EFFECTS OF SOYBEAN PROCESSING AND PACKAGING ON THE QUALITY OF COMMONLY CONSUMED LOCAL DELICACY TEMPE

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ABSTRACT

Tempe is a processed food, produced by fermenting soybeans with fungus *Rhizopus oligosporus*. In this study, the effects of soybean processing (soaking, cooking and fermentation) and packaging (food grade wrapping paper, polyethylene bags and low density polyethylene cling wrap) on physicochemical properties, antioxidant activities and microbial count were evaluated. In addition, shelf life study of tempe samples (packed) was conducted for 1 week. Soaking and cooking significantly decreased polyphenols, flavonoids and antioxidant activities of soybeans. After fermentation, tempe samples exhibited significant improvement in physicochemical properties, antioxidant activities and extractability of polyphenols and flavonoids, when compared with unfermented soybeans. Food grade wrapping paper and perforated polyethylene bags were selected as suitable materials for packaging tempe as there were no significant changes in quality and the shelf life of tempe was extended for 3 days. Results obtained support the use of fermentation to improve the quality of soybeans along with safety standards.

PRACTICAL APPLICATIONS

Tempe processing and packaging plays an important role in extending the shelf-life of the product. The results of this study suggest that tempe samples exhibited significant improvement in antioxidant activities and extractability of polyphenols and flavonoids, after fermentation. Food grade wrapping paper and perforated polyethylene bags were selected as suitable materials for packaging tempe. This study could serve to improve the progress of processing and packaging for maintaining the quality of tempe.

INTRODUCTION

Fermented foods have improved the quality of diets and enriched dishes worldwide, thus representing approximately one-third of total food consumption (Nout and Kiers 2005). Tempe (or tempeh) is an example of traditional Indonesian fermented food that is widely consumed in Malaysia as a local delicacy. Tempe is not consumed raw, but fried, boiled, steamed or roasted (Nout and Kiers 2005; Balqis et al. 2013). Tempe is produced by solid-state fermentation (SSF) of soybeans (*Glycine max*) using *Rhizopus oligosporus*. SSF represents a technological alternative used in the food industry to improve the nutritional value, organoleptic properties, and digestibility of the product, as well as enhance the bioavailability of proteins, carbohydrates and lipids (Cuevas-Rodriguez et al. 2005; Reyes-Bastidas et al. 2010). Generally, tempe is known as a compact, sliceable mass of precooked soybeans that are bound together by a dense, uniform, white mycelium of mould (*R. oligosporus*). The process of making
Tempe is rapid in comparison to other soya fermentations that involved fungi, yeasts and bacteria. Tempe processing includes soaking, cooking, inoculation, and fermentation (Hesseltine et al. 1963). Tempe has superior nutritive qualities and essential characteristics of a dietary staple. Consumption of tempe has been linked to prevention of certain types of cancer, cardiovascular disease and osteoporosis, owing to its antioxidant properties (Babu et al. 2009). In addition, tempe is an excellent source of high-quality, low-cost protein, thus serving as an alternative to meat for vegetarian diet (Shurtleff and Aoyagi 2001). Tempe also has the advantage of containing Vitamin B-12, which is a by-product of SSF (Liem et al. 1977).

Tempe is highly perishable due to microbial enzymatic activities, thus it should be stored appropriately to prolong its shelf life. Freshly made, raw tempe stored at room temperature is best consumed within 1–2 days (Shurtleff and Aoyagi 2001; Babu et al. 2009). Tempe spoilage is obvious when soybeans are bound together only by a sparse white mycelium, foul or rotten smelling, wet, slimy and moulds grow in sparse patches (Steinkraus et al. 1960). Tempe packaging plays an important role in extending the shelf-life of the product. This is mainly because packaging provides an optimum oxygen supply and maintains temperature for fermentation to occur (Haron and Raob 2014).

In Malaysia, tempe has been gaining popularity among health-conscious consumers as the major production of tempe is made traditionally by local producers (small scale industrialists). Therefore, this study was undertaken as part of efforts to evaluate the effects of soybean processing and packaging on the quality parameters of tempe such as physicochemical properties (pH and total soluble solids), antioxidant activities and microbial count. Besides that, shelf life study of tempe samples (packed) stored at 25 ± 1°C was conducted for 1 week. Tempe processing includes soaking, cooking, and fermentation. Additionally, tempe samples were packed in three different packaging, namely food grade wrapping paper, polyethylene (PE) bags, and low density polyethylene (LDPE) cling wrap. The information obtained from this study could serve to improve the progress of processing and packaging for maintaining the quality of tempe.

