehs obtained from all cultivars decreased, except for BRS 257 (Table 1). The average oil content in tempeh was reported as being 25.04% (LEITAO; MENEZES; TANGO, 1967).

The mineral (ash) content in the samples of grains and tempeh did not differ ( $P \leq 0.05$ ). In the soybean grains, the average value for all cultivars was 6.13% (Table 1), higher

**Table 1.** Protein, oil, and ash content (g.100 g<sup>-1</sup>) based on dry weight in soybean grains and in tempeh prepared with different cultivars specially developed for human consumption.

Cultivars	Ash		Protein		Oil	
	Soybean grains	Tempeh	Soybean grains	Tempeh	Soybean grains	Tempeh
BRS 216	5.66 (±0.40) <sup>Ba</sup>	4.06 (±0.5B) <sup>b</sup>	36.81 (±0.80) <sup>Ab</sup>	51.99 (±0.09) <sup>Aa</sup>	21.98 (±1.25) <sup>Ba</sup>	20.95 (±0.48) <sup>Ba</sup>
BRS 232	6.17 (±0.09) <sup>Aa</sup>	5.22 (±0.08) <sup>Ab</sup>	34.73 (±1.04) <sup>Bb</sup>	41.62 (±0.55) <sup>Da</sup>	23.99 (±1.37) <sup>Aa</sup>	22.18 (±0.98) <sup>Bb</sup>
BRS 257	6.33 (±0.13) <sup>Aa</sup>	5.44 (±0.2A) <sup>b</sup>	36.43 (±0.51) <sup>Ab</sup>	44.63 (±0.29) <sup>Ca</sup>	22.90 (±0.30) <sup>Ab</sup>	24.06 (±0.73) <sup>Aa</sup>
BRS 267	6.35 (±0.10) <sup>Aa</sup>	5.42 (±0.2) <sup>Ab</sup>	36.69 (±1.13) <sup>Ab</sup>	46.40 (±0.39) <sup>Ba</sup>	23.94 (±0.92) <sup>Aa</sup>	21.35 (±0.28) <sup>Bb</sup>
Mean	6.13 <sup>a</sup>	5.04 <sup>b</sup>	36.16 <sup>b</sup>	43.16 <sup>a</sup>	23.20 <sup>a</sup>	22.13 <sup>b</sup>

Same capital letters in the columns and same small letters in the lines for each group of compounds are not significantly different by Tukey test at  $P \leq 0.05$ .

than the 5% described by Liu (1997). For tempeh, the average ash value was 5.04%, similar to that reported by Vaidehi and Rathnamani (1990), 5.2%.

Phytoestrogens are important compounds in nutrition and health due to their association with reduced incidence of some chronic diseases. Among these compounds, isoflavones present a non-steroidal structure that makes them function as estrogens in biological systems. These compounds act as antioxidants, enzymatic inhibitors, anti-inflammatories, and antimicrobials that affect several biochemical and physiological processes (SETCHELL, 1998; AGUIAR, 2002).

Isoflavones are affected by different processing techniques (COWARD et al. 1998; CARRÃO-PANIZZI; GOÊS-FAVONI; KIKUCHI, 2004), and changes in chemical composition modifying the isoflavones forms (glycosides, malonyl and aglycons) are common (AGUIAR, 2002). Isoflavones are also highly influenced by genetic variability and environmental conditions (CARRÃO-PANIZZI et al., 2009b).

Observing the overall content of isoflavone, the concentration was reduced in tempehs as compared to those of the grains. Nakashima et al. (2005) also observed reduction in isoflavone content in tempeh, and Haron et al. (2009) reported 43% reduction in total isoflavone in raw tempeh compared to that of fried isoflavone.

In tempeh preparation, grain soaking, dehulling, and cooking can result in loss of isoflavones. In general, dehulling does not cause significant losses of isoflavones because soybean hulls do not present isoflavones. However, isoflavone loss increase when dehulled grains are cooked (MURPHY, 1982).

The isoflavone malonyl forms are unstable at high temperatures and are converted to their respective  $\beta$ -glycoside forms (CARRÃO-PANIZZI; SIMÃO; KIKUCHI, 2003; CARRÃO-PANIZZI; GOÊS-FAVONI; KIKUCHI, 2004). In tempeh processing, soybean grains presented a significant reduction in the isoflavone malonyl forms after cooking (Table 2).

