Nutritional values of tempe inoculated with different strains of *Rhizopus*: its  $\gamma$ -aminobutyric acid content and antioxidant property

(Nilai pemakanan tempe yang diinokulasi dengan pelbagai strain *Rhizopus*: Kandungan asid γ-aminobutirik dan ciri antioksida)

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Keywords: soybean, Rhizopus sp., amino acid, GABA, sensory

#### Abstract

The y-aminobutyric acid (GABA) content and antioxidant profile of fermented soybean inoculated with eight different strains of *Rhizopus* sp. were studied. The ability of these strains, which were obtained from the Centre of Functional Food Cultures (CFFC) collection at MARDI, to produce GABA were compared to wild strains obtained from commercial tempe. Results showed that tempe inoculated with Rhizopus strains of MARDI contained higher GABA, mostly above 0.060 g/100 g dry weight compared to commercial tempe. The highest GABA content was seen in the tempe inoculated with *Rhizopus* 5351 strain with a concentration of up to 0.154 g/100 g dry weight at 48 h fermentation. The amount of beneficial free and essential amino acids of this tempe were also more than 1.70 g and 0.50 g/100 g dry weight respectively. Tempe inoculated with Rhizopus 5351 strain had the highest sensory score in organoleptic acceptability as evaluated by 14 experienced panellists. In addition, the antioxidant content of this tempe was within the range of commercial tempe. Overall, tempe inoculated with Rhizopus 5351 strain had better nutritional value compared to current commercial tempe available in Malaysia. Obviously, Rhizopus 5351 strain can be introduced as a commercial starter culture for making tempe in Malaysia.

### Introduction

Currently, more and more consumers are demanding for healthier food that is produced *via* eco-friendly processes. Soybean, which has several anticarcinogens, including phenolic acids, flavonoids and isoflavonoids, has gained considerable attention for their potential role in reducing several types of cancer and some chronic diseases like cardiovascular disease, osteoporosis and Alzheimers disease (Anderson and Garner 1998; Clarkson and Anthony 1998; Messina and Bennick 1998; Messina 1999; Taie et al. 2008).

Tempe is one of the famous fermented soybean, which serves as a cheap foodstuff with high nutrient content in the Southeast Asia region. It is a staple food that is consumed daily by millions of people in Indonesia because tempe is the main source

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of protein, calories, minerals and vitamins in their daily diet (Nout and Rombouts 1990; Bisping et al. 1993).

Tempe is mainly produced by fermentation of boiled and dehulled soybean using Rhizopus oligosporus. The fungus grows through the beans and forms thick white mycelia which can be cut into slices and cooked, fried or used as protein-enriched meat substitute, which is a popular food among vegetarians. It is well known that fermentation of soybean brings changes in texture, aroma, flavour of the product and improves the nutritional quality. Some lipids, protein profile, the amount of oligosaccharides and several vitamins like vitamin  $B_{12}$  are improved after fermentation (Liem et al. 1977; Winarno and Reddy 1986; Nout and Rombouts 1990; Bisping et al. 1993). Tempe is also reported to be antioxygenic by Hoppe et al. (1997).

Gamma-aminobutyric acid (GABA), an important non-protein constituent amino acid, is also produced during tempe fermentation and this compound has been proven to have pharmaceutical effects on the human body (Nakamura et al. 2000; Aoki et al. 2003; Oh and Oh 2004). GABA, which is present in both prokaryotic and eukaryotic organisms, is known to be biosynthesized via decarboxylation of glutamic acid by glutamate decarboxylase when induced by various stresses. Presently, many reports claim that GABA is present in a variety of fermented foods such as kimchi, red-mould rice, yogurt and other fermented foods (Kohama et al. 1987; Komatsuzaki et al. 2005; Park and Oh 2007; Ran et al. 2007).

