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Submission date: 28-Aug-2023 03:14PM (UTC+0700)
Submission ID: 2152638248
File name: fluence_of_traditional_stirfrying_with_oil_on_acceptability,.pdf (205.21K)
Word count: 6983
Character count: 36117

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The influence of traditional stir-frying with oil on acceptability, antioxidant activities, nutrients, and the phytic acid content of fermented soybean (tempeh)

Received 11 September 2015
Revised 25 November 2015
Accepted 3 December 2015

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Abstract

Purpose – The purpose of this paper was to investigate the acceptability of processed tempeh and the effect of stir-frying on uncooked tempeh composition, total phenolic content (TPC), antioxidant (AO) activities and the phytic acid (PA) concentration.

Design/methodology/approach – Fermentation was performed in the solid-state using soybean (*Glycine max*) inoculated with *Rhizopus oligosporus*. The acceptability of tempeh was evaluated by administering a questionnaire. The TPC of uncooked and stir-fried tempeh was examined using Folin-Ciocalteu's method, and PA was analyzed by high-performance liquid chromatography. AO activities were measured by the thiobarbituric acid reactive substance (TBARS) and ferric ion reducing/antioxidant power methods. The stir-fried tempeh was more acceptable than other preparations to the panelists.

Findings – In comparison with the uncooked tempeh, stir-fried tempeh showed higher fat composition, in addition to decreased levels of minerals, PA and TBARS.

Originality/value – Soy foods are an important source of protein. However, conventional cooking methods could change the chemical properties in soy foods. To avoid additional oil that adds calories, consumers might opt for other cooking methods, such as steaming.

Keywords Fermentation, Sensory, Phenolic, FRAP, TBARS, Uncooked

Paper type Research paper

Nutrition & Food Science
Vol. 46 No. 2, 2016
pp. 259-271

0034-6659
DOI 10.1108/NFS-09-2015-0105

Introduction

Soybean is a legume with high economic value because of its nutritional composition. The nutritional composition of soybean ranges from 32 to 43.6 per cent of crude proteins, 15.5 to 24.7 per cent of lipids and 31.7 to 31.85 per cent of carbohydrates on a dry matter basis (Banaszkiewicz, 2011). The protein composition of soybean is higher than that found in other types of legumes (20 to 30 per cent) as well as that found in cereals (8 to 15 per cent). The mineral found in large quantities in soybean is potassium, followed by phosphorus, magnesium, sulfur, calcium, chloride and sodium. On the other hand, iron, silicon, zinc, copper, manganese and copper are found in lower concentrations (Banaszkiewicz, 2011; Hassan, 2013; Mateos-Aparicio *et al.*, 2008; Mozeika *et al.*, 2013).

In their natural form, soybeans contain anti-nutrients that might be harmful to human health such as phytic acid (PA) (Chen *et al.*, 2013; Mozeika *et al.*, 2013). The PA found in soy food has been considered unfavorable for dietary metal ions like Ca, Fe, K, Mg, Mn and Zn due to its strong binding capacity with its multivalent cations (Bohn *et al.*, 2008). Furthermore, PA has also been suggested to form strong complexes with macronutrients, and these complex formations might affect protein digestibility as well as the utilization of carbohydrate and lipid (Kumar *et al.*, 2010). On the other hand, several beneficial effects of PA for human health have been proposed such as prevention and treatment for various cancers (Fox and Eberl, 2002).

Soybean possesses antioxidant (AO) activities that have been shown to play an important role in diseases such as cancer, dyslipidemia, postmenopausal osteoporosis and coronary heart diseases (Michelfelder, 2009). Common sources of AOs are phenolic group phytochemicals, such as flavonoids, tocopherols, lignans, carotenoids and ascorbic acid (Katekan Dajanta, 2009; Mozeika *et al.*, 2013). Phenolic compounds are often found in both consumable and inedible plants and have been confirmed to have various biological effects, including AO activities (Kähkönen *et al.*, 1999).