**MATERIALS AND METHODS**

**Materials**

Soybean (Glycine max sp.) and tempe starter (Raprima brand, Indonesia) were purchased from a local tempe producer (Johor, Malaysia), located about 260 km from the Postharvest Biotechnology Laboratory, University of Malaya.

Gallic acid, L-ascorbic acid, (+)-catechin, potassium ferrocyanide, ferric chloride, Folin-Ciocalteu reagent, sodium hydroxide, trichloroacetic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma (MO, The United States). Aluminum chloride and peptone water were purchased from R & M Chemicals (Essex, United kingdom). Sodium bicarbonate, sodium nitrite and sodium carbonate were purchased from BDH (Poole, United kingdom). All chemical solvents used were analytical reagent grade and purchased from Sigma (MO, The United States).

**Tempe Processing and Packaging**

Tempe was prepared in laboratory scale according to Egounlety and Aworh (2003) with minor modifications, as shown in Fig. 1. Whole soybeans were washed, soaked in water for 24 hours at room temperature (25 ± 1°C), and hand-dehulled. After washing, the dehulled soybeans were boiled in water for one hour at 100°C, and then drained. The boiled soybeans were air-dried and allowed to cool to room temperature. Tempe starter (or R. oligosporus) was added to dried soybeans with a ratio of 1:100 by weight. The mixture of soybeans and R. oligosporus mould (25 g) were packed in three different packaging (Fig. 2):

a) Tempe A (packed using brown food grade wrapping paper; 38 cm length; 30 cm width)
b) Tempe B (packed using perforated PE bags; 23 cm length; 15 cm width and heat sealed)
c) Tempe C (packed using perforated LDPE cling wrap; 30 cm length; 30 cm width)

The packages were incubated at 30 ± 1°C for 48 hours with good ventilation. The tempe produced should be a compact white cake (soybeans are completely covered in a dense mass of white mycelium) that can be lifted out as a whole piece (Hedger 1982).

**Sample Preparation**

Samples included soybean (raw, soaked and cooked), tempe A (fermented for 24 and 48 hour), tempe B (fermented for 24 and 48 hour), and tempe C (fermented for 24 and 48 hour). All samples were ground and stored at −20°C until further analysis. All analysis was carried out in triplicate.

**Physicochemical Analysis (pH and Total Soluble Solids)**

pH of samples was determined using a pH meter (Hanna Microprocessor pH 211, Italy) at 25 ± 1°C. Total soluble solids (TSS) was determined using a digital refractometer (Atago PR-1 digital refractometer, Tokyo, Japan) at 25 ± 1°C and results were expressed in standard Brix unit.
Soybeans
↓
Washing
↓
Soaking (24 hours)
↓
Dehulling (by hand)
↓
Cooking (boiling for 1 hour)
↓
Air-drying, draining and cooling
↓
Adding tempe starter and mixing
↓
Packing (using food grade wrapping paper, perforated PE bags and perforated LDPE cling wrap)
↓
Fermenting (48 hours)
↓
Tempe

FIG. 1. FLOW SHEET OF TEMPE PRODUCTION.
PE (polyethylene); LDPE (low density polyethylene)

Antioxidant Activity (Total Polyphenol Content, Total Flavonoid Content, DPPH Radical Scavenging Assay, Reducing Power Assay and Total Antioxidant Capacity)

Antioxidants were extracted according to Santhirasegaram et al. (2013). Equal parts of sample were added to 80% methanol. The mixture was placed in a shaking incubator (Shellab Orbital Shaking Incubator S14, OR) at 250 rpm for 30 minutes at room temperature, and then centrifuged. The supernatant was concentrated using a rotary evaporator (Buchi Rotavapor R-215 Postfach Flawil, Switzerland). The crude extracts were collected and used for the antioxidant activity analysis.