Previous studies showed that feeding tempe containing GABA at 0.1% level had significant antihypertensive effect in spontaneously hypertensive rats (Aoki et al. 2003). Dietary GABA from tempe has also been reported to protect the filtration function of the kidneys from damage induced by high blood pressure (Nakamura et al. 2000; Aoki et al. 2003). This GABArich tempe is also rich in various free amino acids and phenolic compounds. Moreover, these compounds not only improve the taste of food, but also have nutritional advantages such as rapid absorption, antioxidant activity and anticancer (McCue and Shetty 2004; Watanabe et al. 2007; Watanabe et al. 2008; Babu et al. 2009). Some reports also stated that GABA is a strong secretagogue of insulin from pancreas, therefore, effectively preventing diabetic conditions (Adeghate and Ponery 2002; Hagiwara et al. 2004).

Knowing the nutritive value of this fermented soybean, MARDI has collected a few strains of *Rhizopus* sp. from local resources. To date, there is no study conducted to evaluate these *Rhizopus* strains in improving local tempe quality. Therefore, this study examined the capability of these *Rhizopus* strains to produce GABA and also other biological active compounds like phenolic acids. Thus, the presence of the bioactive compounds in the tempe production inoculated with MARDI's *Rhizopus* strains collection were compared with commercial tempe obtained from various markets in Malaysia.

## Materials and methods

All the eight strains of *Rhizopus* sp. were obtained from MARDI's Culture Functional Food Centre (CFFC). These cultures were kept in potato dextrose agar slant and stored at 4 °C. Raw soybean was bought from a local supermarket. The phenolic acids standard solution of protocatechuic, β-hydroxybenzoic, vanillic acid, syringic acid, caffeic acid, p-coumaric acid and ferulic acid standards were purchased from Sigma-Aldrich (USA). The amino acids mix standards (2.5 mM of amino acids and 1.25 mM of cysteine) were bought from Waters (USA) including histidine (His), serine (Ser), arginine (Arg), glycine (Gly), aspartic acid (Asp), glutamic acid (Glu), threonine (Thr), alanine (Ala), proline (Pro), cysteine (Cys), lysine (Lys), tyrosine (Tyr), methionine (Met), valine (Val), isoleucine (Ile), leucine (Leu) and phenylalanine (Phe). The  $\gamma$ -aminobutyric acid (GABA) was purchased from Sigma-Aldrich (USA). All

the solvents used were either of analytical or HPLC grade.

### Collection of commercial tempe

A total of 15 commercial tempe samples with unknown *Rhizopus* strains were collected from various states in Malaysia including Selangor (9), Perak (4), Pahang (1) and Negeri Sembilan (1), which were labeled as SE001, SE002, SE003, SE004, SE005, SE006, SE007, SE008, SE009, N001, N002, N003, N004, PA001 and PE001. These samples were subjected to GABA, amino acids profile and a few antioxidant profile analyses as described below.

### Preparation of tempe inoculated with Rhizopus strains from MARDI's culture collection

The soybean was soaked overnight in distilled water (1:3 w/v) for 18 h. The dehulled beans were washed a few times and boiled in water (1:6 w/v) for 60 min, drained and cooled to room temperature. The cooked beans were inoculated with eight different strains of pure Rhizopus sp., namely, 5375, 5347, 5351, 5376, 5346, 5377, 5408 and 5410, at the level of 2 g/kg beans and packed in perforated polyethylene bags (thickness: 15 - 20 mm, perforation distance: 1 cm apart, 100 g cooked beans per pack). The inoculated beans were aerobically incubated at room temperature (30 °C) for 48 h. The tempe was then dried at 70 °C for 2 days and ground into fine powder using an ultra centrifugal mill (Retsch, model: ZM200). All tempe fermentation with different Rhizopus strains were carried out under standardized conditions.

# Extraction of water soluble free amino acids and GABA

The water soluble free amino acids and GABA were extracted from non-fermented (control) and fermented beans using distilled water. A total of 1 g fine tempe powder was mixed with 20 ml distilled water in a 100 ml

conical flask. The mixture was vigorously shaken at 300 rpm (30 °C) for 30 min, followed by centrifugation at 10,000 rpm for 5 min. The supernatant was further filtered with filter paper (Advantec, No. 1) to obtain the final extract for GABA and the amino acids profile analyses.