A reduction in the malonyl forms of about 43% on average (Table 2) was observed for the tempehs prepared with all cultivars, except for cultivar BRS 232, which presented a lower content of these compounds. Cultivar BRS 232 had the lowest

amount of total isoflavones in the grains and tempeh (Table 2). Isoflavones  $\beta$ -glycosides in soybean grains were also significantly low (35.98 mg.100 g<sup>-1</sup>), which made the tempeh prepared with this cultivar to present the lowest value (22.27 mg.100 g<sup>-1</sup>) of these isoflavone forms (Table 2).

Total isoflavone content expressed as aglycons in the grains and tempehs prepared with grains of soybean cultivars BRS 216, BRS 232, BRS 257, and BRS 267 are presented in Table 2. Grains of the cultivar BRS 257 presented the highest concentration of total de isoflavones (200.24 mg.100 g<sup>-1</sup>), and cultivar BRS 232 presented the lowest content (89.63 mg.100 g<sup>-1</sup>). The same tendency was observed for tempeh prepared with cultivar BRS 257 (176.72 mg.100 g<sup>-1</sup>) and cultivar BRS 232 (86.41 mg.100 g<sup>-1</sup>). Analyzing the overall data of total isoflavone content, it can be observed that isoflavone concentration was reduced in tempeh as compared with the grains.

During tempeh fermentation, the glycoside forms are reduced, while the aglycon forms increases (COWARD et al., 1998). Fermentation or moisture activates the  $\beta$ -glycosidase enzyme causing hydrolyze of isoflavone glycosides forming the aglycon isoflavones (MATSUURA; OBATA, 1993). Concentrations of isoflavone aglycons in fermented soybean foods (natto, misô, shoyu and tempeh) are higher when compared with those of non-fermented foods (PARK et al., 2001).

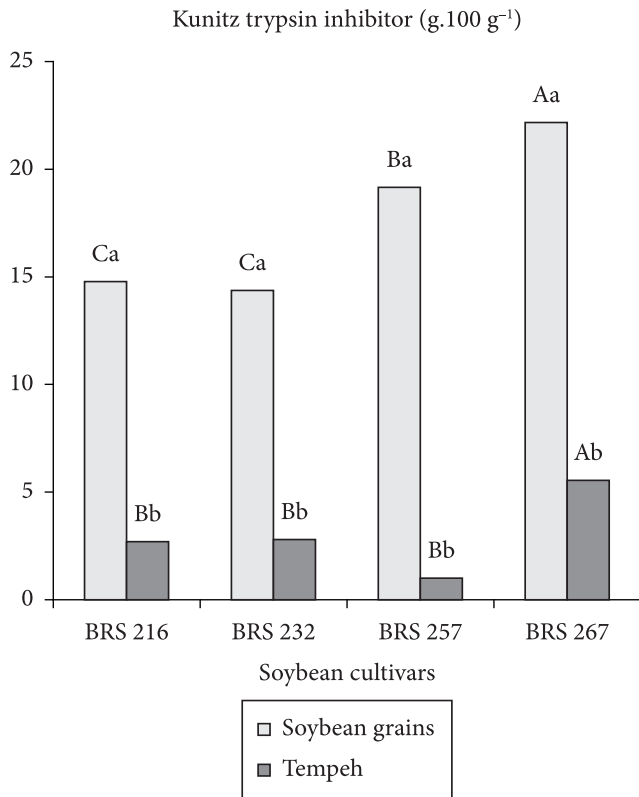
In general, it has been observed that isoflavone aglycons are not formed in fresh harvested soybean grains (CARRÃO-PANIZZI; SIMÃO; KIKUCHI, 2003). It is assumed that seeds should have some damage to favor water absorption giving conditions for  $\beta$ -glycosidases to catalyze aglycons formation.

