The effect of fermented soybean (tempeh) supplementation among active pulmonary tuberculosis patients with standard therapy in Indonesia

Dissertation submitted to the Faculty of Agricultural, Nutritional Sciences and Environmental Management, Justus-Liebig-University Giessen, Germany for the degree of Dr. oec. troph.

by
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Born in Surabaya, Indonesia

Gießen, January 2016
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*The thesis is structured in the form of a general introduction (1) and discussion (4 - 6) whilst the specific methods and results are described and also discussed in two chapters (2, 3) in the form of two manuscripts being submitted for publication.*

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<th>Description</th>
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<tbody>
<tr>
<td>6MWT</td>
<td>6-minute walk test</td>
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<tr>
<td>6MWORK</td>
<td>6-minute distance body weight product</td>
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<tr>
<td>6MWD</td>
<td>6-minute walk distance</td>
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<tr>
<td>ALA</td>
<td>Alpha linoleic acid</td>
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<tr>
<td>ATS</td>
<td>American thoracic society</td>
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<tr>
<td>BHT</td>
<td>Butylated hydroxyl toluene</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>C3</td>
<td>Complement C3</td>
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<tr>
<td>CAE</td>
<td>Catechin equivalent</td>
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<tr>
<td>CHD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>CVD</td>
<td>Coronary heart disease</td>
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<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>DNA</td>
<td>Deoxyribo nucleic acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ETEC</td>
<td>Enterotoxigenic escherichia coli</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>F–C</td>
<td>Folin–Ciocalteu’s</td>
</tr>
<tr>
<td>FFM</td>
<td>Free fat mass</td>
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<tr>
<td>FRAP</td>
<td>Ferric reducing/antioxidant power</td>
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<tr>
<td>GAE</td>
<td>Gallic acid equivalent</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycosylated hemoglobin</td>
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<tr>
<td>HCL</td>
<td>Hydrogen chloride</td>
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<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
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<tr>
<td>HHW</td>
<td>Home-Heart-Walk</td>
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<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>ICC</td>
<td>intra-class correlation coefficient</td>
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<tr>
<td>ICP-AES</td>
<td>Inductively coupled plasma-atomic emission spectrometer</td>
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<tr>
<td>IL-1β</td>
<td>Interleukine-1β</td>
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<tr>
<td>IL-6</td>
<td>Interleukine-6</td>
</tr>
<tr>
<td>LA Chemie</td>
<td>Landesanstalt für Landwirtschaftliche Chemie</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density Lipoprotein</td>
</tr>
<tr>
<td>LP</td>
<td>Lipid peroxidation</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharides</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<td>-----------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>MDR-TB</td>
<td>Multidrug-resistant TB</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MTC</td>
<td>Mycobacterium tuberculosis complex</td>
</tr>
<tr>
<td>MUAC</td>
<td>Muscle upper arm circumference</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>PA</td>
<td>Phytic acid</td>
</tr>
<tr>
<td>PGE2</td>
<td>Prostaglandin E2</td>
</tr>
<tr>
<td>PMCC</td>
<td>Pearson product-moment correlation coefficient</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended daily allowance</td>
</tr>
<tr>
<td>RSA</td>
<td>Radical scavenging activity</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
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<tr>
<td>SSF</td>
<td>Solid-state fermentation</td>
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<tr>
<td>SF</td>
<td>Social functioning</td>
</tr>
<tr>
<td>TB</td>
<td>Tuberculosis</td>
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<tr>
<td>TBA</td>
<td>Thiobarbituric acid</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid reactive substances</td>
</tr>
<tr>
<td>TCA</td>
<td>Trichloroacetic acid</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td>TPC</td>
<td>Total phenolic content</td>
</tr>
<tr>
<td>TPTZ</td>
<td>2, 4, 6-Tripyridyl-s-triazine</td>
</tr>
<tr>
<td>UNSCN</td>
<td>United nations standing committee on nutrition</td>
</tr>
<tr>
<td>USFDA</td>
<td>United states food and drug administration</td>
</tr>
<tr>
<td>VAD</td>
<td>Vitamin A deficiency</td>
</tr>
<tr>
<td>WHO</td>
<td>World health organization</td>
</tr>
<tr>
<td>HbA1C</td>
<td>Hemoglobin A1C</td>
</tr>
<tr>
<td>VO₂max</td>
<td>Maximal oxygen consumption</td>
</tr>
<tr>
<td>NF-KB</td>
<td>Nuclear factor kappa-light-chain-enhancer of activated B cells</td>
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1 Introduction of the study

1.1 Background

1.1.1 Etiology and pathogenesis of tuberculosis

Tuberculosis (TB) has become a serious concern all the way through the history of humanity. TB has been known as a wasting disease since centuries ago as described by John Bunyan (1628 -1688) as “The Captain among these men of death” (Lawn et al. 2011). It has been a leading cause of death throughout the world, and still is especially in developing countries. The modern point of view regarding TB is traced back to the work of Robert Heinrich Herman Koch, a German physician and microbiologist. His contribution such as “Koch postulate” is still considered as a standard in modern microbiology. In spite of the first anti tuberculosis drugs were discovered since 1950s (Zumla et al. 2013), tuberculosis recently still killed an estimated 1.5 million people worldwide in the year 2013 (WHO 2014). World Health Organization (2014) reported 510,000 and 80,000 of global estimation number of deaths in the same year due to TB among women and children. South-East Asia and Western Pacific Regions showed the highest rate of TB incidence which was 56% out of estimated 9 million people who developed TB.

TB is a contagious disease that often affects the lungs (pulmonary TB), though it can also attack other organs (extra pulmonary TB). It is caused by an airborne pathogen, acid fast, rod-shaped, and aerobic bacteria, called *Mycobacterium tuberculosis*. The disease spreads through aerosol transmission of droplets containing the bacteria for example by coughing, sneezing, shouting, or singing from infected person to a healthy one (Sasindran et al. 2011; WHO 2014). Mycobacteria that cause TB in human and/or animal are gathered in a genetically related group called *Mycobacterium tuberculosis* complex (MTC). *Mycobacterium tuberculosis* complex consist of *M. Tuberculosis*, *M. bovis*, *M. africanum*, *M. canetti*, *M. microti*. Among all members of the complex, *M. tuberculosis* is the most important cause of TB worldwide (Brosch et al. 2002; Huard et al. 2003).

Once reaching the respiratory tract, most of the bacilli are trapped in the superior segments of airways where goblet cells present and secrete the mucus. The mucus traps the bacilli, and then the cilia clear respiratory tract by waving them up for removal (Frieden et al. 2003). Alveolar macrophages engulf the bacteria in droplets that pass through this mucociliary system and reach the alveoli of the lung (Frieden et al. 2003). Macrophages produce proteolytic enzymes and cytokines in an attempt to degrade the
bacteria (Nicod 2007; van Crevel et al. 2002). The majority of these bacilli are eradicated, but a small number of them may survive intracellularly and they are released when macrophages decease. (Frieden et al. 2003). The mycobacteria which survive from phagocytosis attract blood monocytes and inflammatory cells to the lung. These monocytes have ability to differentiate themselves into tissue macrophages. The macrophages engulf once again the bacilli but do not kill them. The mycobacteria multiply inside of the macrophage exponentially. In this phase, the cell-mediated immunity is involved by the arrival and proliferation of antigen-specific lymphocytes in the lesions (van Crevel et al. 2002). It stimulates the macrophages to destroy the bacilli intracellularly. Individuals with adequate immune system, the subsequent defense mechanism is to form granulomas around the *M. tuberculosis* (Rosenkrands et al. 2002). These nodular-type lesions are formed by macrophages and activated T lymphocytes to create conditions that prevent further mycobacteria growth (Frieden et al. 2003; Nicod 2007). The lesions have a caseous necrotic environment which lack of resources for their multiplication (Knechel 2009). Later on, it can be either latent tuberculosis or active progression, known as primary progressive tuberculosis (Frieden et al. 2003). Individuals with ineffective immune systems, the lesions can develop to primary progressive tuberculosis. In immunocompromised condition, the necrotic tissue results in liquefaction and cavity formation (Knechel 2009). TB bacteria can be transported by the lymphatic system from the lesion sites into the tracheobronchial lymph nodes and new granulomas may occur (Dheda et al. 2005). If the discharge drain into a blood vessel, extra pulmonary tuberculosis is more likely occurred (Knechel 2009). For several reasons, the bacilli reactivate in about 10% of the individuals with latent tuberculosis and produce clinically active TB symptoms (Silva Miranda et al. 2012).