Total polyphenol content of samples was determined using Folin-Ciocalteu assay (Singleton et al. 1965) modified to a microscale (Bae and Suh 2007). Sample extract (10 μL) was added to 790 μL sterilized distilled water (SDW) and 50 μL Folin–Ciocalteu reagent in a 1.5 mL microcentrifuge tube, and mixed. After 1 minute, 150 μL of 20% sodium carbonate solution was added, and the solution was mixed by inverting the tubes. The mixture was allowed to stand at room temperature (25 ± 1°C) for 120 minutes (in dark). Absorbance was measured at 750 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank. A standard curve of gallic acid ($y = 0.00566x$, $r^2 = 0.9955$) was prepared and results were reported as milligrams of gallic acid equivalent (GAE) per 100 g sample.

Total flavonoid content of samples was determined using a colorimetric method described by Sakanaka et al. (2005). Sample extract (250 μL) was added to 1.25 mL of SDW and 75 μL of a 5% sodium nitrite solution in a test tube, and mixed. After 5 minutes of incubation at room temperature (25 ± 1°C), 150 μL of a 10% aluminum chloride solution was added to the mixture. The mixture was allowed to stand for another 5 minutes and then, 500 μL of 1 N sodium hydroxide was added. The mixture was made up to 2.5 mL with SDW and vortexed. Absorbance was measured at 510 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank. A standard curve of (+)-catechin ($y = 0.0135x$, $r^2 = 0.9943$) was prepared and results were reported as milligrams of catechin equivalent (CE) per 100 g sample.

DPPH radical scavenging assay was carried out as described by Oyaizu (1986) and Bae and Suh (2007). Sample extract (500 μL) was added to 1 mL of 80% methanolic 0.1 mM DPPH solution in a 2 mL amber microcentrifuge tube. The mixture was vortexed and incubated in the water bath (Memmert, Germany) at 37°C for 30 minutes (in dark). Absorbance was measured at 517 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank and a control. A standard curve of ascorbic acid ($y = 10.145x$, $r^2 = 0.9907$) was prepared and results were reported as micrograms of ascorbic acid equivalent (AAE) per g sample.

A spectrophotometric method by Oyaizu (1986) was used for measuring the reducing power of samples. Sample extract (50 μL) was added to 200 μL of 0.2M phosphate buffer (pH 6.6) and 200 μL of 1% potassium ferricyanide in a 1.5 mL microcentrifuge tube. The mixture was incubated in the water bath (Memmert, Germany) for 20 minutes at 50°C (in dark). Next, 250 μL of 10% trichloroacetic acid was added to the mixture, and then centrifuged (UEC Micro 14/B, New York) at 1,000 rpm for 5 minutes at room temperature (25 ± 1°C). The supernatant of the centrifuged
sample (500 μL) was mixed with 500 μL of SDW and 100 μL of 0.1% ferric chloride. The mixture was allowed to stand at room temperature (25 ± 1°C) for 5 minutes. Absorbance was measured at 700 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank and a control. A standard curve of ascorbic acid \( y = 0.0014x, r^2 = 0.9906 \) was prepared and results were reported as micrograms of ascorbic acid equivalent (AAE) per g sample.

Total antioxidant capacity of samples was determined using the phosphomolybdenum method described by Prieto et al. (1999). Sample extract (100 μL) was added to 1 mL of reagent solution in a 1.5 mL microcentrifuge tube. The reagent solution consists of equal volume of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The tubes were incubated in a water bath (Memmert, Germany) at 95°C for 90 minutes (in dark). After cooling to room temperature (25 ± 1°C), absorbance was measured at 695 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank. A standard curve of ascorbic acid \( y = 0.0018x, r^2 = 0.9981 \) was prepared and results were reported as micrograms of ascorbic acid equivalent (AAE) per g sample.