Tempeh is a traditional fermented soybean product with high nutritional value and originally from Indonesia (Handoyo and Morita, 2006). The fermentation period of tempeh is shorter (approximately two days). The fermentation process for tempeh comprises four different phases: soaking, boiling, inoculating with yeast and incubating at room temperature. *Rhizopus oligosporus* strains are commonly used as an inoculum during the fermentation to make soybeans more palatable. Fermentation improves acceptability, reduces cooking time and decreases anti-nutrients (Nout and Kiers, 2005).

The fermented soybean (tempeh) usually undergoes a further process of heating before consumption. Frying, boiling and steaming are the most common heating process used to prepare tempeh further for a variety of dishes or for consumption with rice. These cooking methods are intended to improve the acceptability and digestibility of the food as well as to kill harmful microbes. Besides, high-temperature can deactivate the anti-nutrients and, consequently, improve the nutritional quality of foods (Chau *et al.*, 1997; Vijayakumari *et al.*, 1998). However, the high-temperature processing might contribute to loss of nutrients and decreased AO levels in the food.

The appreciation of palatable healthier food has been increasing among consumers. On the other hand, consumers consider that traditional cooking process with high temperature could have deleterious effects on the chemical composition in food. The current study sought to analyze the acceptability of tempeh and the influence of traditional cooking on the nutritional composition, micronutrient content (iron and zinc), AO activities and the PA concentration.

Materials and methods

Materials and reagents

Soybeans [*Glycine max* (L.) Merr] were purchased from a local Asian market in Giessen, Hessen, Germany. The tempeh starter containing "*R. oligosporus* and rice flour", for solid state of fermentation was bought from the local market, Jogjakarta, in Indonesia. The brand name of tempeh starter was "Raprima™", and it was produced by Aneka Fermentasi Industri, Bandung, Indonesia. All reagents used were of analytical grade.

Preparation of tempeh samples

Tempeh was prepared following a traditional method from Indonesia in which the soybeans were boiled twice before fermentation. Two hundred grams of yellow soybeans were boiled at 100°C for 30 min and, after discarding the water, they were de-hulled by hand until approximately 90 per cent were freed from their skin. The soybeans were left to soak overnight in fresh tap water at room temperature for 12 h with the water level being 5 cm over the beans. The second boiling of the soybeans was done at 100°C for 30 min and the water drained. The soybeans were then laid uniformly on a clean cloth to cool for 30 min to provide a suitable temperature for *R. oligosporus* inoculum. Five milliliters of cooking vinegar (10 per cent solution) was added to create an acid environment for the tempeh starter. The beans were inoculated with 0.4 g of tempeh starter, which contains *R. oligosporus* and stirred gently to distribute the tempeh uniformly. Tempeh starter absorption required 20 min at normal room temperature, and then the soybeans were placed into perforated sealed polyethylene bags. The beans were placed in an incubator (Heraeus Instruments, Hanau, Germany, Model No. UT 20) at 29°C for 48 and 72 h, respectively. Finally, the fermented soybeans were refrigerated for 24 h at 4°C.

Sensory evaluation

The tempeh samples were processed using traditional methods namely: stir-fried, steamed, boiled, dried and uncooked. The samples were prepared 2 h before the sensory test that were conducted at Local International Hall, Eichendorffring Giessen, Hessen, Germany. Stir-fried tempeh was prepared by frying the fermented soybeans in 250 mL of sunflower oil in a Teflon-coated pan (24 cm in diameter) at 160°C for three minutes. Steamed tempeh was performed by steaming the fermented soybeans in a steamer pot at 98°C for 10 min with the 200 mL boiling tap water. Boiled tempeh was processed by boiling in a pot at 100°C for 10 min in 300 mL tap water. Dried tempeh was carried out by drying in the incubator (Heraeus Instruments, Hanau, Germany, Model No. UT 20) with 60°C temperature for six hours. An electric stove was used with a hot plate (Maybaum, W. Germany, Type 551H No. 8,135) to produce high-temperature for the cooking procedures at the "middle" position on its heat control panel.