# Determination of GABA content and amino acid profiles

The  $\gamma$ -aminobutyric acid (GABA) content and amino acid profiles were determined using ultra performance liquid chromatography (UPLC). The stock standard solution of GABA with a concentration of 2.5 mM was prepared by dissolving pure GABA with 0.1 N HCl. A working standard solution including all amino acids and GABA standard was prepared by mixing the amino acids stock standard solution with deionized water. The final concentration was 100 pmole amino acid/µl of each amino acid except for cysteine, which was 50 pmole/µl. The working standard solution was further derivatized with 70 µl of AccQ-Tag<sup>TM</sup> Ultra borate buffer and 20 µl of AccQ<sup>TM</sup> Fluor reagent as described in the UPLC amino acid analysis application solution.

The GABA and amino acid profiles of tempe were separated using AccQ-Tag<sup>TM</sup> Ultra column (2.1 mm x 100 mm,  $1.7 \mu$ m) at a flow rate of 0.7 ml/min with column temperature controlled at 55 °C under the UV spectra of 260 nm. The gradient elution consists of AccQ-Tag<sup>TM</sup> Ultra Eluent A and AccQ-Tag<sup>TM</sup> Ultra Eluent B with injection volume of 1 µl. Gradient elution was conducted as follows: from 0 to 0.54 min, maintained at 99.9% A; from 0.54 to 5.74 min, linear gradient from 99.9 to 90.9% A; from 5.74 to 7.74 min, linear gradient from 90.9 to 78.8% A; from 7.74 to 8.50 min, linear gradient from 78.8 to 40.4% A and then hold for 0.3 min at 40.4% A; from 8.80 to 8.90 min, linear gradient from 40.4 to 99.9% A and then maintained at 99.9% for another 2.1 min.

Quantification was made using calibration curves obtained by injecting

known amounts of amino acids standard and GABA as external standards with known retention times. The total essential amino acids were calculated based on the sum of phenylalanine, threonine, methionine, leucine, isoleucine, lysine and valine. All analyses were performed in triplicate.

# Extraction and determination of soluble phenolic acid compounds

Soluble phenolic acid compounds in nonfermented and fermented beans (5 g each) were extracted with 70% ethanol (4 x 50 ml, 10 min each) according to Tian et al. (2004). Each extract was pooled and evaporated to 10 ml at 30 °C under reduced pressure and then lyophilized to dryness. Dried samples were dissolved in 2 ml of 2.5% methanol and were subjected to HPLC analysis.

Analyses of phenolic compounds were carried out with a high performance liquid chromatography (HPLC), Alliance Separation Module (Waters, 2695), equipped with a diode array detector (Waters, 2996). A 10 µl aliquot of sample solution was separated using a reverse-phase analytical column (100 mm x 34.6 mm Chromolith Performance RP-18e, Merck) with the temperature controlled at 30 °C. The mobile phase consisted of mobile phase A (methanol and distilled water mixtures at 2.5:97.5, v/v) and mobile phase B (methanol and distilled water mixtures at 50:50, v/v) with a flow rate of 2.1 ml/min. Both mobile phases A and B were adjusted to pH 3 with H<sub>3</sub>PO<sub>4</sub>.

Gradient elution was performed as follows: from 0 - 5 min, 100% A; from 5 - 6 min, linear gradient from 100 - 90% A; from 6 - 10 min, linear gradient from 90 - 82% A; from 10 - 15 min, linear gradient from 82 - 75% A; from 15 - 20 min, linear gradient from 75 - 65% A; from 20 - 22min, linear gradient from 65 - 0% A; and from 22 - 24 min, linear gradient from 0 - 100% A. Peak identification was made by comparing retention times and UV spectra (280 nm) with authentic compounds. Quantification was made using calibration curves obtained by injecting known amounts of pure compounds as external standards. The total soluble phenolic acids content was calculated based on the sum of protocatechuic acid,  $\beta$ -hydroxybenzoic acid, vanillic acid, syringic acid, caffeic acid, p-coumaric acid and ferulic acid. All analyses were performed in triplicate.

## **Determination of antioxidant profile assay** A total of 20 g non-fermented and fermented beans were extracted by boiling with 200 ml distilled water at 100 °C for 10 min. The residue samples were then extracted with another 100 ml boiled distilled water as described earlier. The combined water extracts were filtered and freeze-dried. The freeze dried samples were subjected to three different antioxidant assays as described below.