### 1.1.2 Standard therapy of tuberculosis

TB treatment uses a combination of antibiotics to kill the bacteria *M. tuberculosis*. The standard therapy for TB differ in comparison with other common infections. The structure and chemical composition of the cell wall of the bacteria is able to inhibit the entry of drugs and causes antibiotics to become ineffective. Since 2010 the recommended therapy for new patients with drug-sensitive TB is a six-month course treatment of first-line drugs combination: isoniazid, rifampicin, ethambutol and pyrazinamide for the first two months (intensive phase). For the next four months,
continuation phase uses rifampicin and isoniazid regimen. WHO global tuberculosis report 2014 showed that this regimen produced success rates up to 85% or more and was also effective in TB patients with HIV co-infected (Maartens et al. 2007; Lawn et al. 2011; WHO 2014). Therapy for multidrug-resistant TB (MDR-TB) needs longer time (20 months), is more expensive and more toxic medications and has higher failure rates. Novel TB combination drugs, including new agents and vaccines are being tested in clinical trials (WHO 2014).

1.1.3 Nutritional status of people with tuberculosis
The relationship between tuberculosis and undernutrition has bidirectional link. Undernutrition predisposes individuals to the development of TB clinical manifestation, and TB contributes to malnutrition (Cegielski et al. 2004). TB makes undernutrition worse and undernutrition weakens immunity, consequently stimulating latent TB to evolve into active disease. In addition, TB is often related to poverty and food insecurity, especially in low-income and middle-income countries (Cegielski et al. 2004; WHO 2013). Studies have described that body mass index (BMI) of active TB patients is lower than healthy people (Karyadi et al. 2000; Kassu et al. 2005; Zachariah et al. 2002).

The recovery of nutritional parameters has been reported as an indicator for effective anti-TB therapy (Onwubalili 1988; Harries et al. 1988). Of these parameters, body weight gain has been proposed as an inexpensive clinical marker of standard TB treatment outcome (Vasantha et al. 2009; Krapp et al. 2008). Related study described that body weight gain of 5% or less was found as one of the factors which correlate with ineffective TB treatment outcomes such as failures and relapses (Khan et al. 2006; Krapp et al. 2008). More recent study also reported that the degree of weight gain during 6 months anti TB therapy was associated with outcomes like death, defaulted, or failed treatment in multidrug-resistant tuberculosis (MDR-TB) patients (Gler et al. 2013). Beside of body weight gain, BMI change during the first of TB treatment has been identified having a strong association with mortality (Benova et al. 2012).

1.1.4 Physical function in tuberculosis
Loss of fat and lean mass (wasting) is a well-known manifestation of active TB. Wasting produces a deterioration on the physical ability of the patients in their daily activities. Physical function and role social functioning (SF) scale scores in active TB patients
were found lower compared to the healthy population (Chamla 2004). On the contrary, higher physical health of SF scores was indicated in patients who completed standard TB therapy than in patients who are in the early phase of treatment (Babikako et al. 2010). Beside of health quality score, handgrip strength in patients starting standard TB therapy was found lower than age and sex matched neighborhood controls (PrayGod et al. 2011). Additionally, a significant decrease was found in regard to maximal oxygen uptake (VO$_2$ max) and 6-minute walking test (6MWT) in people with pulmonary TB compared with an age-matched healthy volunteers (Sivaranjini et al. 2010). Though physical ability could improve after TB standard therapy but body mass repletion has been described predominantly in fat mass instead of lean mass (Schwenk et al. 2004).

1.2 Rationale of the study

1.2.1 The benefit of additional protein intake

Protein intakes above the recommended daily allowance or RDA (0.8 g/kg/day) was suggested to give positive effect for physical ability (Wolfe 2006) and in the several diseases such as metabolic syndrome, osteoporosis, obesity, sarcopenia, and heart disease (Layman 2009). People with common infections were advised to consume 20-25% extra protein of the daily-recommended intake for common infections (Kurpad 2006). These recommendations were in line with the suggestion for critically ill patients with severe underweight. The critical ill patients were suggested to consume more than 1.5 g/kg/day protein, even when adequate energy is provided (Hoffer 2003). A study of nitrogen balance has demonstrated that a protein intake between 1.2 and 1.8 g/kg/day produced a positive nitrogen balance in HIV patients (Selberg et al. 1995). Higher protein intake has been shown to associate positively with age-related loss of skeletal muscle in older adults (Houston et al. 2008). Consumption of 30 g protein has resulted in approximately 50% increase protein synthesis in young and older healthy people (Symons et al. 2009). Higher proportion protein consumption has been suggested also to have positive effects on glycemic regulation, including postprandial glucose, and insulin responses (Layman et al. 2008). Even distribution of protein intake over the day could give more beneficial in protein synthesis than skew pattern protein intake (Mamerow et al. 2014). In the skew pattern protein intake, most adults were considered to consume less than 10 g of protein, especially at breakfast (Layman 2009). Higher amount protein intake than 30 g was found ineffective to increase protein
synthesis when it consumed only once per day (Symons et al. 2009). Distribution of protein intakes over the day was also associated with frailty in older adults (Bollwein et al. 2013).

1.2.2 Soy food as a functional food
Soybean (Glycine max (L.) Merrill) is an essential vegetable belong to the Leguminosae family. The bean is one of protein sources and contains significant amounts of phytoprotectants, which are responsible for its potential health benefits (Setchell 2001). Nuts, soy, and lentils, which are consumed widely, especially in the Asia-Pacific region. They are well known for positive health values because of their favorable fatty acid composition, low glycemic indices, high contents of dietary fiber, folate and vitamin B12, and, especially for soy, high levels of isoflavones (Lukito 2001). High soy diet along with low animal protein consumption are considered as a functional food to lower risks of malignancies such as prostate and breast cancers. Whole soy protein intake was suggested to reduce levels of total cholesterol, low-density lipoproteins, and triglycerides. Moreover, soybean ingestion reliefs menopausal hot flashes, preserves bone density and reduces fractures in postmenopausal women (Cassidy 2003; Michelfelder 2009).

1.2.3 The effect of soy isoflavones and soy food on inflammatory markers
Soy isoflavones are type of sources of phytoestrogens in the diet of the people. Soy isoflavones have been found to down regulate pro-inflammatory cytokines and exert anti-inflammatory activity due to their PPARα/γ agonists properties (Medjakovic et al. 2010). In the animal model, soy isoflavones inhibited inflammation induced by LPS (lipopolysaccharides) and decreased levels of IL-1β, IL-6, NO and PGE2 (Kao et al. 2007). In the human study, soy-isoflavones-enriched food consumption for eight weeks have resulted in reduction of C-reactive protein (CRP) level in postmenopausal women (Hall et al. 2005).

1.2.4 Fermented soybeans and insulin resistance
The increasing level of inflammatory cytokines like IL6 and TNFα in response to M. tuberculosis infection could lead to a rise of insulin resistance and a reduction of insulin secretion (Pickup 2004). This notion is supported by several studies that described high prevalence rates of impaired glucose tolerance test ranged between 10.8 to
18.77% in cases of pulmonary tuberculosis (Ramesh et al. 2013; Jain et al. 2006; Yamagishi et al. 2000; Firsova et al. 2000). Also, glycosylated hemoglobin (HbA1c) level elevated in TB patients during first three months of standard treatment (Tabarsi et al. 2014). On the other hand, various types of fermented soybeans have demonstrated a positive effect on insulin sensitivity. As an example, a Korean fermented soybean (Natto) combined with viscous vegetables decreased peak glucose and insulin concentrations and the incremental areas under the curve for glucose in healthy volunteers (Taniguchi et al. 2008). This positive effect has been supported also by in vitro studies of different types of fermented soybean on insulin sensitivity and insulinotropic activity (Kwon et al. 2011; Yang et al. 2013).