The panelists for the sensory evaluation were 28 persons. Prior to the commencement of the sensory evaluation, the panelists were given detailed information on the procedures and methods used to prepare the tempeh samples. The panelist also gave signed informed consent to participate in the study. The tempeh was cut to the size of $2 \times 2 \times 2\ cm^3$. Then, they divided into five different containers labeled A-E in random. The panelists to taste all the samples used toothpicks. Each time, the tasting was followed by filling the questionnaire. Panelists who had already performed the sensory test were instructed not to communicate to each other so as not to affect personal

opinions. The questionnaires consisted of the four-point Likert-type scales (Chang, 1994). The Likert-type scales comprised four measuring levels, ranging one to four where one represented for "strongly dislike" and four for "strongly like". The evaluation of the different tempeh samples based on appearance, aroma, texture, mouth feel, aftertaste and overall. From the result of sensory evaluation, the sample with the most acceptable cooking method was further analyzed for the chemical composition and compared with the uncooked variety.

Preparation of methanolic extracts

All the processed samples (uncooked tempeh and stir-fried tempeh) were freeze dried (Virtis, Freeze mobile 25 EL, Gardiner, New York) at −80°C for two days. Then the samples were pulverized in a mixer grinder (Philips, Germany). Once done, the concerned samples were then stored at 4°C, prior to further usage. Next, 0.5 g of each sample was treated with 5 mL of n-hexane. The samples were vortexed for a minute and were placed on a rolling mixer (RM-810) f 5 min, followed by centrifugation (Hettich Mikro 22R, Type 1,110, Germany) using 5,000 × g for 20 min at 4°C. After carefully discarding the n-hexane, the defatted samples were extracted with methanol acidified with 1 per cent conc. HCL, then repeat the sequential processes vortex, roller mixer and centrifugation as aforementioned. Final pool after three consecutive extractions was stored at 4°C until further analysis of total phenolic content (TPC) and AO activity.

Analysis of macronutrient, iron and zinc composition in tempeh

Stir-fried and uncooked tempeh samples were analyzed at LA Chemie, Universität Hohenheim, Germany, to determine their macronutrient composition. Crude protein composition and crude lipid content of the samples were determined using the Kjeldahl and Soxhlet fat extraction methods, respectively (AOAC International, 2000). Carbohydrate content was estimated by the difference method. Iron and zinc levels were asses using the LA Chemie method (P22-3-115, P12-3-088). Iron and zinc composition were determined by inductively coupled plasma-atomic emission spectrometer (Varian, Darmstadt, Germany, Model Vista Pro.ggt5).

Determination of total phenolic content

C was determined using Folin Ciocalteu's method (Singleton and Rossi, 1965). In all, 0.1 mL extract and 0.5 mL Folin ciocalteu's reagent were mixed with pure H_20 (1:1) and placed in a tube, vortexed and allowed to stand for 8 min. Then, 4.5 mL of 2 per cent sodium carbonate solution was added, vortexed and incubated in a dark room for 1 h at room temperature. The absorbance of the resulting blue complex was measured at 765 nm using a spectrophotometer [Genesys 20, Thermo Fisher scientific spectrophotometer (400/14)]. Methanol was used as the blank, and catechin was used as the standard (Singleton and Rossi, 1965).

Ferric ion reducing/antioxidant power assay

Ferric ion reducing/AO power (FRAP) assay was carried out according to the procedure described by Benzie and Strain (1996) and modified by Pulido *et al.* (2000). The freshly pared FRAP reagent contained 3.5 mL of 20 mmol/L 2,4,6-Tripyridyl-Triazine solution in 40 mmol/L HCL plus 3.5 mL of 20 mmol/L $FeCl_3 \cdot 6H_2O$ and 35 mL of 0.3M acetate buffer, pH 3.6. Later on, the FRAP reagent was incubated at 37°C for 30 min. Then 1,800 μL of this FRAP reagent was mixed with 180 μL of pure water and 60 μL of

test sample or pure water (blank). Then, the samples and blank solution were incubated at 37°C for 30 min in a water bath. At the end of the incubation period, the absorbance was recorded immediately at 593 nm using spectrophotometer [Genesys 20, Thermo Fisher Scientific spectrophotometer (4001/4)]. Methanolic solution of known Fe (II) concentration ranging between spectrophotometer 200 and 2000 μm/L ($FeSO_4 \cdot 7H_2O$) was used for the preparation of the standard calibration curve. Butylated hydroxytoluene was used for the positive control. Finally, the concentration of each extract having ferric-TPTZ reducing ability was expressed in μmol/g (Benzie and Strain, 1996; Pulido *et al.*, 2000).