**Microbial Analysis (Coliform Count, Aerobic Plate Count, Lactic Acid Bacteria, Total Yeast and Mould Count)**

The 3M Petrifilm plate methods are recognized as AOAC International Official Methods of Analysis (3M Food Safety 2010). Microbial count of samples were determined using Petrifilm plates (3M Center, MN) for coliform, aerobic...
bacteria, lactic acid bacteria, yeast and mould according to Santhirasegaram et al. (2013). Serial dilution bottles were filled with 0.1% peptone water and then autoclaved. Samples were appropriately diluted (10^{-1} to 10^{-3}) and the pH was adjusted for optimum growth. Then, 1 ml diluted sample was placed on the Petrifilm plates. The coliform Petrifilms were incubated at 35 ± 1C for 24 hours. The aerobic and lactic acid bacteria Petrifilms were incubated at 35 ± 1C for 48 hours. The yeast and mould Petrifilms were incubated at 25 ± 1C for 3–5 days. The coliform, aerobic bacteria, lactic acid bacteria, yeast and mould counts in samples were calculated as colony-forming units (CFU) per gram of sample according to the equation:

\[
\text{CFU per mL} = \frac{\text{number of colonies \times dilution factor of plate}}{\text{aliquot plated}}
\]

The results will be expressed as log (CFU/g).

**Shelf Life Study**

Shelf life of tempe samples were evaluated according to the Public Health Laboratory Service, PHLS (2000). Tempe samples A, B and C were stored at room temperature (25 ± 1C) for 1 week. Tempe samples were analyzed daily for aerobic bacteria, yeast and mould count using Petrifilm plates (3M Center, MN) according to Santhirasegaram et al. (2013), as described in the previous section. Serial dilution bottles were filled with 0.1% peptone water and then autoclaved. Samples were appropriately diluted (10^{-1} to 10^{-3}) and the pH was adjusted for optimum growth. Then, 1 ml diluted sample was placed on the Petrifilm plates. The coliform Petrifilms were incubated at 35 ± 1C for 24 hours. The aerobic and lactic acid bacteria Petrifilms were incubated at 35 ± 1C for 48 hours. The yeast and mould Petrifilms were incubated at 25 ± 1C for 3–5 days. The coliform, aerobic bacteria, lactic acid bacteria, yeast and mould counts in samples were calculated as colony-forming units (CFU) per gram of sample according to the equation:

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\text{CFU per mL} = \frac{\text{number of colonies \times dilution factor of plate}}{\text{aliquot plated}}
\]

The results will be expressed as log (CFU/g).

**Statistical Analysis**

Data obtained were subjected to statistical analysis using SPSS 22.0 software (SPSS Inc., IBM). Overall, nine different batches of soybean and tempe samples were used for this study (n = 9). For each batch, processing and packaging was conducted in triplicate. In addition, analyses of quality parameters (physicochemical properties, antioxidant activities and microbial count) and shelf-life were also conducted in triplicate for each sample. In this study, data were represented as mean values ± standard deviation (SD). The significant differences between mean values of samples were determined by analysis of variance (one way-ANOVA) using Tukey’s HSD (Honestly Significant Difference) test at a significance level of P < 0.05. The relationship between variables was determined using Pearson’s correlation test at a significance level of P < 0.01.

**RESULTS AND DISCUSSION**

**Tempe Processing and Packaging**

Tempe samples A, B and C (fermented for 48 hours) are shown in Fig. 2. According to Steinkraus et al. (1960) and Hedger (1982), low quality tempe is indicated by a soft cake which tend to break up, and the spaces between soybean are partly filled by mycelium. In this study, the quality of tempe produced were good as the soybeans are bound together by a dense, uniform, white mycelium of *R. oligosporus* into a firm, compact white cake. In addition, the mycelium permeates the entire cake, enabling it to be lifted out as a whole piece. Besides that, black coloration or patches of spores forming on the surface of tempe samples were not observed. This is a good indication that there was no bacterial contamination.