1.2.5 The Indonesian fermented soybean (tempeh)
Tempeh is a traditional fermented soybean product with high nutritional value. It originated from Indonesia (Handoyo et al. 2006). Yellow soybean (Glycine max) and black soya bean (Glycine soya) are two common types to be consumed in Indonesia. Glycine max is common to make tempeh and Glycine soya is usually used to produce a soybean sauce due to its natural black color (Shurtleff et al. 2012). Fermentation improves acceptability, reduces cooking time, and decreases anti-nutrients (Nout et al. 2005). The fermentation period of tempeh is approximately two days. Rhizopus oligosporus strains are commonly used as an inoculum during the fermentation. Tempeh is not only healthy food but it can easily be prepared without any sophisticated utensil, which is suitable for resource-limited households in Indonesia.

1.2.6 Tempeh and human health
Studies performed in Indonesia have described the hypolipidemic properties of tempeh. In several intervention studies, tempeh consumption resulted in reduction of low-density lipoprotein (LDL) and total cholesterol as well as improvement of high-density lipoprotein (HDL) cholesterol (Astuti et al. 2000; Karyadi et al. 1996; Karyadi et al. 1996). Tempeh supplementation in menopause women for 4 weeks could improve the lipid profile, antioxidant enzyme superoxide dismutase (SOD) and decreased malondialdehyde (MDA) level (Utari et al. 2011). Tempeh as a fermented soybean product also contains folate, which is beneficial for cognitive. Tempeh consumption could show a protective effect on cognitive function in middle-aged and elderly participants better than tofu as an unfermented soybean (Hogervorst et al. 2008;
Hogervorst et al. 2011). Children under age of five who were treated using tempeh-based food had a significantly shorter time of diarrhea compared with children fed milk-based food (Mahmud et al., 1985).

1.2.7 Beneficial effects of tempeh on nutritional status
Nutritional intervention studies of under-five children, conducted in Indonesia, have shown preliminary evidences of tempeh’s potential benefits. A food supplement consisting of 70% rice flour and 30% tempeh or soybean flour could provide a beneficial effect on weight gain in children under the age of three (Hermana 1983). Children under the age of five years with poor nutritional status who received 50 grams of tempeh’s powder for six months showed a better weight gain and lower prevalence of diarrhea and anemia compared to milk as a control (Irawati et al. 1994; Muljati et al. 1995). In addition, a significant increase in body weight change has been described in under five children with moderate underweight treated with 50 g tempeh-dates combination biscuit for four weeks compared to placebo biscuit group (Fatmah 2012).

1.3 Objectives
Suggestions for nutritional supplementation for mitigation of undernutrition among TB active patients have been proposed by several authors (Zachariah et al. 2002; Coker et al. 2005; Gupta et al. 2009; Hood 2013). Soy-based supplements have been considered having positive effects among active TB patients under standard therapy with antimicrobial drugs to treat tuberculosis (Mel'nyk et al. 2006). Thus, considering the potential of tempeh as a low cost, locally available food supplement for TB patients in Indonesia, this study aimed to:

- determine the difference between patients with active TB who receive fermented soybean compared to the control group in term of body weight change
- measure the handgrip strength change of the patients supplemented by fermented soybean compared to the control group.
- calculate the 6-minute walk test change of the patients supplemented by fermented soybean compared to the control group.
1.4 Conceptual framework
The conceptual framework of this study is presented in Figure 1. Daily tempeh supplementation is expected to have positive effect in body weight change. Improvement in body weight change might help the TB patients have faster recovery in physical function. Additionally, there are several proposed possible mechanisms that might provide rationales to justify the usage of tempeh in the dietary intervention study. However, this study does not measure all those possible mechanisms.

**Figure 1: conceptual framework of the study**

**Research site: Surabaya, East Java, Indonesia**
The official site of Surabaya is www.surabaya.go.id and it describes Surabaya as a multiethnic city, officially established on 31st May 1293. The city situated in East Java province, Indonesia (Figure 2) and it is the second largest city after the capital city, Jakarta. The regency of Surabaya, with area coverage of 33,306.30 km², consists of 31 districts, which are subdivided into 163 sub districts. The number of registered
residents per 1st March 2015 is 2,869,916 with a population growth of 1.2 % per year.

Surabaya borders with Madura strait (at the east and north side), Gresik district (west side), and Sidoarjo district (south side). Geographically, it is located between 07° 21’ south latitudes and 112° 36’ - 112° 54’ east longitudes. As a city, Surabaya has developed into a center of economic activity in the East Java province. The majority of its populations works of service, industry, and trading sectors, so that agriculture land is hardly to be found. The manufacture and trading industries are the main contributors for Surabaya’s main economic activities. The study site was in an area which covered three adjacent districts namely: kenjeran, semampir and pabean cantian. It was done at one hospital and four public health centers serving as the integrated referral system of health services for active TB patients. The main ethnic groups in these districts are Javanese and Madurese.

![Figure 2: Map of Surabaya, Indonesia](image-url)
2 The influence of traditional stir-frying with oil on acceptability, antioxidant activities, nutrients, and the phytic acid content of fermented soybean (tempeh)

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The manuscript has been accepted by Nutrition and Food Science with ID NFS-09-2015-0105.R1

2.1 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% of crude proteins, 15.5 to 24.7% of lipids and 31.7 to 31.85% 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%) as well as that found in cereals (8 to 15%). 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 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 like prevention and treatment for various cancers (Fox et al. 2002).

Soybean possesses 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 et al. 2006). The fermentation period of tempeh is shorter (approximately two days). The fermentation process for tempeh comprises of 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 et al. 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, improves 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 of 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.

2.2 Materials and Methods

2.2.1 Materials and reagents

Soybeans [Glycine max (L.) Merr] were purchased from a local Asian market in Giessen, Hessen, Germany. The tempeh starter containing “Rhizopus oligosporus and rice flour”, for solid state of fermentation (SSF) 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 (AFI), Bandung, Indonesia. All reagents used were of analytical grade.

2.2.2 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 minutes and, after discarding the water, they were de-hulled by hand until approximately 90% were freed from their skin. The soybeans were left to soak overnight in fresh tap water at room temperature for 12 hours with the water level being 5 cm over the beans. The second boiling of the soybeans was done at 100°C for 30 minutes and the water drained. The soybeans were then laid uniformly on a clean cloth to cool for 30 minutes in order to provide a suitable temperature for \textit{R. oligosporus} inoculum. Five mL of cooking vinegar (10\% 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 \textit{R. oligosporus} and stirred gently to distribute the tempeh uniformly. Tempeh starter absorption required 20 minutes 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 hours respectively. Finally, the fermented soybeans were refrigerated for 24 hours at 4°C.

2.2.3 Sensory evaluation
The tempeh samples were processed using traditional methods namely: stir-fried, steamed, boiled, dried, and uncooked. The samples were prepared 2 hours 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 minutes with the 200 mL boiling tap water. Boiled tempeh was processed by boiling in a pot at 100°C for 10 minutes 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. 8135) 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 2x2x2 cm³. 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 4-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 to the uncooked variety.

2.2.4 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) for 15 min, followed by centrifugation (Hettich Mikro 22R, Type 1110, Germany) using 5000 x g for 20 minutes at 4 °C. After carefully discarding the n-hexane, the defatted samples were extracted with methanol acidified with 1% conc. HCL, then repeat the sequential processes vortex, roller mixer, centrifugation as aforementioned. Final pool after three consecutive extractions was stored at 4 °C until further analysis of total phenolic content and AO activity.

2.2.5 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, 2000). Carbohydrate content was estimated by the difference method. Iron and zinc levels were assessed 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).
2.2.6 Determination of total phenolic content

Total Phenolic Content (TPC) was determined using Folin Ciocalteu’s method (Singleton et al. 1965). 0.1 mL extract and 0.5 mL Folin Ciocalteu’s reagent were mixed with pure H₂O (1:1) and placed in a tube, vortexed and allowed to stand for 8 minutes. Then 4.5 mL of 2% sodium carbonate solution was added, vortexed and incubated in a dark room for 1 hour 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 et al. 1965).