## Determination of total phenolics content

The total phenolics content of water extract of non-fermented and fermented beans were determined according to Marina et al. (2009) with some modifications. Aliquots of one ml water extract (2 mg/ml) were taken in a test tube and mixed with 5 ml Folin-Ciocalteau reagent (1:10 with water) and allowed to stand at room temperature for 5 min. A total of 4 ml of sodium carbonate solution (7.5%, w/v) were added sequentially and the mixture was vortexed. The tubes were placed in the dark for 2 h and the absorbance was read at 765 nm against the reagent blank. The amount of total phenolics content was calculated as gallic acid equivalents (mg GAE/g extract) from a gallic acid calibration curve.

# Determination of free radical scavenging activity

The free radical scavenging activity of non-fermented and fermented beans using the 1,1-diphenyl-2-picryl-hydrazil (DPPH) was determined according to Thaipong et al. (2006) with minor modifications. The beans were extracted by boiling with distilled water at 100 °C for 10 min. The DPPH stock solution was prepared by dissolving 24 mg DPPH in 100 ml methanol and stored at -20 °C. The solution was obtained by mixing 10 ml DPPH stock solution with 45 ml methanol to obtain an absorbance of  $1.1 \pm 0.02$  units at 515 nm using the spectrophotometer (Cary 50 Conc, Varian). To 150 µl of the water extract sample, 2,850 µl of freshly prepared methanolic DPPH solution was added and shaken vigorously. The decolourizing process was recorded after the mixture was allowed to stand for 30 min in the dark. The absorbance (Abs) was measured at 515 nm and compared with a blank control.  $IC_{50}$  (Inhibitory concentration of 50%) was determined from the DPPH calibration curve, indicating the concentration of sample required to scavenge the 50% of the DPPH free radicals. The scavenging ability was calculated as follows:

Scavenging activity (%) = [1 - (Abs<sub>sample</sub>/Abs<sub>control</sub>)] x 100

# Determination of ferric reducing antioxidant power (FRAP)

The FRAP assay was done according to method of Thaipong et al. (2006). The working solution of FRAP was prepared by mixing 25 ml acetate buffer (300 mM acetate buffer, pH 3.6), 2.5 ml TPTZ (2, 4, 6-tripyridyl-s-triazine) solution (10 mM TPTZ in 40 mM HCl) and 2.5 ml FeCl<sub>3</sub>.6H<sub>2</sub>O solution (20 mM) and incubated at 37 °C. A total of 150 µl of water extract (5 mg/ml) was allowed to react with 2,850 µl of FRAP solution, shaken vigorously and left to stand for 30 min in the dark. The coloured product (ferrous tripyridyltriazine complex) formed was read at 593 nm. The ascorbic acid was used as a standard and was linear in the range between 20 and 200 ppm. The results were expressed as ascorbic acid equivalent (mg AAE)/g extract.

### Sensory evaluation of tempe snacks

The organoleptic quality of tempe snacks, which were prepared from tempe inoculated with eight strains of *Rhizopus* sp. (MARDI's culture collection), was studied in the sensory analysis. The fried slices were evaluated for colour, aroma, texture, taste and overall acceptability based on ninepoint hedonic preference test (1 = Dislike extremely, 3 = Dislike moderately, 5 = Neither like or dislike, 7 = Like moderately, 9 = Like extremely; Koh et al. 2011). All these attributes were evaluated by 14 panellists with good experiences in the tempe field.

### Statistical analysis

Data were statistically analysed by one-way analysis of variance using PAWS statistics (version 18). Significant differences (p <0.05) between means were determined by Duncan multiple range test (DMRT).