**Physicochemical Analysis (pH and TSS)**

The pH and TSS of soybean and tempe samples are shown in Table 1. After soaking, there was a significant decrease in pH of soybeans. This could be explained by the early stages of germination, and active pre-fermentation of soybeans due to the presence of lactic acid bacteria. This process creates a slightly acidic condition (pH 5.02), thus making it difficult for pathogenic bacteria to grow (Shurtleff and Aoyagi 2001; Mo et al. 2013). Cooking did not induce any significant changes in pH of soybeans. After fermentation (24 and 48 hours) by *R. oligosporus*, a significant increase in pH value (from pH 5.06–6.72) was observed in tempe A. Similarly, tempe B and C exhibited a significant increase in pH after fermentation. This observation is in agreement with previous studies reporting a gradual increase in pH of velvet bean tempe (Egounlety 2003) and soybean tempe (Mo et al. 2013) during fungal fermentation. Moreover, tempe fermented with *R. oligosporus* is at its peak of quality when the pH value was in the range of 6.3–6.8 (Shurtleff and Aoyagi 2001; Omosebi and Otunola 2013). The results also indicated that different packaging did not affect the pH value of tempe. After soaking and cooking, there was no significant change in TSS of soybeans. In contrast, fermentation (24
et al. Therefore, contributing to the TSS value. According to Iljas et al. (2003), fermentation that will convert carbohydrate to simple sugars, this could be attributed to the enzymatic reactions during fermentation. A significant increase in TSS (from 1 to 19Brix) was reported on velvet bean tempe fermented by fungal cultures, where the TSS of tempe A, B, and C (from 3.5 to 8.2–8.5Brix). This indicates that different packaging maintained the quality of tempe.

**Antioxidant Activity (Total Polyphenol Content, Total Flavonoid Content, DPPH Radical Scavenging Assay, Reducing Power Assay and Total Antioxidant Capacity)**

The antioxidant activity of soybean and tempe samples are shown in Table 2. Fermented foods, especially soybean tempe, contain numerous bioactive compounds that contribute to their antioxidant activity. Since most natural antioxidants are multifunctional, thus more than one method is necessary to measure antioxidant properties according to their ability to chelate metal ions and scavenge specific radicals (Martinez et al. 2012).

The Folin-Ciocalteu assay is based on the detection of phenolic compounds by reduction of the reagent, which contains tungsten and molybdenum oxides (Waterhouse 2002). On the other hand, aluminum chloride colorimetric assay is used for the determination of flavonoids by measuring the formation of acid labile complexes. The DPPH assay measures the ability of antioxidants to scavenge the stable free radical DPPH (Shon et al. 2003). Besides that, antioxidant activity may be evaluated by reductive capacity by Fe3+ to Fe2+ transformation (Rama Prabha and Vasantha 2011). The phosphomolybdenum method or also known as total antioxidant capacity assay is evaluated by the reduction of Mo (VI) to Mo (V) in an acidic medium (Prieto et al. 1999).

In this study, significant reduction in total polyphenols (15–27%) and total flavonoids (29–56%) were observed in soybean samples after soaking and cooking. The highest decrease (27%) was from 42.58 mg GAE/100 g to 31.22 mg GAE/100 g in soaked soybean sample for phenolic and 48 hours) by *R. oligosporus* induced a significant increase in TSS of tempe A, B, and C (from 3.5 to 8.2–8.5Brix). This is consistent with the study conducted by Egounlezy (2003) on velvet bean tempe fermented by fungal cultures, where significant increase in TSS (from 1 to 19Brix) was reported. This could be attributed to the enzymatic reactions during fermentation that will convert carbohydrate to simple sugars, therefore contributing to the TSS value. According to Iljas et al. (1973), the increase in TSS clarifies the higher digestibility of tempe compared with cooked soybeans. In addition, no significant modification in TSS was observed in tempe samples A, B, and C, thus indicating that different packaging maintained the quality of tempe.

<table>
<thead>
<tr>
<th>TABLE 1. EFFECTS OF PROCESSING AND PACKAGING ON PHYSICOCHEMICAL ANALYSIS (pH AND TOTAL SOLUBLE SOLIDS) OF SOYBEAN AND TEMPE SAMPLES</th>
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<tbody>
<tr>
<td><strong>Samples</strong></td>
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<td>Soy bean</td>
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<tr>
<td>Raw</td>
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<tr>
<td>Soaked</td>
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<tr>
<td>Cooked (unfermented)</td>
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<tr>
<td>Tempe A</td>
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<td>Fermented 24 hour</td>
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<td>Fermented 48 hour</td>
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<td>Tempe B</td>
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<td>Fermented 24 hour</td>
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<td>Fermented 48 hour</td>
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<td>Tempe C</td>
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<td>Fermented 24 hour</td>
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<td>Fermented 48 hour</td>
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Values followed by different letters within the same column are significantly different (p < 0.05) (n = 9).

Tempe A (packed using brown food grade wrapping paper); Tempe B (packed using PE bags and heat sealed); Tempe C (packed using LDPE cling wrap).