Thiobarbituric acid reactive substance assay

Thiobarbituric acid reactive substance (TBARS) assay in the current study was performed according to the experiments of Chun *et al.* (2005). The preparation of linoleic acid emulsion was carried out by mixing 1 per cent linoleic acid and 1 per cent Tween 20 in 100 mL of pure water. Then, 0.8 mL of this emulsion mixture was added to 0.2 mL of extract, and the vortexed samples incubated for an hour at 50°C. To this, 1 mL mixture, 2 mL of TBA reagent (100 mL of stock TCA-TBA-HCL solution containing 15 per cent TCA, 0.375 g TBA, 3 mL of 2 per cent BHT in ethanol and final volume with 0.25M HCL) was added and vortexed thoroughly. The mixture was placed in boiling water for 10 min. The mixture was then centrifuged at 6,000 × g for about 15 min. The absorbance of the supernatant was measured at 532 nm using spectrophotometer. The rate of inhibition of TBARS was then calculated from a standard curve prepared using 1,1,3,3-tetraethoxypropane. Pure water and BHT were used as blank and positive control, respectively (Chun *et al.*, 2005).

Estimation of the phytic acid content

PA extraction of the treatment samples was performed by the method described by Kwanyuen and Burton (2005) with modifications. In all, 0.5 g of each freeze-dried testing samples was placed in a screw-capped 15-mL falcon tubes. The samples then were extracted with 5 mL of 1 M HCL and were vortexed for around 2 min. Later, they placed on a roller for about 20 min. Once done, they were centrifuged (Hettic Mikro 22R, Type 1,110, Germany) at 16,000 rpm for 15 min at 4°C. Supernatant was carefully collected in a labeled centrifuge tubes and aliquot of approximately 2 mL of the supernatant was subjected to centrifugation for the second time in a micro centrifuge (Eppendorf 5,415-D) at 13,000 × g for 20 min at 4°C. After collecting the supernatant, they were filtered using cellulose filters of 0.4 μm. Finally, obtained clear filtered samples were stored at 4°C prior to the high-performance liquid chromatography (HPLC) analysis (Kwanyuen and Burton, 2005).

Chromatographic analysis was performed on an HPLC system with a 50 × 4.6 mm PL-SAX 1000A (particle size 5 μm) strong anion-exchange column (Agilent Technologies) equipped with a 20 × 4 mm pre-column (GROM-SIL100 ODS-2FE, particle size 12 μm). Evaluation of PA was achieved with a 30-min linear gradient separation of 0.01 M 1-methylpiperazine pH 4.3, 0.5 M KNO_3 in 0.01 M 1-methylpiperazine, pH 4.3, injection volume 50 μL, at flow rate of 1 mL/min, pressure 70 bars according to Rounds and Nielsen (1993) with modifications. Wade reagent (0.015 per cent (wt/vol) $FeCl_3$ and 0.15 per cent (wt/vol) 5-sulfosalicylic acid) at a flow of 0.5 mL/min, pressure 8 bars and PA eluted from the column were mixed in a mixer

(Kontrom Instruments M800). The final absorbance was measured at 500 nm, and the detector signals showing the peaks displayed on a real-time monitor that was integrated into the data acquisition system. PA dipotassium salt (≥95 per cent) of known concentration ranging from 0.21 to 1.7 mg/mL was used for the preparation of the standard calibration curve.