### **Results and discussion**

GABA and amino acids profiles of tempe Both commercial tempe and tempe prepared using different Rhizopus sp. from MARDI had higher amounts of beneficial free and essential amino acids present in the fermented soybean than in the control. The same phenomenon was also observed by Handoyo and Morita (2006). However, the results showed that commercial tempe had lower amounts of free and essential amino acids content (below 1.70 g and 0.55 g/100 g dry weight respectively) than tempe inoculated with MARDI's Rhizopus sp. (Figures 1a and 2a). Tempe inoculated with strains of MARDI's Rhizopus sp. showed significant increment in yield of free and essential amino acids (p < 0.05), particularly those inoculated with Rhizopus strains of 5347, 5351, 5408 and 5410 (Figure 1a). These four strains had the capability to produce more proteases than the other Rhizopus strains. The rest of MARDI's Rhizopus strains exhibited amino acid profiles similar to the commercial tempe. According to Watanabe et al. (2008),



Figure 1. (a) Total free and essential amino acids (AAs) profile and (b) GABA content of tempe inoculated with 8 different **Rhizopus** strains. Each value in the figure represents the mean  $\pm$  standard deviation from triplicate analyses. Means within each bar with different letters are significantly different (p <0.05)

fermented soybean using *Rhizopus* assisted in the improvement of calcium absorption ratio in the animal model. It is believed that the high calcium absorption might be contributed by both low phytate content and peptides that were produced during the soybean fermentation. Based on the high amount of free amino acids present in the tempe, it is believed that tempe inoculated with MARDI's *Rhizopus* collection may also contain high peptides.

Among all the *Rhizopus* strains, tempe fermented with *Rhizopus* 5351 strain had the highest GABA content, 0.154 g/100 g dry weight (*Figure 1b*). This means that this *Rhizopus* strain was capable of producing higher glutamate decarboxylase enzyme than the other *Rhizopus* strains under the same tempe preparation procedure (Bouche and Fromm 2004). *Rhizopus* 5347 strain, which is currently sold commercially by MARDI, had lower GABA content (0.062 g/100 g dry weight) than some of the other *Rhizopus* strains (*Figure 1b*). These findings showed that some of the *Rhizopus* strains from MARDI's culture collection favoured the GABA production in tempe.

In addition, the findings also indicated that tempe inoculated with MARDI's *Rhizopus* strains had higher GABA content (more than 0.060 g/100 g dry weight) than the commercial tempe (*Figures 1b* and 2b). In fact, they also had higher GABA content as opposed to other fermented foods such as yogurt (0.042 g/100 g dry weight) fermented using lactic acid bacteria (Park and Oh 2007). Overall, the results obtained indicated that tempe prepared using *Rhizopus* strains from MARDI's culture collection had better benefits compared to commercial tempe.

### Antioxidant profiles of tempe

In general, the total phenolics content (TPC), IC<sub>50</sub> value, FRAP value and total soluble phenolic acids content varied with the different commercial tempe samples. The values ranged from 9.9 - 22.9 mg gallic acid equivalent/g extract, 13.6 - 61.5 mg extract/ ml, 7.2 - 14.8 mg ascorbic acid equivalent/g extract and  $17.22 - 59.11 \mu g/g$  extract respectively (*Figure 3a*).

Some of the commercial tempe samples had better antioxidant activities than tempe inoculated with MARDI's Rhizopus strains, especially samples SE001, SE003, SE005, SE006, SE007, SE008, SE009, PA001, N002 and N004 which had lower IC<sub>50</sub> values (below 20 mg extract/ml) and higher FRAP values (above 9 mg AAE/g extract) (Tables 1 and 2). Among all the Rhizopus strains, tempe inoculated with Rhizopus 5351 strain exhibited the highest antioxidant activities (p < 0.05). However, when compared to commercial tempe, the antioxidant profile of this tempe was lower but still within the commercial acceptable range.



Figure 2. (a) Total free and essential amino acids (AAs) profile and (b) GABA content of 15 commercial tempe collected from various states in Malaysia. Each value in the figure represents the mean  $\pm$  standard deviation from triplicate analyses. Means within each bar with different letters are significantly different (p < 0.05)

The total soluble phenolic acids content in some of the commercial tempe were higher than tempe inoculated with MARDI's *Rhizopus* strains (*Figures 3a* and *3b*). Commercial tempe samples such as SE003, SE004, SE006, SE007, PA001, N001, N003 and N004 were observed to have total soluble phenolic acids content above 40  $\mu$ g/g extract. Most of the tempe prepared using MARDI's *Rhizopus* strains had total phenolic acids content between 20 and 40  $\mu$ g/g extract. However, tempe inoculated with *Rhizopus* 5408 strain showed significantly high soluble phenolic acids content (p < 0.05) at above 40 µg/g extract.