<table>
<thead>
<tr>
<th>TABLE 2. EFFECTS OF PROCESSING AND PACKAGING ON ANTIOXIDANT ACTIVITY OF SOYBEAN AND TEMPE SAMPLES</th>
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<tbody>
<tr>
<td><strong>Samples</strong></td>
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</table>

Values followed by different letters within the same column are significantly different (p < 0.05) (n = 9).

GAE, gallic acid equivalent; CE, catechin equivalent; AAE, ascorbic acid equivalent.

Tempe A (packed using brown food grade wrapping paper); Tempe B (packed using PE bags and heat sealed); Tempe C (packed using LDPE cling wrap).
Correlation is significant at the 0.01 level.

Total antioxidant capacity 0.970* 0.951* 0.913* 0.902* 1
Reducing power 0.918* 0.903* 0.804* 1
DPPH assay 0.922* 0.858* 1
Flavonoid content 0.964* 1
Polyphenol content 1

Assays Polyphenol content Flavonoid content DPPH assay Reducing power Total antioxidant capacity
Polyphenol content 1
Flavonoid content 0.964* 1
DPPH assay 0.922* 0.858* 1
Reducing power 0.918* 0.903* 0.804* 1
Total antioxidant capacity 0.970* 0.951* 0.913* 0.902* 1

**Correlation is significant at the 0.01 level.

TABLE 3. PEARSON’S CORRELATION COEFFICIENTS BETWEEN POLYPHENOL CONTENT, FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY MEASURED BY DIFFERENT ASSAYS (DPPH, REDUCING POWER AND TOTAL ANTIOXIDANT CAPACITY)

After fermentation (24 and 48 hours) by R. oligosporus, significant enhancement in extraction yield of polyphenols (from 31.2 to 63.1–65.3 mg GAE/100 g) and flavonoids (from 3.5 to 63.1–65.3 mg CE/100 g) was observed in tempe samples A, B and C. Likewise, significant increase in polyphenol content of fermented common bean was reported by Reyes-Bastidas et al. (2010). During fungal fermentation, β-glucosidase is produced, thus catalyzing the release of aglycones from the bean substrate and contributing to the increase in polyphenol content (Reyes-Bastidas et al. 2010; Mona Rashad et al. 2011).

Soaking and cooking induced a significant decrease in antioxidant activities (DPPH radical scavenging activity, reducing power and total antioxidant capacity) of soybean samples. As explained previously, the leaching of polyphenols and flavonoids into the soaking and boiling water, may be responsible for the reduction of antioxidant activity (Boateng et al. 2008; Haron and Raob 2014).

After fermentation (24 and 48 hours), significant enhancement in DPPH inhibition, reducing power and total antioxidant capacity was observed in tempe samples A, B and C. Results obtained are in agreement with previous studies that reported increased antioxidant activity in fermented foods, such as yellow and black soybeans (Xu and Chang 2008), soybean curd (Mona Rashad et al. 2011), and common bean flour (Reyes-Bastidas et al. 2010). This is mainly due to the antioxidant peptides of starter organism, aglycones and isoflavone derivatives generated during fermentation, thus accounting for the enhanced antioxidant activity. In addition, the increase in reducing power could be attributed to the formation of reductants that react with radicals by donating hydrogen during fermentation (Yang et al. 2000; Mona Rashad et al. 2011). The materials used for packaging tempe A, B and C did not induce any significant alteration of antioxidant activities in temple samples, thus indicating that different packaging maintained the nutritional quality of tempe.

The correlation between polyphenol content, flavonoid content, and antioxidant assays (DPPH, reducing power and total antioxidant capacity) are shown in Table 3. Total polyphenols and total flavonoids exhibited a strong positive correlation ($P < 0.01$) with the antioxidant assays studied. Polyphenol content strongly correlated with total antioxidant capacity ($R^2 = 0.970$) and DPPH ($R^2 = 0.922$), while flavonoid content strongly correlated with RPA ($R^2 = 0.903$) and total antioxidant capacity ($R^2 = 0.951$). This is a clear indication that phenolic compounds (including flavonoids) are the major contributor of antioxidant activity, in terms of reducing capacity and radical scavenging ability. The correlation between phenolic content and antioxidant activity has been previously reported (Gorinstein et al. 2004; Rama Prabha and Vasantha 2011). Overall, the results of total antioxidant capacity correlated well with those obtained by other antioxidant assays ($P < 0.01$), therefore it serves as a good estimate of antioxidant capacity in soybean and tempe samples.