Statistical analyses

The study results were expressed as means and 95 per cent confidence intervals. The measurements of tempeh were made in triplicate per sample. The univariate analysis of variance and a mixed procedure was used to test repeated measurements of 28 panelists in sensory evaluation and to assess the interaction between cooking processes and sensory characteristics. The means comparison of macronutrient, iron and zinc composition were measured by the *t*-test. The content of total phenolic, FRAP assay and PA concentration were assessed using the univariate analysis of variance, followed by Sidak post-hoc test. TBARS assay values was analyzed by the univariate analysis of variance followed by Dunnett T3 post-hoc test. The level of significance among different samples was set as $p < 0.05$. All statistical analyzes were performed using the SPSS software package (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

Result

The sensory evaluation of different characteristics from traditional cooking methods is given in Table I. The hedonic scale means of stir-fried tempeh were statistically superior, especially in terms of its aroma and mouthfeel compared with other traditional cooking methods. Steaming produced comparable acceptability with stir-frying in appearance, texture, aftertaste and overall indices, respectively. Stir-frying resulted in comparable sensory characteristics as boiling and drying in the aftertaste means. Figure 1 summarizes the analysis of the estimated marginal means of every cooking method after controlling characteristic indices. The estimated marginal mean of stir-frying represented the highest scale, followed by steaming, boiling and drying.

Table II summarizes the changes in protein, fat, carbohydrate, iron and zinc composition due to stir-frying. Protein composition of stir-fried tempeh was slightly

Table I.
The scale means for tempeh samples with different conventional preparations*

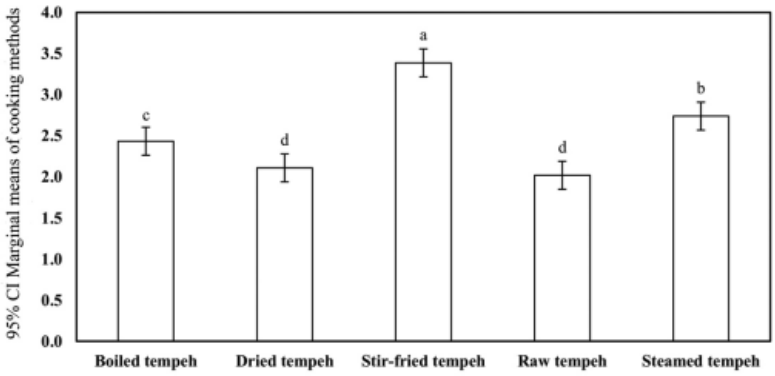
Parameters	Cooking methods: Steamed Mean (95% CI)	Stir-fried Mean (95% CI)	Roasted Mean (95% CI)	Dried Mean (95% CI)	Boiled Mean (95% CI)
Appearance	2.9 (2.6, 3.3)$^{a, b}$	3.3 (2.9, 3.6)^{a}	2.5 (2.1, 2.9)$^{a, b}$	2.2 (1.8, 2.6)^{b}	2.2 (1.8, 2.6)^{b}
Aroma	2.8 (2.4, 3.1)^{b}	3.6 (3.3, 4.0)^{a}	1.6 (1.2, 2.0)^{c}	2.0 (1.6, 2.4)$^{b, c}$	2.5 (2.1, 2.9)^{b}
Texture	2.8 (2.4, 3.2)$^{a, b}$	3.3 (3.0, 3.7)^{a}	2.4 (2.0, 2.8)$^{b, c}$	2.0 (1.6, 2.4)^{c}	2.1 (1.8, 2.5)$^{b, c}$
Mouthfeel	2.6 (2.2, 2.9)^{b}	3.5 (3.2, 3.9)^{a}	2.0 (1.7, 2.4)^{b}	2.0 (1.7, 2.4)^{b}	2.6 (2.3, 3.0)^{b}
Aftertaste	2.7 (2.3, 3.1)^{a}	3.2 (2.8, 3.6)^{a}	1.8 (1.4, 2.2)^{b}	2.3 (1.9, 2.7)$^{a, b}$	2.6 (2.2, 3.0)$^{a, b}$
Overall	2.71 (2.4, 3.1)$^{a, b}$	3.4 (3.0, 3.7)^{a}	1.8 (1.5, 2.2)^{c}	2.2 (1.8, 2.5)$^{b, c}$	2.5 (2.1, 2.9)$^{b, c}$

Notes: *UNIANOVA followed by Sidak post-hoc test; superscript letters (a-c) in the same row indicate a significantly different ($p < 0.05$)

above that of uncooked tempeh. The carbohydrate composition of fried tempeh was inferior than that of uncooked tempeh. The fat composition of stir-fried tempeh was higher than that of uncooked tempeh. The iron and zinc composition of stir-fried tempeh samples were lower in comparison to the value in uncooked tempeh varieties.