In general, fermentation process is known to improve the antioxidant profile of soybean (Watanabe et al. 2007). The antioxidant activities of tempe might be attributed by various groups, namely free amino acids, peptides and phenolic compounds as claimed by Watanabe et al. (2007). The simple molecular structure of phenolic acids allowed them to be easily absorbed into the human system and offered an anti-aging benefit which was related to increasing antioxidant Nutritional values of tempeh



Figure 3. Total soluble phenolic acids content of (a) 15 commercial tempe and (b) tempe inoculated with 8 different **Rhizopus** strains. Each value in the figure represents the mean  $\pm$  standard deviation from triplicate analyses. Means within each bar with different letters are significantly different (p <0.05)

activity and prevention of growth of abnormal cells (Dykes and Rooney 2007). These compounds are known to pose a wide spectrum of biochemical activities such as antioxidant, antimutagenic and anticarcinogenic which play an important role in human health (Tapiero et al. 2002; Nakamura et al. 2003).

An overview on the total phenolics content,  $IC_{50}$  values, FRAP values and total soluble phenolic acids content showed that all prepared tempe exhibited different antioxidant capability when inoculated with different *Rhizopus* strains under the same fermentation condition. In summary, all antioxidant assays indicated that tempe inoculated with MARDI's *Rhizopus* strains collection was less competitive in terms of antioxidants profile when compared with commercial tempe.

## Sensory evaluation of tempe prepared using eight different Rhizopus strains

A nine-point hedonic scale with 14 experienced panellists was used to evaluate the organoleptic acceptability of tempe prepared using *Rhizopus* strains from MARDI's culture collection. The tempe was evaluated based on the appearance, texture, colour, odour, taste and overall acceptability (*Table 3*). In general, tempe inoculated with *Rhizopus* strains of 5351, 5376, 5346 and

Commercial	TPC	IC <sub>50</sub>	FRAP
Tempe	(mg GAE/g extract)	(mg extract/ml)	(mg AAE/g extract)
SE001	19.889 ± 0.294g	$16.087 \pm 0.031c$	$11.903 \pm 0.014i$
SE002	11.778 ± 0.309b	61.517 ± 0.611j	$8.025 \pm 0.017c$
SE003	22.926 ± 0.210j	$16.223 \pm 0.060c$	$11.555 \pm 0.038$ g
SE004	9.889 ± 0.111a	32.283 ± 0.549i	$7.193 \pm 0.019a$
SE005	$15.870 \pm 0.306c$	$18.360 \pm 0.053 d$	11.583 ± 0.019g
SE006	$20.389 \pm 0.167h$	$13.633 \pm 0.021b$	$13.462 \pm 0.031$ k
SE007	19.981 ± 0.032g,h	$19.467 \pm 0.035e$	$9.977 \pm 0.020d$
SE008	$19.204 \pm 0.274 f$	$16.403 \pm 0.023c$	$11.577 \pm 0.016$ g
SE009	20.037 ± 0.169g,h	17.957 ± 0.093d	$12.685 \pm 0.017$ j
PA001	22.278 ± 0.255i	$16.380 \pm 0.052c$	$11.182 \pm 0.008 f$
PE001	17.111 ± 0.193d	$31.500 \pm 0.096h$	$7.355 \pm 0.017b$
N001	19.926 ± 0.339g	$24.347 \pm 0.280 f$	$10.057 \pm 0.016e$
N002	$20.370 \pm 0.225h$	$12.647 \pm 0.106a$	$14.842 \pm 0.0281$
N003	$18.741 \pm 0.169e$	26.497 ± 0.137g	$8.000 \pm 0.017c$
N004	$17.333 \pm 0.200d$	$17.957 \pm 0.015d$	$11.772 \pm 0.016h$
N004	$17.333 \pm 0.200d$	$17.957 \pm 0.015d$	$11.772 \pm 0.016h$