The results presented in Table 2 showed reduced content of aglycon isoflavones in grains as compared with tempeh. Except for cultivar BRS 257, which presented an increase of about four times the aglycon content in tempeh; in all the other cultivars, the aglycon content increased by twofold. Cultivar BRS 232 presented the lowest content of aglycons in both tempeh and the grains (Table 2). According to Liu (1997), soybean fermented foods contain high content of aglycons, while non-fermented foods have mostly the conjugated forms.

**Table 2.** Malonylglycosides,  $\beta$ -glycosides, aglycons, and total isoflavones expressed as aglycon equivalents (mg.100 g<sup>-1</sup>) ( $\pm$ SD) in soybean grains of cultivars specially developed for human consumption and in tempehs prepared with these cultivars.

Grains	Malonylglycosides	$\beta$ -Glycosides	Aglycons	Total isoflavones
BRS 216	91.85 ( $\pm$ 0.30) <sup>a</sup>	72.50 ( $\pm$ 0.12) <sup>b</sup>	26.74 ( $\pm$ 0.33) <sup>a</sup>	191.09 ( $\pm$ 0.32) <sup>ab</sup>
BRS 232	38.64 ( $\pm$ 0.41) <sup>c</sup>	35.98 ( $\pm$ 0.32) <sup>c</sup>	15.01 ( $\pm$ 0.42) <sup>c</sup>	89.63 ( $\pm$ 0.32) <sup>c</sup>
BRS 257	81.76 ( $\pm$ 0.53) <sup>b</sup>	95.70 ( $\pm$ 0.53) <sup>a</sup>	22.77 ( $\pm$ 0.51) <sup>b</sup>	200.24 ( $\pm$ 0.51) <sup>a</sup>
BRS 267	86.56 ( $\pm$ 0.44) <sup>b</sup>	73.96 ( $\pm$ 0.96) <sup>b</sup>	21.44 ( $\pm$ 0.12) <sup>b</sup>	181.96 ( $\pm$ 0.73) <sup>b</sup>
Tempehs				
BRS 216	48.32 ( $\pm$ 0.21) <sup>a</sup>	30.01 ( $\pm$ 0.12) <sup>b</sup>	45.58 ( $\pm$ 0.16) <sup>b</sup>	123.91 ( $\pm$ 0.12) <sup>b</sup>
BRS 232	36.91 ( $\pm$ 0.42) <sup>b</sup>	22.27 ( $\pm$ 0.52) <sup>c</sup>	27.22 ( $\pm$ 0.24) <sup>c</sup>	86.41 ( $\pm$ 0.41) <sup>c</sup>
BRS 257	38.51 ( $\pm$ 0.33) <sup>b</sup>	55.50 ( $\pm$ 0.49) <sup>a</sup>	82.72 ( $\pm$ 0.51) <sup>a</sup>	176.72 ( $\pm$ 0.52) <sup>a</sup>
BRS 267	26.51 ( $\pm$ 0.52) <sup>c</sup>	52.93 ( $\pm$ 0.33) <sup>a</sup>	40.49 ( $\pm$ 0.42) <sup>b</sup>	119.93 ( $\pm$ 0.43) <sup>b</sup>

Same letters for grains and for tempeh in the columns for each group of compounds are not significantly different by Tukey test at  $P \leq 0.05$ .