The TPCs of both uncooked tempeh samples were higher than stir-fried tempeh samples (Table III). Uncooked tempeh with a three-day fermentation period (6.40, 95 per cent CI 5.84; 6.95 mg Catechin equivalents/g dry weight) was not statistically different from uncooked tempeh which was fermented for a two-day period (6.22, 95 per cent CI 5.89; 6.56 mg CAE/g dry weight). The TPC of stir-fried tempeh fermented for two days (4.50, 95 per cent CI 3.71; 5.28 mg CAE/g dry weight) and three days (4.22, 95 per cent CI 3.41; 5.04 mg CAE/g dry weight) were not statistically different.

Uncooked tempeh with a three-day fermentation period (30.07, 95 per cent CI 26.85; 33.29 μmol $FeSO_4$/g) demonstrated higher FRAP assay value among the samples, but lower compared to BHT as a positive control (Table III). Uncooked tempeh fermented for two days (25.13, 95 per cent CI 21.87; 28.39 μmol $FeSO_4$/g), stir-fried tempeh fermented for two days (22.36, 95 per cent CI 19.91; 24.81 μmol $FeSO_4$/g) and stir-fried tempeh fermented for three days (24.36, 95 per cent CI 22.87; 25.85 μmol $FeSO_4$/g) were not significantly different.



Note: Letters a-d indicate statistically significant different ($p < 0.05$)

Figure 1. Estimated marginal means of the different cooking processes in sensory evaluation

Nutritional content	Uncooked tempeh Mean	95% CI Lower bound	95% CI Upper bound	Stir-fried tempeh Mean	95% CI Lower bound	95% CI Upper bound	*P*-value*
Protein, %	41.6	41.4	41.8	42.1	41.9	42.3	0.015
Fat, %	25.1	25.0	25.2	34.5	34.4	34.6	< 0.001
Carbohydrate, %	33.3	33.1	33.6	23.5	23.2	23.7	< 0.001
Fe, mg/kg	22.0	20.0	24.1	18.6	16.6	20.6	< 0.001
Zn, mg/kg	16.3	15.9	16.8	14.0	13.5	14.4	0.001

Note: *Independent *t*-test for comparison between uncooked tempeh and stir-fried tempeh

Table II. Macronutrients composition (dry weight basis), iron and zinc levels in uncooked and stir-fried tempeh samples

Table III.
Total phenolic content, FRAP assay, TBARS assay and the phytic acid concentration of methanolic extracts of uncooked and stir-fried tempeh samples

Treatments	Total phenolics* (mg CAE/g dry weight) Mean (95% CI)	FRAP* (μmoL Fe(II)/g) Mean (95% CI)	TBARS** (μM MDA Equi/ 100 g) Mean (95% CI)	Phytic acid* (mg/g) Mean (95% CI)
Uncooked two day fermented tempeh	6.2 (5.9, 6.6)[a]	25.1 (21.9, 28.4)[a]	0.8 (0.7, 0.9)[a]	13.1 (10.8, 15.4)[a]
Uncooked three day fermented tempeh	6.4 (5.8, 7.0)[a]	30.1 (26.9, 33.3)[b]	0.7 (0.6, 0.7)[a, b]	12.4 (10.4, 14.5)[a]
Stir-fried two day fermented tempeh	4.5 (3.7, 5.3)[b]	22.4 (19.9, 24.8)[a]	0.6 (0.5, 0.7)[b]	8.2 (7.6, 8.8)[b]
Stir-fried three day fermented tempeh	4.2 (3.4, 5.0)[b]	24.4 (22.9, 25.9)[a]	0.5 (0.3, 0.7)[a, b, c]	7.3 (6.0, 8.7)[b]
BHT	NA	40.1 (33.6, 48.0)[c]	0.2 (0.2, 0.3)[c]	NA

Notes: *UNIANOVA followed by Dunnett T3 post-hoc test; **UNIANOVA followed by Sidak post-hoc test; superscript letters (a-c) in the same column indicate a significantly different ($p < 0.05$); NA = not available