Table 1. Antioxidant profiles of 15 samples of commercial tempe collected from various states in Malaysia

Each value in the table represents the mean  $\pm$  standard deviation from triplicate analyses. Means within each column with different letters are significantly different (p < 0.05) TPC = Total phenolics content; IC<sub>50</sub> (Inhibitory concentration at 50%) = Concentration of sample required to scavenge 50% of the DPPH free radicals; FRAP = Ferric reducing antioxidant power; GAE = Gallic acid equivalent; AAE = Ascorbic acid equivalent

Table 2. Antioxidant profile of tempe inoculated with 8 different <i>Rhizopus</i> s	strains
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<i>Rhizopus</i> Strain	TPC (mg GAE/g extract)	IC50 (mg extract/ml)	FRAP (mg AAE/g extract)
Control	$4.586 \pm 0.017a$	$88.175 \pm 0.636h$	$2.924 \pm 0.013b$
5375	$14.802 \pm 0.081b$	53.913 ± 1.347g	$4.832 \pm 0.009$ g
5347	$21.162 \pm 0.078 f$	$24.273 \pm 0.028b$	$4.051 \pm 0.009d$
5351	$14.698 \pm 0.102b$	$20.659 \pm 0.142a$	5.813 ± 0.012i
5376	$16.848 \pm 0.080c$	$24.118 \pm 0.105b$	$4.357 \pm 0.001e$
5346	$18.835 \pm 0.091e$	$37.392 \pm 1.064 f$	$5.741 \pm 0.009h$
5377	$14.753 \pm 0.076b$	33.252 ± 0.110d	$2.371 \pm 0.009a$
5408	$17.882 \pm 0.224d$	25.693 ± 0.402c	$4.452 \pm 0.010 f$
5410	$14.457 \pm 0.467b$	35.971 ± 1.279e	$3.843 \pm 0.009c$

Each value in the table represents the mean  $\pm$  standard deviation from triplicate analyses. Means within each column with different letters are significantly different (p < 0.05) TPC = Total phenolics content; IC<sub>50</sub> (Inhibitory concentration 50%) = Concentration of sample required to scavenge 50% of the DPPH free radicals; FRAP = Ferric reducing antioxidant power; GAE = Gallic acid equivalent; AAE = Ascorbic acid equivalent

5377 had sensory scores of more than 8 in the overall acceptability attribute indicating there were no significant differences in the acceptability of these tempes. However, tempe inoculated with *Rhizopus* 5351 strain scored the highest sensory points in terms of appearance, taste and overall acceptability in comparison to other *Rhizopus* strains. Based on these findings, it showed that *Rhizopus* 5351 strain had higher commercial value as this strain was found to be able to ferment soybean with higher GABA content (*Figure 1b*) compared to other *Rhizopus* strains.

Table 3.	Sensory scores of te	empe inoculated with 8 e	different Rhizopus strair	IS		
	Appearance	Texture	Colour	Aroma	Taste	Overall acceptability
5375	$7.67 \pm 1.00$ a,b	$7.44 \pm 0.53a,b,c$	$7.56 \pm 0.88$ a,b,c	$7.56 \pm 0.53 a, b$	$7.89 \pm 0.60a$	$7.78 \pm 0.67$ a,b,c
5347	$7.62 \pm 1.12a,b$	$6.62 \pm 1.66a$	$7.15 \pm 1.57a,b$	$7.46 \pm 1.27$ a,b	$6.92 \pm 1.19a$	$7.08 \pm 1.44$ a,b
5351	$8.32 \pm 0.84b$	$8.27 \pm 1.01$ d,e	$8.18 \pm 0.87$ b,c	$7.91 \pm 0.94$ a,b	$8.14 \pm 1.19a$	$8.32 \pm 0.84c$
5376	$8.30 \pm 0.95b$	$7.90 \pm 0.99$ b, c, d	$8.10 \pm 1.45$ b,c	$7.30 \pm 1.06a,b$	$8.00 \pm 1.63a$	$8.05 \pm 1.42b,c$
5346	$8.30 \pm 0.48b$	$8.60 \pm 0.70e$	$8.40 \pm 0.52c$	$8.00 \pm 1.05b$	$8.10 \pm 1.37a$	$8.10 \pm 0.88$ b,c
5377	$8.00 \pm 0.47a,b$	$8.10 \pm 0.99$ b, c, d	$7.80 \pm 0.79$ a,b,c	$7.80 \pm 0.63 a, b$	$7.70 \pm 1.06a$	$8.10 \pm 0.88$ b,c