**Microbial Analysis (Coliform Count, Aerobic Plate Count, Lactic Acid Bacteria, Total Yeast and Mould Count)**

The microbial analysis of soybean and tempe samples are shown in Table 4. In this study, aerobic bacteria, yeast and mould counts in raw soybeans were 5.21 log CFU/g and 3.21 log CFU/g, respectively. In contrast, lactic acid bacteria was below detection limit (< 1 log CFU/g) in raw soybeans. Coliform count was not detected in all soybean and tempe samples. The absence of coliform indicates that the samples were free from fecal contamination. Hence, the microbial status of soybean and tempe samples conform to the accepted standards.
standard and can be classified as safe (Omoregie and Otunola 2013; Oyeniyi et al. 2014).

After soaking, there was a significant decrease (13%) in aerobic plate count but the growth of lactic acid bacteria increased significantly. This is mainly due to pre-fermentation of soybeans by lactic acid bacteria. Pre-fermentation lowers pH of soybeans (as discussed earlier) making it unfavorable to spoilage bacteria and bacterial pathogens, thereby extending its shelf-life (Shurtleff and Aoyagi 2001; Mo et al. 2013). In addition, the abundant growth of lactic acid bacteria is known to produce vitamin B during fermentation (Omoregie and Otunola 2013), thus being an added advantage in tempe.

Aerobic bacteria, lactic acid bacteria, yeast and mould counts in soybeans were significantly reduced after cooking. This could be explained by the high temperature used during cooking, thus causing adverse effects on microbes by denaturing essential proteins, eventually reducing microbial count. In addition, cooking also stops pre-fermentation, therefore decreasing the growth of lactic acid bacteria (Shurtleff and Aoyagi 2001).

After fermentation (24 and 48 hours) by *R. oligosporus*, significant increase in aerobic bacteria (1.55–1.81 log CFU/g), and total yeast and mould counts (1.89–2.74 log CFU/g) in tempe A, B and C were observed. In contrast, there was no significant change in the growth of lactic acid bacteria. The results of aerobic plate count agrees with pH values of tempe A, B and C (Table 1), whereby the increase in pH (from 5.06 to 6.67–6.75) denoted favorable condition for the growth of aerobic bacteria in tempe samples (Oyeniyi et al. 2014). Besides that, the increase in total yeast and mould count is mainly due to the growth of *R. oligosporus* to facilitate tempe fermentation, as reported by Omoregie and Otunola (2013). In addition, no significant modification in microbial count was observed in tempe samples A, B and C, thus indicating that different packaging maintained the microbiological quality of tempe.

**Shelf Life Study**

Changes in microbial counts (aerobic bacteria, yeast and mould) in tempe A, B and C stored at room temperature (25 ± 1°C) for 1 week are shown in Figs. 3 and 4. Shelf life of tempe samples were evaluated according to the Public Health Laboratory Service, PHLS (2000). Generally, there is no guideline for acceptable microbial limit for fermented