Stir-fried tempeh through a two-day fermentation period (0.57, 95 per cent CI 0.49; 0.65 μM Malondialdehyde Equi/100g) and a three-day fermentation period (0.51, 95 per cent CI 0.30; 0.72 μM MDA Equi/100g) were not significantly inferior compared with uncooked tempeh through a three-day fermentation period (0.69, 95 per cent CI 0.64; 0.73 μM MDA Equi/100g) in TBARS assay (Table III). However, stir-frying tempeh with a two-day fermentation period revealed a lower MDA level, in contrast with uncooked tempeh with a two-day fermentation period (0.80, 95 per cent CI 0.69; 0.91 μM MDA Equi/100g). In comparison with BHT as a positive control, the TBARS values of all samples, except for stir-fried tempeh with a three-day fermentation period, were significantly higher.

In the present study, stir-frying reduced PA concentration in tempeh (Table III). PA concentration of both stir-fried tempeh fermented for two days (8.20, 95 per cent CI 7.57; 8.83 mg/g dry weight) and three days (7.31, 95 per cent CI 5.96; 8.65 mg/g dry weight) were statistically significantly lower in comparison with uncooked tempeh fermented for two days (13.12, 95 per cent CI 10.82; 15.42 mg/g dry weight) and three days (12.42, 95 per cent CI 10.38; 14.46 mg/g dry weight).

Discussion

Acceptability of fried tempeh

The present study showed that stir-fried tempeh had high acceptability among the panelists. This result was similar with previous findings in various settings. Fried tempeh made from a mixture of soybean and sunflower seed products with different flavors showed 90 per cent acceptability among 100 Indian children aged between five and seven years (Vaidehi *et al.*, 1985). In Nigeria, fried tempeh with flavor was tested among health workers, produced 77.17 per cent acceptability (Aderibigbe and Osegboun, 2006). Also, in a related study from Indonesia, the panelists showed higher

acceptance of fried tempeh made from black soybean (*Glycine soja*) compared with other traditional methods (Nurhidajah and Nurrahman, 2009).

Nutrient composition

In the present study, crude protein composition of uncooked tempeh comprised 41.55 per cent on a dry weight basis. Another prior study has described that the range of tempeh's crude protein composition prepared in a laboratory setting in Indonesia and Japan was from 46.9 to 56.9 per cent (Murata *et al.*, 1967). The current study also revealed that the composition of tempeh's crude protein increased slightly after stir-frying. However, this small difference might not justify the increase of protein composition in tempeh samples. Marginal increase could be due to operational technique. Different studies have also shown that the protein composition increased after frying in filet of various fishes compared with the raw variety (Ghelichpour *et al.*, 2012; Zhang *et al.*, 2013). This increase may be due to the development of novel products similar to protein during the frying process and affects the evaluation of protein composition using the Kjeldahl method (DeMan, 1999). The reduction of moisture in food has been proposed also as a cause for the increase (Ersoy and Özeren, 2009; Bordin *et al.*, 2013). On the contrary, several findings suggested that frying could cause a reduction to the protein composition (Steiner-Asiedu *et al.*, 1991), certain amino acids and protein quality (Henry, 1998).

The stir-fried tempeh sample in this study resulted in a 29 per cent decrease in carbohydrate content. The decrease can be explained by the transformation of starch into sugar and acrylamide caused by high-temperature, especially for food with high carbohydrate composition (Damodaran *et al.*, 2007; Palazoğlu *et al.*, 2010). Besides, frying can augment the proportion of resistant starch and, to some extent, contributes to the formation of the amylose-lipid complex, thereby increasing fiber content (Bordin *et al.*, 2013). Another possibility was because of the diffusion of free sugars from food to oil during frying process (Inocent *et al.*, 2011). Furthermore, storage prior stir-frying could lead also to the decrease in the carbohydrates, due to the action of variety of enzymes produced by the *R. oligosporus* during fermentation. Later on, the mold would have used these sugars as a source of carbon for their energy and structural growth (Egounlety *et al.*, 2003).