2.2.7 Ferric reducing ability of plasma assay

Ferric 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 prepared FRAP reagent contained 3.5 mL of 20 mmol/l TPTZ solution in 40 mmol/L HCL plus 3.5 mL of 20 mmol/L FeCl₃·6H₂O and 35 mL of 0.3M acetate buffer, pH 3.6. Later on the FRAP reagent was incubated at 37 ºC for 30 minutes. Then 1800 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 ºC for 30 minutes 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 to 2000 µm/L (FeSO₄·7H₂O) was used for the preparation of the standard calibration curve. BHT was used for the positive control. Finally, the concentration of each extract having ferric-TPTZ reducing ability was expressed in µmol/g (Benzie et al. 1996; Pulido et al. 2000).

2.2.8 Thiobarbituric acid reactive substances assay

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% linoleic acid and 1% 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 ºC. To this 1 mL mixture, 2 mL of TBA reagent (100 mL of stock TCA-TBA-HCL solution containing 15% TCA, 0.375 g TBA, 3 mL of 2% BHT in ethanol and final volume with 0.25M HCL) was added and vortexed thoroughly. The mixture was
placed in boiling water for 10 minutes. The mixture was then centrifuged at 6000 x g for about 15 minutes. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The rate of inhibition of thiobarbituric acid reactive substances 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).

2.2.9 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. 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 minutes. Later they placed on a roller for about 20 minutes. Once done, they were centrifuged (Hetetic Mikro 22R, Type 1110, Germany) at 16,000 rpm for 15 minutes at 4 ºC. 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 5415-D) at 13,000 x g for 20 minutes at 4 ºC. After collecting the supernatant, they were filtered using cellulose filters of 0.4 µm. Finally obtained clear filtered samples were stored at 4 ºC prior to the HPLC analysis (Kwanyuen et al. 2005). Chromatographic analysis was performed on an HPLC system with a 50 x 4.6 mm PL-SAX 1000A (particle size 5 µm) strong anion-exchange column (Agilent Technologies) equipped with a 20 x 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 KNO₃ 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% (wt/vol) FeCl₃ and 0.15% (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%) of known concentration ranging from 0.21 to 1.7 mg/mL was used for the preparation of the standard calibration curve.
2.2.10 Statistical analyses

The study results were expressed as means and 95% 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 twenty-eight 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 the 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.).

2.3 Results

The sensory evaluation of different characteristics from traditional cooking methods is given in Table 1. The hedonic scale means of stir-fried tempeh were statistically superior, especially in terms of its aroma and mouthfeel compared to 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.

Table 1: The scale means for tempeh samples with different conventional preparations*

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

*UNIANOVA followed by Sidak post-hoc test
Superscript letters (a-c) in the same row indicate a significantly different ($p < 0.05$).
Figure 3 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.

Figure 3: Estimated marginal means of the different conventional cooking methods among healthy volunteers. Letters (a-d) indicate statically significant different ($p < 0.05$).

Table 2: Macronutrients composition (dry weight basis), iron and zinc levels in uncooked and stir-fried tempeh samples.

<table>
<thead>
<tr>
<th></th>
<th>Uncooked tempeh</th>
<th>Stir-fried tempeh</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean 95% CI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein, %</td>
<td>41.55 41.35 41.75</td>
<td>42.06 41.86 42.27</td>
<td>0.015</td>
</tr>
<tr>
<td>Fat, %</td>
<td>25.12 25.03 25.21</td>
<td>34.48 34.39 34.57</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Carbohydrate, %</td>
<td>33.33 33.09 33.57</td>
<td>23.46 23.22 23.69</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fe, mg/kg</td>
<td>22.03 20.00 24.07</td>
<td>18.60 16.57 20.63</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Zn, mg/kg</td>
<td>16.33 15.86 16.81</td>
<td>13.97 13.49 14.44</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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

The total phenolic contents of both uncooked tempeh samples were higher than stir-fried tempeh samples (Table 3). Uncooked tempeh with a three-day fermentation period (6.40 95% CI 5.84, 6.95 mg CAE/g dry weight) was not statistically different
from uncooked tempeh which was fermented for a two-day period (6.22 95% CI 5.89, 6.56 mg CAE/g dry weight). The total phenolic content of stir-fried tempeh fermented for two days (4.50 95% CI 3.71, 5.28 mg CAE/g dry weight) and three days (4.22 95% CI 3.41, 5.04 mg CAE/g dry weight) were not statistically different.

Table 3: Total phenolic content, FRAP assay, TBARS assay and the phytic acid concentration of methanolic extracts of uncooked and stir-fried tempeh samples

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

*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

Uncooked tempeh with a three-day fermentation period (30.07 95% CI 26.85, 33.29 µmol FeSO₄/g) demonstrated higher FRAP assay value among the samples, but lower compared to BHT as a positive control (Table 3). Uncooked tempeh fermented for two days (25.13 95% CI 21.87, 28.39 µmol FeSO₄/g), stir-fried tempeh fermented for two days (22.36 95% CI 19.91, 24.81 µmol FeSO₄/g) and stir-fried tempeh fermented for three days (24.36 95% CI 22.87, 25.85 µmol FeSO₄/g) were not significantly different. Stir-fried tempeh through a two-day fermentation period (0.57 95% CI 0.49, 0.65 µM MDA Equi/100g) and a three-day fermentation period (0.51 95% 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% CI 0.64, 0.73 µM MDA Equi/100g) in TBARS assay (Table 3). However, stir-frying tempeh with a two-day fermentation period revealed a lower MDA level, in contrast with uncooked tempeh for a two-day fermentation period (0.80 95% 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 3). PA concentration of both stir-fried tempeh fermented for two days (8.20 95% CI 7.57, 8.83 mg/g dry weight) and three days (7.31 95% 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% CI 10.82, 15.42 mg/g dry weight) and three days (12.42 95% CI 10.38, 14.46 mg/g dry weight).

2.4 Discussion

2.4.1 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% 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% acceptability (Aderibigbe et al. 2006). Also, a related study from Indonesia, the panelists showed higher acceptance of fried tempeh made from black soybean (Glycine soja) compared to other traditional methods (Nurhidajah et al. 2009).

2.4.2 Nutrient composition

In the present study, crude protein composition of uncooked tempeh comprised 41.55% 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% (Murata et al. 1967). The current study also revealed that the composition of tempeh’s crude protein increased slightly after stir-frying. Probably, this small different could not justify the increase of protein composition in tempeh samples. It might because of operational techniques during this study. However, different studies have also shown that the protein composition increased after frying in fillets 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 et al. 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% 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). Besides, storage prior stir-frying could lead also to the decrease in the carbohydrates, due to the action of variety of enzymes produced by the Rhizopus oligosporus during fermentation. Later on, the mold would have utilized 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% 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 et al. 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% respectively, compared to those in uncooked tempeh samples. Since 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 et al. 1998).

2.4.3 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 minutes caused a 45% reduction of total isoflavones in tempeh (Haron et al. 2009). Similar results in the total phenolic content reduction due to traditional frying have been presented for tomatoes and onions (Crozier et al. 1997; Price et al. 1997).

Stir-frying decreased the ferric reducing/antioxidant power (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 ferric reducing ability of plasma antioxidant activity (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 antioxidant activity can be decreased (Shahidi, 2015). Tempeh samples with three-day fermentation period decreased more reducing power compared with the two-day fermentation period varieties. This might be due to frying resulted in a higher reduction of polyphenols and flavonoids especially among uncooked samples with higher antioxidant activity (Segev et al., 2012).

Contrary to the results obtained through FRAP, TBARS assay revealed that the application of the stir-frying process to two-day fermented tempeh samples 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).

2.4.4 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% reduction in PA concentration (Sutardi et al. 1985a). Similar to the present study, stir-frying also reduced PA concentration in tempeh. There were 37% and 41% reduction in PA
concentration of uncooked tempeh with a two-day fermentation and a three-day fermentation after stir-frying respectively.

2.5 Conclusion
Tempeh was found not only to be a good source of nutrients, but also other important health components such as antioxidant. 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.