Each value in the table represents the mean  $\pm$  standard deviation from 14 observations. Means within each column with different letters are significantly different (p < 0.05) 1 = Dislike extremely, 3 = Dislike moderately, 5 = Neither like or dislike, 7 = Like moderately and 9 = Like extremely

Conclusion

Tempe made using *Rhizopus* strains from MARDI's culture collection as starter culture has been proven to have better nutritional value in comparison to commercial tempe sold in Malaysia. In general, tempe inoculated with Rhizopus 5351 strain had higher nutritive value than other Rhizopus strains and commercial tempe in terms of GABA, free and essential amino acids content. However, this tempe had less antioxidants activity compared to commercial tempe. Nevertheless, tempe inoculated with Rhizopus 5351 strain was highly acceptable and this Rhizopus strain had a higher market value since it was shown to produce tempe with better nutritive quality compared to currently available tempe in Malaysia.

### Acknowledgements

The authors gratefully acknowledge the Ministry of Agriculture and Agro-based Industry (MOA) for the financial support of this work and express gratitude to fellowships from MARDI and Universiti Putra Malaysia for their kind technical assistance.

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 $7.33 \pm 1.41a.b.c$ 

 $6.82 \pm 1.33 \mathrm{a}$ 

 $7.09 \pm 1.14a$ 

 $7.00 \pm 1.22a$ 

 $7.22 \pm 1.09$ a,b

 $8.11 \pm 0.60$ b,c  $7.00 \pm 1.41a$ 

 $7.89 \pm 0.60$  b, 2.00 b, 2.00 $7.09 \pm 1.30$ a.b

 $7.78 \pm 1.48a,b$  $7.00 \pm 1.41a$ 

5410 5408

 $7.00\pm0.77a$ 

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## Abstrak

Kandungan asid y-aminobutirik (GABA) dan profil antioksida tempe yang diinokulasi dengan lapan strain Rhizopus yang berbeza telah dikaji. Keupayaan semua strain ini yang diperoleh daripada koleksi Pusat Kultur Makanan Berfungsi (CFFC), MARDI untuk menghasilkan GABA telah dibandingkan dengan strain yang diguna untuk menghasilkan tempe komersial. Hasil kajian menunjukkan tempe yang diinokulasi dengan strain Rhizopus MARDI mengandungi GABA yang lebih tinggi daripada tempe komersial dengan kandungan GABAnya melebihi 0.060 g/100 g berat kering. Kandungan GABA yang tertinggi diperoleh daripada tempe yang diinokulasi dengan strain Rhizopus 5351 dengan tempoh fermentasi selama 48 jam. Dalam tempoh ini, kepekatan GABA telah meningkat sehingga 0.154 g/100 g berat kering. Kandungan asid amino bebas dan perlu yang berfaedah diperoleh daripada tempe ini juga masing-masing melebihi 1.70 g dan 0.50 g/100 g berat kering. Tempe yang diinokulasi dengan Rhizopus 5351 juga menunjukkan skor penerimaan yang tertinggi ke atas ujian penerimaan organoleptik yang telah dinilai oleh 14 panel yang berpengalaman. Sebagai tambahan, kandungan antioksida dalam tempe ini juga diukur dan didapati kandungannya berada dalam julat tempe komersial. Keseluruhannya, tempe yang diinokulasi dengan strain Rhizopus 5351 mempunyai nilai pemakanan yang lebih baik berbanding dengan tempe komersial yang terdapat di Malaysia. Strain Rhizopus 5351 dengan jelas boleh diperkenalkan secara komersial sebagai kultur pemula untuk penghasilan tempe di Malaysia.