Stir-frying was found to increase the fat composition of the tempeh by 38 per cent in the present study. The increase of fat composition can be explained by the absorption and retention of oil during frying, which implies an increase in the calorie density of the food (Fillion and Henry, 1998). Foods of plant origin that have more water and less fat absorb more oil than foods of animal origin. This higher fat absorption may be due to the higher content of fat in animal-origin foods decreasing moisture evaporation. Additionally, the oil is absorbed into plant tissue filled with air, compared to intercellular space, which is occupied by fluid in animal-origin foods. This results in greater absorption of oil in foods of plant origin (Fillion and Henry, 1998; Ghidurus *et al.*, 2010).

Zinc and iron levels in fried tempeh samples declined by 15 and 16 per cent, respectively, compared to those in uncooked tempeh samples. As most minerals are non-volatile, the content of minerals, on wet weight, would be expected to rise. On the other hand, the uptake of the oil at the same time increases the weight of fried food. A slight decrease in mineral content might be found when the mineral level is stated on a

dry weight basis. High-temperature or frying does not affect or decrease mineral levels significantly, but minerals are frequently leached if cooked in boiling water (Fillion and Henry, 1998).

Total phenolic content and antioxidant activities

Stir-frying the tempeh samples in sunflower oil significantly decreased the content of polyphenols. The attenuation can be explained by the effective breakdown of flavonoids during cooking. This study outcome showed compatibility with the result obtained from a study of the frying effect on tempeh isoflavones belonging to polyphenol groups. In the study, frying for 30 min caused a 45 per cent reduction of total isoflavones in tempeh (Haron *et al.*, 2009). Similar results in the TPC reduction due to traditional frying have been presented for tomatoes and onions (Crozier *et al.*, 1997; Price *et al.*, 1997).

Stir-frying decreased the FRAP assay only on three-day fermented tempeh, but not on two-day fermented tempeh in contrast with uncooked samples. A related study suggested that frying decreased the FRAP chickpea with the black seed coat, but not for chickpea with the cream seed coat (Segev *et al.*, 2012). The decline can be due to depletion of the moisture in the vegetables/fruits, the bioactive components are inactivated, and subsequently, the AO activity can be decreased (Shahidi, 2015). Tempeh samples with three-day fermentation period decreased more reducing power compared with the two-day fermentation period variety. This might be due to frying resulted in a higher reduction of polyphenols and flavonoids especially among uncooked samples with higher AO activity (Segev *et al.*, 2012).

Contrary to the results obtained through FRAP, TBARS assay revealed that the application of the stir-frying process to a two-day fermented tempeh would increase AO activity, which is superior to the corresponding uncooked variety. In particular conditions, heating might stimulate the oxidation of polyphenols to a transitional substance, which can exhibit higher AO activity than the nonoxidized one (Miglio *et al.*, 2008). Furthermore, the matrix unstiffening effect and extractability substances during cooking can be transformed into AO chemical species. Therefore, cooked vegetables do not always exhibit lower nutritional and physicochemical properties (Miglio *et al.*, 2008).

Phytic acid composition

Frying and storage have been suggested as being able to reduce the PA concentration in tempeh. The reduction can be explained by the heat instability of PA, and phytase activity might have been continued during the storage of tempeh samples prior to their frying. In a related study, tempeh fried in peanut oil resulted in a 50 per cent reduction in PA concentration (Sutardi and Buckle, 1985). Similar to the present study, stir-frying also reduced PA concentration in tempeh. There were 37 and 41 per cent reduction in PA concentration of uncooked tempeh with a two-day fermentation and a three-day fermentation after stir-frying, respectively.

Conclusion

Tempeh was found to be a good source of not only nutrients but also other important health components such as AO. Stir-frying was the most preferred conventional method for preparing tempeh. However, stir-frying was associated with a significant increase in caloric density due to oil absorption. Therefore, choosing alternative cooking methods which do not include addition of oil and very high temperature would be more favorable options.

Declaration of interest

The authors report no conflicts of interest to disclose. The authors alone are responsible for the content and writing of this article. This work was partially supported by the post-graduate scholarship from Directorate General of Higher Education (DGHE) of Indonesia.

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