Acknowledgement
The authors greatly acknowledge the support from Dr. Herrmann who contributed with statistical analysis advices.

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.

2.6 References
References are presented for the whole thesis at the end of the thesis.
3 The effect of fermented soybean (tempeh) supplementation among active pulmonary tuberculosis patients with standard therapy in Indonesia

3.1 Introduction
Tuberculosis (TB) is still highly prevalent among infectious diseases and considered a major global public health problem, causing high rates of morbidity and mortality in developing countries. According to WHO (2014) the estimation of global TB incidence in the year 2013 was 9.0 million, which was equivalent to 126 cases per 100,000 people. The number of mortality was estimated to be 1.5 million, equivalent to 16 deaths per 100,000 people. Most of the incidence occurred in Asia (56%) and Africa (29%). Indonesia ranked fifth in the estimation of TB incidence after India, China, Nigeria, and Pakistan with an estimated number of 460,000. Wasting is considered as one of the common features of TB (Zachariah et al. 2002). It reflects the loss of fat and lean mass and may persist for months, even after the introduction of TB standard therapy (Onwubalili 1988). There is evidence for a strong and consistent log-linear relationship between TB incidence and low BMI (weight (kg)/height (m)^2) across various settings especially within 18.5-30 BMI range (Lonnroth et al. 2010). TB is also considered as a stronger leading factor in wasting than HIV in patients with coinfection (Paton et al. 2006). Wasting is associated with TB due to a combination of several factors, such as decreased appetite, nutrient malabsorption, altered metabolism related to inflammation, and immune response (Gupta et al. 2009). In addition, the elevation of proinflammatory cytokines with lipolytic and proteolytic activities may increase energy expenditure of the patient. A reduction in lean body mass due to wasting can cause impairment in physical function, such as decreasing the ability to carry out daily activities in patients with tuberculosis (Paton et al. 2006; Villamor et al. 2006). Decreased muscle mass and weight loss are commonly associated with fatigue and physical inactivity (Poulsen 2012). Impairment of TB patient’s physical function might play an important role, especially in countries where many people are unable to have long leave from work. Therefore, faster restoration of physical function might help to shorten the convalescent period and facilitate earlier return to productive work (Paton et al. 2004). Beside body weight, the severe deficit of handgrip has been found in TB patients starting standard therapy (PrayGod et al. 2011). Handgrip strength is an assessment
that has been used by occupational therapists in a range of clinical settings, particularly hand therapy and occupational rehabilitation. It is fast, easy to perform, reliable, and produces a result that can be used as a measurement of performance (Innes 1999). Handgrip strength is a proxy measure of physical health and muscle function; therefore, its increase may reflect physical function improvement (Ramlagan et al. 2014). Increase handgrip strength may lead to improved ability to produce or procure foods, and hence patients may be able to care for their families better.

The 6-minute walk test (6MWT) has been known as an approach to objectively evaluate functional capacity in patients (Pollentier et al. 2010). The 6-minute walk test is easy to manage, well tolerated, and reflects activities of daily living better than other walking tests (Solway et al. 2001). Most activities of daily living are more likely performed at submaximal levels of exertion so that the 6MWT may better reflect the physical functional level of daily physical activities (ATS 2002). The 6MWT has also been already applied to measure functional ability of TB patients (Adedoyin et al. 2010; Di Naso et al. 2011). Other tools also have been used to assess functional ability in TB patients, such as timed stand test, quality of life-scale questionnaire, St George’s respiratory questionnaire, and forced expiratory volume in 1 second (Jahnavi et al. 2010; Paton et al. 2004; Ralph et al. 2013).

Supplementation of macronutrients among active TB patients is considered to produce an increase in weight gain and quality of life (Sinclair et al. 2011). The required additional intake of macronutrients for a patient can be achieved by following nutritional advice. However, especially in low and middle-income countries, TB patients may not be able to acquire the daily requirement of food. This inability can be caused by local food insecurity and / or due to economic hardship through illness and loss of work. Besides, active TB patients are more likely to be associated with lower socioeconomic status, particularly in Asia and the Pacific (J. Wu et al. 2012).

Soy based supplements might have positive effects among TB patients under standard therapy with antimicrobial drugs to treat tuberculosis (Mel’nyk et al. 2006). Tempeh is a fermented soybean product, originally made by central Javanese people in Indonesia. *Glycine max* is a species of legume used to make tempeh and it is usually fermented by *Rhizopus oligosporus* as a starter. The fermentation period is rapid, taking only two days to be completed. The tropical climate characteristic of Indonesia also facilitates tempeh fermentation, allowing incubation at room temperature without consumption of energy or heating. The production of tempeh requires only simple or low-level technology, no machines are necessary, and production costs are low.
(Shurtleff et al. 1979). Tempeh is easy to prepare for consumption and requires only several minutes of cooking.

Various macronutrients supplements have been applied among adult TB patients in trials such as cholesterol-rich foods (Pérez-Guzmán et al. 2005), locally available foods (Jahnavi et al. 2010; Martins et al. 2009; Sudarsanam et al. 2011), energy-protein biscuit (PrayGod et al. 2012), high-energy oral nutritional supplement (Paton et al. 2004), and soy protein extract powder (Taslim 2004). A previous study using tempeh-dates biscuit for supplementation to under-five children with TB demonstrated no evidence of anthropometric improvement (Fatmah 2013). It could be due to the high dropout rate and resulted in small sample size in the end of the study. However, to the extent of our knowledge, the randomized efficacy study of cooked tempeh without any combination as a supplement for adult active pulmonary TB patients has not been established.

Thus, considering the potential of tempeh as a low cost, locally available food supplement for TB patients in Indonesia, this study aimed to analyze the efficacy of tempeh supplementation on the changes of body weight and physical function among newly diagnosed active pulmonary TB patients with standard therapy.

3.2 Materials and methods

3.2.1 Location of the study

This study was carried out at the outpatient department building, Surabaya Lung Hospital, Indonesia. As a national health referral system in TB program, the hospital was related to four local sub district health centers that were involved in the recruitment of participants in this study. Tempeh was prepared at local household in Surabaya. Ethical clearances were obtained from the committee on health research ethics JLU Giessen, Germany (research project no. 147/13) and Wijaya Kusuma University Surabaya ethic committee (no.13/SLE/FK/UWKS/III/2013). The protocol of this study has been presented and approved by the medical committee of Surabaya Lung Hospital prior the enrollment. Later on, the hospital issued a formal confirmation letter after the study has been completed (no. 070/419.03/101.13/2015). This study has been publicly registered in the ClinicalTrials.gov site with identifier: NCT02554318.

3.2.2 Sample size

Windows version G*Power 3.1.5 software was used to calculate the sample size for the intervention study (Faul et al. 2007). A minimum sample size of 64 patients per
group was determined to detect a mean difference in body weight change of $\geq 1.1$ kg between groups. In a previous trial, it was found that the pooled standard deviation of body weight change in the first 3 months was 2.2 kg (Jahnavi et al. 2010). The design set a confidence level of 95%, a medium effect size of $d = 0.5$ (Cohen 1992), and a power of 0.8.

3.2.3 Randomization
The study was designed as a randomized, controlled dietary intervention trial. The patients were divided into two groups, namely the intervention and control group. Both groups received TB standard therapy. In addition, the intervention group received tempeh as daily supplement for two months. The randomization procedure was carried out using the site http://www.randomization.com and with permuted blocks of ten. Randomization took place with sealed, unmarked opaque envelopes that are allocated to patients participating in the study. The envelope contained an allocation paper corresponding to the study. To participate in the study, patients had to give a written consent to the investigator. Participation in the study was on a voluntary basis and participants were not coerced to participate in the study and were allowed to leave the study at any time.