### TABLE 4. EFFECTS OF PROCESSING AND PACKAGING ON MICROBIAL ANALYSIS OF SOYBEAN AND TEMPE SAMPLES

<table>
<thead>
<tr>
<th>Samples</th>
<th>Coliform count (log CFU/g)</th>
<th>Aerobic plate count (log CFU/g)</th>
<th>Lactic acid bacteria (log CFU/g)</th>
<th>Yeast and mould count (log CFU/g)</th>
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<tr>
<td>Soy bean</td>
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<td>Raw</td>
<td>ND a</td>
<td>5.21 ± 0.05 a</td>
<td>ND a</td>
<td>3.21 ± 0.05 a</td>
</tr>
<tr>
<td>Soaked</td>
<td>ND a</td>
<td>4.52 ± 0.03 b</td>
<td>5.37 ± 0.05 b</td>
<td>3.18 ± 0.04 a</td>
</tr>
<tr>
<td>Cooked</td>
<td>ND a</td>
<td>3.89 ± 0.06 c</td>
<td>4.60 ± 0.03 c</td>
<td>2.61 ± 0.02 b</td>
</tr>
<tr>
<td>Tempe A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fermented 24 hour</td>
<td>ND a</td>
<td>5.44 ± 0.02 d</td>
<td>4.31 ± 0.06 d</td>
<td>4.50 ± 0.04 c</td>
</tr>
<tr>
<td>Fermented 48 hour</td>
<td>ND a</td>
<td>5.68 ± 0.05 e</td>
<td>4.38 ± 0.04 d</td>
<td>5.35 ± 0.06 d</td>
</tr>
<tr>
<td>Tempe B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fermented 24 hour</td>
<td>ND a</td>
<td>5.53 ± 0.07 d</td>
<td>4.46 ± 0.05 d</td>
<td>4.65 ± 0.05 d</td>
</tr>
<tr>
<td>Fermented 48 hour</td>
<td>ND a</td>
<td>5.74 ± 0.05 e</td>
<td>4.39 ± 0.05 d</td>
<td>5.30 ± 0.02 d</td>
</tr>
<tr>
<td>Tempe C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fermented 24 hour</td>
<td>ND a</td>
<td>5.49 ± 0.02 d</td>
<td>4.38 ± 0.08 d</td>
<td>4.62 ± 0.05 c</td>
</tr>
<tr>
<td>Fermented 48 hour</td>
<td>ND a</td>
<td>5.70 ± 0.05 e</td>
<td>4.42 ± 0.03 d</td>
<td>5.29 ± 0.03 d</td>
</tr>
</tbody>
</table>

Values followed by different letters within the same column are significantly different (p < 0.05) (n = 9).

ND, not detected; CFU, colony-forming unit.

Tempe A (packed using brown food grade wrapping paper); Tempe B (packed using PE bags and heat sealed); Tempe C (packed using LDPE cling wrap).

![FIG. 3. CHANGES IN AEROBIC PLATE COUNT OF TEMPE SAMPLES DURING STORAGE AT 25°C.](image)
food. However, acceptability is based on appearance, smell, texture, and the levels or absence of indicator organisms. The acceptable maximum microbial load was selected according to preliminary studies on tempe processing to achieve quality retention and avoid microbial spoilage. Hence, the acceptable maximum microbial load including aerobic bacteria, and total yeast and mould in tempe samples is about 7 log CFU/g. The rate of microbial growth observed in tempe C was higher than tempe A and B samples during 1 week storage.

The shelf life of fresh tempe stored at room temperature is 2 days (Shurtleff and Aoyagi 2001; Babu et al. 2009). The shelf life of tempe sample, A and B (5 days) was longer than tempe C (3 days), in terms of microbial load (aerobic plate count, and yeast and mould count) limit. Hence, the shelf life of tempe samples A and B (packed using food grade wrapping paper or perforated PE bags) stored at 25 ± 1°C was extended for 3 days longer than fresh tempe. Besides that, the shelf life of tempe samples C (packed using perforated LDPE cling wrap) stored at 25 ± 1°C was extended for 1 days longer than fresh tempe. Tempe packaging plays an important role in extending the shelf-life of the product. Hence, appropriate packaging provides an optimum oxygen supply and controls temperature for fermentation to occur (Haron and Raob 2014).

CONCLUSIONS

Soaking significantly increased the growth of lactic acid bacteria, which lowers the pH of soybeans. Cooking was effective in reducing the microbial count in soybeans samples. After fermentation (48 hours) by R. oligosporus, tempe samples exhibited significant improvement in physicochemical properties, antioxidant activities and extractability of polyphenols and flavonoids, when compared with unfermented soybeans. The materials used for packaging did not induce any significant alteration in physicochemical and antioxidant properties of tempe samples. Food grade wrapping paper and perforated PE bags were selected as suitable materials for packaging tempe, as the shelf life was extended for 3 days longer than fresh tempe (stored at 25 ± 1°C). The fermentation (48 hours) of soybean by R. oligosporus may have potential application in improving its bioavailable nutraceutical and nutritional properties. In addition, the increased level of antioxidant activities in tempe may reduce the need for fortification during preparation of soybean products. Further research work is required to characterize the chemical compositions that contribute to the antioxidant activity of soybean tempe.

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