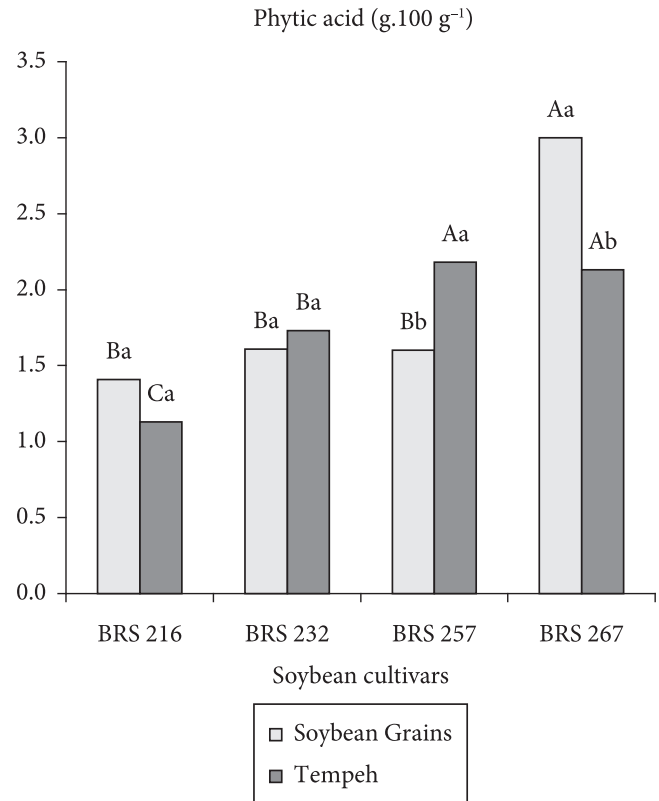


**Figure 1.** Kunitz trypsin inhibitor ( $\text{mg}\cdot\text{g}^{-1}$ ) in soybean grains and tempeh prepared with different cultivars specially developed for human consumption. (Same capital letters among cultivars and small letters between grains and tempeh are not significantly different by Tukey test at  $P \leq 0.05$ ).

The content of Kunitz trypsin inhibitor was remarkable reduced in tempeh (Figure 1). Thermal treatments reduced about 80% of the trypsin inhibitor activity in the soybean grains (MANDARINO, 2010). In the present study, it was observed an overall reduction of 83%, with some differences among the cultivars. Although this compound presents similar trend for all cultivars, the lowest content observed was in the tempeh prepared with cultivar BRS 257 ( $1.01 \text{ mg}\cdot 100 \text{ g}^{-1}$ ). Cooking, soaking, and dehulling soybean reduced 82.2% of trypsin inhibitor (EGOUNLETY; AWORH, 2003),

Phytic acid has been reported to interfere with absorptions of mineral, especially zinc. However, phytic acid can also reduce the risk of kidney stone formation and heart disease, and it can act as anticancer agent by inhibiting the formation of endothelial cells (VUCENIK; SHAMSUDDIN, 2003).

Soybean grains contain about  $1.0 \text{ g}\cdot 100 \text{ g}^{-1}$  to  $2.30 \text{ g}\cdot 100 \text{ g}^{-1}$  of phytic acid (ANDERSON; WOLF, 1995). Fermentation processing mobilizes phytates by the action of phytase enzyme reducing phytic acid content (MANDARINO, 2010). Phytic acid in tempeh is in the range of  $0.69 \text{ g}\cdot 100^{-1} \text{ g}$  to  $0.96 \text{ g}\cdot 100 \text{ g}^{-1}$  (ANDERSON; WOLF, 1995). Egounlety and Aworh (2003) reported phytic acid reduction of 30% in tempeh fermentation for 36 hours. In the present study, differences for phytic acid content among cultivars were observed in grains and in tempehs (Figure 2). However, the decrease in this compound



**Figure 2.** Phytic acid content ( $\text{g}\cdot 100 \text{ g}^{-1}$ ) in soybean grains and in tempeh prepared with different cultivars specially developed for human consumption. (Same capital letters for cultivars and same small letters for each product are not significantly different by Tukey test at  $P \leq 0.05$ ).

was observed only for cultivars BRS 216 and BRS 267. Cultivar BRS 267, which presented the highest content of phytic acid in the grains had a significant reduction in phytic acid in tempeh (Figure 2). Conditions for phytase activity (pH or temperature), which were not measured; it is supposed that they were not ideal for reducing phytic acid in cultivars BRS 232 and BRS 257.

Tempeh prepared with soybean cultivars with high content of protein and isoflavone aglycons can be a good source of high nutritive food. Among the analyzed cultivars, BRS 216, which presents the highest content of protein and isoflavones, could be a good raw material for tempeh production. Trypsin inhibitor was similar to what has been observed for other soybean products, which means that trypsin inhibitor is reduced with thermal treatment. Phytic acid, however, presented different performance among the cultivars.

#### 4 Conclusion

Tempeh prepared with soybean cultivars especially developed for human consumption, BRS 216, BRS 232, BRS 257, and BRS 267, presented different chemical composition. This traditional food from Indonesia with high nutritive value and biological functions can be directly consumed or used as raw-material (ingredient) for the preparation of other foods for Brazilians.

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