3.2.4 Population under study
Between November 2013 and February 2015, patients who attended the lung clinic for newly diagnosed pulmonary TB were screened for study enrollment (Figure 4). Subjects were selected based on secondary data from the newly diagnosed TB patients at the lung hospital in Surabaya and from four sub district health centers. Patients were examined by hospital pulmonologists at the time of diagnosis. The inclusive criteria were as follows: (1) newly diagnosed adult male and female pulmonary TB active patients aged between 18 and 55 years; (2) having clinical evidences of active TB symptoms; (3) those who had positive sputum smears or a positive chest X-ray that showed changes compatible with a diagnosis of tuberculosis. Patients with extra pulmonary TB, pregnancy, lactation, heavy smoker (>20 cigarettes per day), and having clinical evidences of any underlying diseases were excluded. Based on the Indonesia national standard guideline, chest X-rays of patients were taken at the time of diagnosis for newly diagnosed TB active patients only if sputum smear were not very convincing (Aditama et al. 2011).
Three specimens of early morning sputum from the patients were examined by direct microscopy after staining sputum smears at the early intensive phase. The Ziehl Neelsen procedure was used to evaluate sputum smear that is based on the number of acid-fast bacilli (AFB) visible in oil immersion by microscope. A patient was considered smear-positive if one or more of the three specimens tested were positive. In regard to assess the sputum smear conversion, three sputum specimens were examined again at the end of the intensive phase. When a patient still had smear positive after two months with standard therapy, the intensive phase was repeated for an additional month (extended intensive phase). In such case, at the fifth month during an extended intensive phase, two sputum smears were examined.

**Figure 4: Study profile**

Standard TB drugs dosages were based on national guidelines for newly diagnosed TB patients. In the intensive phase, patients received rifampicin (R), isoniazid (H), pyrazinamide (Z), and ethambutol (E) daily for two months. It was followed by a continuation phase with isoniazid and ethambutol three times per week in appropriate doses based on body weight categories (Aditama et al. 2011). The standard regimen
was given in fixed-dose combination RHZE (150mg/75mg/400mg/ 275mg) per tablet for intensive phase and RH (150mg/150mg) page tablet for continuation phase.

3.3 Dietary interventions

3.3.1 Tempeh preparation

Soybeans local cultivar Grobogan were obtained from Budi Mixed Farming (BMF) in grobogan district, middle Java. Soybeans were further fermented at a local tempeh home industry in Surabaya, according to controlled hygienic condition. Tempeh was prepared following a traditional method from Indonesia. Briefly, 10 kg of soybeans were boiled in preheated fresh tap water, which was preheated at 100º C, for one hour. Then the soybeans were de-hulled by hands until about 90% were freed from their skin. The remnants of the soybean skins floating on the water surface were removed by refreshing the tap water. Boiled soybeans were then soaked in fresh tap water for 12 hours at room temperature ranged between 27-30º C with the water level being 5 cm over the beans. After soaking, another boiling process were carried out using preheated tap water (100º C, 1 h). The water was discarded and the beans were then spread uniformly on a clean cloth to cool for 30 minutes to provide a suitable temperature for R. oligosporus inoculum.

Twenty-five ml of cooking vinegar was added to create an acid environment for the tempeh starter. Twenty grams of the starter yeast (Rhizopus oligosporus) were added to the soybeans. They were stirred gently for a uniform distribution of starter with beans. The inoculated beans (150 g) were then placed in a fermentation container (a sealed perforated plastic bag). The beans with yeast were incubated at room temperature for 48 hours (Figure 5). After fermentation, tempeh was stored at -10ºC until further process. Before distributed, tempeh was boiled along with a commercial seasoning “Bumbu Racik®” to improve the acceptability for savory flavors and softer consistency. After boiling, there were changes on the weight of tempeh to 166.5 g. Frying process was avoided for TB pulmonary patients since fried foods and dairy products are considered as mucus-forming foods (Pelton 2002; Zand et al. 1999).

3.3.2 Provision of tempeh

Every patient in the intervention group received cooked tempeh cake to be consumed as a meal with rice or as a snack. The supplement weight was based on a previous study which suggested a beneficial effect of uncooked 150 to 160 g of daily tempeh supplementation among post menopause women in Indonesia (Utari et al. 2011).
Cooked tempeh cakes were distributed to TB patients in the intervention group three times a week to their homes. Patients in the intervention group were instructed to cut one cake tempeh into three pieces and eat them three times in a day. Other cakes of tempeh for the next days’ supplementation were to store in a refrigerator to keep them fresh. They were also asked not to give the supplement to any family member or other person. Consumption frequencies of supplements were recorded in a logbook by an enumerator during random visits once a week. One of the patient family members was asked to help to supervise compliance. On the other hand, the patients in the control group were instructed not to eat any tempeh for the two-month study period since the food was easily bought from the market.

Figure 5: Uncooked fermented soybean (tempeh)

3.4 Outcome measures
3.4.1 Tempeh composition and isoflavones levels
The cooked tempeh sample was assessed for macronutrient composition and calorie content. Fat was determined by soxhlet extraction with petroleum ether, protein by Kjeldahl methods, and carbohydrate by spectrophotometric with antron-sulphuric acid as a reagent. Ash contents (gravimetric) were determined based on methods outlined in AOAC International (2000). The cooked tempeh was also analyzed by high-performance liquid chromatography (HPLC) for food genistein and daidzein content. Isoflavone standards namely genistein (≥98%) and daidzein (≥98%) were purchased from Sigma-Aldrich chemicals (St Louis, MO, USA). All determinations were made in triplicate. Measurement of macronutrient composition and isoflavones levels was performed at faculty of pharmacy, University of Airlangga, Surabaya.
3.4.2 Body weight and dietary intake
The body weight as well as handgrip strength and 6MWT were measured twice, prior to and immediately after the intervention. The weight measurement of the patients was taken using a digital weight scale (Cariba® Instrument, Model Nr. hd 2006A2, Indonesia) with minimal clothing and no shoes. The weight measurement was assessed to the nearest 0.1 kilogram. Body height was measured to the nearest of 0.5 cm. The measured weight and heights of the patients will be used to calculate their BMI, (kg/m$^2$). For that purpose, the patient’s height will be converted into meters.

The 24-hour dietary recall method was used to assess the dietary intakes of the patients twice, during the first and second months of the intervention period. The calorie and protein intakes of the patients were calculated from the 24-hour dietary recall data using NutriSurvey software version 2005, with the country specific food database for Indonesia.

3.4.3 Handgrip strength
Handgrip strength was measured with a dynamometer (electronic hand dynamometer Smedley®, Model Nr. TL-SLC 100, Trailite Germany; Figure 6 left) and standardized procedures (Mathiowetz et al. 1985). The patient was sitting on a chair with following position: (1) feet on the ground; (2) the shoulder was adducted and neutrally rotated; (3) elbow flexed at 90 degrees; (4) forearm in a neutral position; (5) the wrist between 0 and 30 degrees’ extension and between 0 and 15 degrees’ ulnar deviation. The arm was not sustained by anything (Figure 6 right). Using the dominant hand with a contraction grip time of ≤ 3 seconds, the participant was asked to put maximum force on the dynamometer three times. Thirty seconds time interval was maintained between each handgrip strength test (Trossman et al. 1989). The mean of three values was calculated and considered as the maximum grip strength (Hamilton et al. 1994). Values were recorded in kilograms. Tone and volume of instructions each time a test conducted was maintained remain the same. Measurements took place from 09.00 to 15.00 o’clock. The dynamometer was calibrated before each assessment.
3.4.4 The 6-minute walk test
The materials used to conduct the 6MWT and the preparation of participants was carried out according to American Thoracic Society (ATS) guideline (ATS 2002). The 6MWT was carried out on a track along the 30-meter corridor marked by two colored cones placed at both ends of the track alignment (Figure 7). The air conditioner in the hallway was set at 27°C. Patients were asked to walk at their self-selected pace back and forth between the cones as far as they could for 6 minutes. The distance taken by each participant was measured and then recorded. Instructions were given to every patient by reading a guideline with the same intonations to every patient before performing the test. To minimize intraday variability, the test was done between 10.00 to 14.00 o'clock. An encouraging phrase "do the best" was given at the end of the instruction. The outcome of the 6MWT was expressed in meters.

3.4.5 Statistical analyses
Descriptive data on the main outcomes are presented as mean and standard deviation. Independent sample t-test was used to test for differences in means at baseline and end line between groups. Paired sample t-test was used to test for differences in means within groups before and after treatment. The effect of the intervention was evaluated using independent sample t-tests on the individual changes. This test is identical to the repeated measures ANOVA with the group-by-time interaction. Homogeneity of the sample population was tested using chi-square test of proportions. The statistical significance of the results based on two-tailed p value <0.05. All
statistical analyses were performed using the SPSS software package (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

Figure 7: Six-minute walk test in a 30-m hallway of the hospital.

3.5 Results
Table 4 describes the amount of protein, carbohydrate, fat, and calories in the cooked tempeh for the patients per day in the intervention group. In addition, the two main components of isoflavones in the cooked fermented soybean are also presented in the table.

Table 4: Contents of macronutrient, isoflavones, and calories of 166.5 g edible weight cooked tempeh (n=3)

<table>
<thead>
<tr>
<th>Composition</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, g</td>
<td>24.99 ± 0.17</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>7.82 ± 0.03</td>
</tr>
<tr>
<td>Fat, g</td>
<td>10.83 ± 0.13</td>
</tr>
<tr>
<td>Genistein, g</td>
<td>47.30 ± 0.30</td>
</tr>
<tr>
<td>Daidzein, g</td>
<td>38.37 ± 0.14</td>
</tr>
<tr>
<td>Calories</td>
<td>228.74 ± 0.47</td>
</tr>
</tbody>
</table>

Table 5 shows characteristics of the selected patients on socioeconomic, demographic information as well as sputum smear and BMI. Age tended to be higher in the control group than in the intervention group. All other variables were similar in both groups. The majority had at least a primary school education, followed by high school diploma.
The income of both groups was dominated by less than one million rupiahs and between one to three million rupiahs per month. The patients who had income less than one million included unemployed patients that had no salary. The group without income, such as students, homemakers, and retired was 29.2% in the intervention group and 34.4% in the control group. Informal employment in the private sector was prominent for both two groups. No one was working as a government employee while the rest had an irregular job. Among the participants, there were two main ethnic groups, namely Javanese and Madurese. In terms of sputum smear, the number of patients with smear-positive was higher than smear-negative.

**Table 5: Characteristics of selected patients assessed at baseline** *

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intervention (n = 65)</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
</tr>
<tr>
<td>Age means‡</td>
<td>31.11 ± 9.63</td>
</tr>
<tr>
<td>Gender:</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>35 (53.8)</td>
</tr>
<tr>
<td>Female</td>
<td>30 (46.2)</td>
</tr>
<tr>
<td>Education:</td>
<td></td>
</tr>
<tr>
<td>Elementary school</td>
<td>26 (40.0)</td>
</tr>
<tr>
<td>Middle school</td>
<td>12 (18.5)</td>
</tr>
<tr>
<td>High school</td>
<td>25 (34.2)</td>
</tr>
<tr>
<td>Bachelor</td>
<td>4 (6.2)</td>
</tr>
<tr>
<td>Income (million rupiahs):</td>
<td></td>
</tr>
<tr>
<td>Less than one</td>
<td>38 (58.5)</td>
</tr>
<tr>
<td>Between one to three</td>
<td>26 (40.0)</td>
</tr>
<tr>
<td>More than three</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Occupation:</td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>19 (29.2)</td>
</tr>
<tr>
<td>Part timer</td>
<td>5 (7.7)</td>
</tr>
<tr>
<td>Private</td>
<td>41 (63.1)</td>
</tr>
<tr>
<td>Ethnic:</td>
<td></td>
</tr>
<tr>
<td>Javanese</td>
<td>32 (49.2)</td>
</tr>
<tr>
<td>Madurese</td>
<td>31 (47.7)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (3.1)</td>
</tr>
</tbody>
</table>
Sputum smear:

- Sputum smear positive: 44 (67.7) vs. 44 (68.8)
- Sputum smear negative: 21 (32.3) vs. 20 (31.2)
- Sputum smear mean †: 1.29 ± 1.16 vs. 1.25 ± 1.07

Body mass Index †:

- Mean ± SD: 18.41 ± 3.10 vs. 18.57 ± 3.26

* There were no significant differences between two groups (student’s t-test and chi-square)
† Mean ± SD

Table 6 depicts the means of body weight, handgrip strength, and 6MWT of the intervention and control groups at month zero (baseline) and month two (end line). No variables differed between groups at baseline comparison. At end line, only handgrip strength was statistically significant higher in the intervention group than in the control group. In addition, in the same group, before and after intervention comparison revealed an improvement of all variables in all groups, except for handgrip strength in the control group.

Table 6: Intervention and control group comparison for body weight, handgrip strength, and 6MWT

<table>
<thead>
<tr>
<th>Group</th>
<th>Intervention (n = 65)</th>
<th>Control (n = 64)</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>p-value*</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Body weight month 0, kg</td>
<td>45.82 ± 8.52</td>
<td>&lt; 0.001</td>
<td>46.84 ± 8.93</td>
</tr>
<tr>
<td>Body weight month 2, kg</td>
<td>48.63 ± 8.95</td>
<td></td>
<td>48.27 ± 9.22</td>
</tr>
<tr>
<td>Handgrip strength month 0, kg</td>
<td>25.05 ± 8.61</td>
<td>&lt; 0.001</td>
<td>24.86 ± 8.02</td>
</tr>
<tr>
<td>Handgrip strength month 2, kg</td>
<td>28.94 ± 10.13</td>
<td>&lt; 0.001</td>
<td>25.54 ± 9.04</td>
</tr>
<tr>
<td>6MWT month 0, m</td>
<td>343.95 ± 65.79</td>
<td>&lt; 0.001</td>
<td>357.13 ± 81.80</td>
</tr>
<tr>
<td>6MWT month 2, m</td>
<td>392.67 ± 69.01</td>
<td></td>
<td>382.37 ± 79.46</td>
</tr>
</tbody>
</table>

* Paired t-test for comparison in the same group before and after intervention
† Independent t-test for comparison between two groups

Table 7 shows the comparison of the means in the changes of body weight, handgrip strength, and performance of 6MWT between both groups. The numbers illustrate that the nutritional supplementation resulted in a significant change in all three variables in the intervention group compared to the control group.
Table 7: Changes in body weight, handgrip strength, and 6MWT after two-month tempeh supplementation

<table>
<thead>
<tr>
<th>Group</th>
<th>Intervention (n = 65)</th>
<th>Control (n = 64)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight change, kg</td>
<td>2.80 ± 2.33</td>
<td>1.44 ± 2.42</td>
<td>0.02</td>
</tr>
<tr>
<td>Handgrip change, kg</td>
<td>3.90 ± 4.50</td>
<td>0.84 ± 4.83</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>6MWT change, m</td>
<td>49.67 ± 58.49</td>
<td>25.75 ± 56.85</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Independent t-test for comparison between two groups

The amount of protein and caloric intakes based on the 24-hour dietary recall for the two groups are presented in Figure 8. The dietary intervention did not improve the protein intake of the intervention group compared to the control group (77.52 ± 26.63 vs. 71.42 ± 21.06 gr/day, p=0.16). The supplementary feeding given to the patients displayed a comparable value in calorie in both groups (2113.16 ± 429.41 vs. 1972.12 ± 530.04 kcal, p=0.10).

![Figure 8: Protein and caloric intake of study population at the midterm and end line (mean and standard deviation)](image)

**3.6 Discussion**

In this study, tempeh supplementation led to a significantly higher changes of body weight and physical function in the intervention group than the control group. The intervention group gained 1.36 kg more than the control group in body weight after two-month supplementation. The improvement on body weight change is in line with
previous randomized controlled studies among adult TB patients in various settings. A high-energy nutritional supplementation among TB patients resulted in 1.73 kg greater increase in body weight after six weeks of intervention (Paton et al. 2004). PrayGod et al. (2011) described 1.9 kg more weight gain after two-month energy-protein biscuit supplementation, especially among TB patients with HIV co-infection (+) and CD4 counts ≥ 350 cells/ml. Another dietary intervention study in India showed an increase of 2.6 kg in weight gain after a three-month locally available foods supplementation (Jahnavi et al. 2010). The finding from this study is relatively lower than other corresponding studies, it might be due to the lower content of calorie applied in the supplementation.

The current study described a greater increase of 3.06 kg in handgrip strength among patients in the intervention group compared with the control group. Related studies reported that nutritional supplementation produces higher increase of handgrip strength ranging from 1.5 to 2.3 kg with a duration of supplementation period between 1,5 to 5 months (Jahnavi et al. 2010; Paton et al. 2004; PrayGod et al. 2012). The higher outcome of the current study might come from the potential benefit of soy protein intake for muscle. Hirasaka et al. (2013) has suggested that soy isoflavone has a protective effect against inflammation-related muscle atrophy during catabolic state. Additionally, it is possible that this benefit comes from three divided portions of regular tempeh consumption per day during the intervention period. Mamerow et al. (2014) showed that even distribution of protein intake in a day results in higher muscle protein synthesis than skew pattern of protein consumption.

Another characteristic of physical function determined in this study was the 6MWT change. It was expected to provide confirmative information about the effect of tempeh supplementation on physical function among the patients. Additional information is needed since handgrip strength measures only one aspect of functional ability (Pieterse et al. 2002). The result of the test is in line with the outcome of the handgrip strength change. The 6MWT gain of the intervention group presented significantly farther than in the control group. Patients in the intervention group gained 23.93 m more than in the control group. This result is in agreement with a study that of a two-month supplementation of arginine among TB patients in Indonesia. Arginine consumption resulted in 30.7 m greater increase in 6MWT (Ralph et al. 2013). However, longer distance in 6MWT result in the previous study could be due to the patients in the intervention group walked significantly farther than in the control group
at the base line. On the contrary, in this study the intervention group walked equal distance compared with the control group at the base line.

Tempeh supplementation for two months among patients with active pulmonary TB did not increase caloric and protein intakes significantly even though the compliance report reached 75.69%. TB patients in the intervention group consumed tempeh as a partial replacement of regular intake and not as a food supplement. It might be due to consumption of soy foods leads to satiety, which can depress calorie and protein intake (Leidy et al. 2015; Neacsu et al. 2014). In contrast to healthy persons, TB patients with abnormalities in leptin regulation and inflammation are associated with suppression of eating appetite or low BMI (van Crevel, Karyadi, et al. 2002; Zheng et al. 2013). Therefore, these factors could contribute to ineffectiveness of the supplement to increase caloric and protein intakes among TB patients in the intervention group.

The beneficial effects of supplementation on the study outcomes might be due to the nature of soy foods, which possess "anti-inflammatory" properties. Several evidences showed that soy food supplementation can reduce several inflammation markers in postmenopausal women (Azadbakht et al. 2007; Nasca et al. 2008; S. H. Wu et al. 2012). Moreover, isoflavones isolated from soybean has been described giving a positive effect for postmenopausal women who have elevated C-reactive protein inflammatory marker (Dong et al. 2011). Thus, the wasting process might be suppressed by decreased inflammation so that the body mass can be more efficiently improved.

3.7 Conclusion

In summary, daily consumption of boiled tempeh could provide an alternative for adjunctive nutritional care for active pulmonary TB patients on standard therapy. In this setting, boiled tempeh contributed to improvement of weight gain and physical function change. However, these positive effects might not be directly from increased protein and caloric intakes of tempeh. Therefore, TB patients need additional nutritional counselling to improve overall dietary diversity. Assessments of body composition and inflammatory indices alongside weight gain are recommended during the supplementation period to confirm these findings. In addition, various assessment tools could be used to evaluate the multi-dimensional aspects of physical function. There is a need to evaluate the effects of other fermented soybean products in upcoming similar studies conducted on a larger participant, and over a longer intervention period.
Acknowledgements
We would like to thank Directorate General of Higher Education (DGHE) of Indonesia for a partial financial support. We gratefully acknowledge and appreciate the assistance of the participants and their family members. We thank Kusdiantoro MD for the clinical advices and Dr. Herrmann for statistical counselling.

Author disclosure
The authors declare no conflicts of interest to disclose.

3.8 References
References are presented for the whole thesis at the end of the thesis.

4 Discussion
The effect of fermentation on soybean
According to the additional results obtained, Table 8 shows the effect of fermentation on total phenolic content, antioxidant activities and phytic acid level. Fermentation mediated by *R. oligosporus*, could increase total phenolic content in tempeh. This increase could be due to the release of sugar residues linked to the hydroxyl group of isoflavones by β-glucosidase. This proteolytic enzyme has hydrolytic action and developed by fungus during fermentation (Georgetti *et al.* 2009). Therefore, fermentation results in a rise total phenolic content because in the natural form, isoflavones usually present as conjugated forms bound with a sugar moiety through hydroxyl group. The increase of total phenolic content in tempeh after fermentation, as observed in this study is relevant to previous studies. Similar phenomenon was also observed in fermented black soybean, koji, and fermented soymilk. (Lin *et al.* 2006; Juan *et al.* 2010; Lai *et al.* 2013).

The fermentation effect of soybean on total phenolic content was supported by the results of its AO activities. The outcomes of FRAP assay depicted the improvement of reducing ability of the soybean after fermentation. Several authors indicated that fermentation using various fungi produced higher reducing power than unfermented varieties in soybean (Lin *et al.* 2006) and black bean (Lee *et al.* 2008). TBARS assay results described also higher inhibition of lipid peroxidation in fermented soybean than the unfermented soybeans. Chang *et al.* (2009) showed that fermentation using *Rhizopus oligosporus* on soybean resulted in higher inhibition of lipid peroxidation.
compared with unfermented variety. Additionally, longer period fermentation showed even higher inhibition results in TBARS assay. This positive effect of fermentation has been supported also by in vivo studies (Wang et al. 2008; Chou et al. 2008). In comparison with raw soybean, the current results described a considerable 11% and 18% reduction of PA in tempeh fermented for two and three days respectively. This PA reduction might be due to the catalytic action of phytase enzyme to the phytic acid which was produced during fermentation period (Shivanna et al. 2014). These results showed compatibility with Egounlety et al. (2003), who reported a decrease in PA content after fermentation. The previous study described a 30.7% loss of PA level in soybean after fermentation using R. oligosporus. This discrepancy could be due to different method of soaking, boiling and fermentation (Sandberg 1991).

Table 8: Total phenolic content, FRAP assay, TBARS assay and the phytic acid content of methanolic extracts of raw and different processed soybeans

<table>
<thead>
<tr>
<th></th>
<th>Total phenolics* (mg CAE/g)</th>
<th>FRAP* (µmole Fe(II)/g)</th>
<th>TBARS** (µM MDA Eqv/100g)</th>
<th>Phytic acid* (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw soybean</td>
<td>4.13 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.49 ± 0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.33 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.61 ± 1.53&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Twelve hours soaked soybean</td>
<td>4.33 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.19 ± 1.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.36 ± 0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.09 ± 0.72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Uncooked 2 days fermented tempeh</td>
<td>6.22 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.13 ± 1.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.80 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.12 ± 0.93&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Uncooked 3 days fermented tempeh</td>
<td>6.40 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.07 ± 1.29&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.68 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.42 ± 0.82&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Butylated hydroxyl toluene (BHT)</td>
<td>NA</td>
<td>40.1 ± 2.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.22 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NA</td>
</tr>
</tbody>
</table>

*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

Sensory test

Stir-fried tempeh was more accepted by adult healthy panelists than other traditional preparation methods, especially in its indices of aroma and mouthfeel. Steaming was comparable in hedonic scale outcome with stir-frying in appearance, texture, aftertaste, and overall indices. Mouthfeel indices for steamed, raw, dried and boiled tempeh were not significantly different. However, boiled tempeh was the second highest mouthfeel after stir-fried tempeh. After controlling the characteristics of all indices, stir-frying was more acceptable than other cooking methods in estimated marginal mean results followed by steaming and boiling.
This outcome is in agreement with Nurhidajah et al. (1998), who showed that fried tempeh made from black soybean (Glycine soja) was higher in organoleptic evaluation result than varieties prepared using other traditional cooking methods. Abu-Salem et al. (2014) observed that fried tempeh made from soybean was better in tested sensory attributes than fried tempeh made from kidney bean. However, Vaidehi et al. (1985) described fried tempeh chips made from combination of soybean and sun flower was more acceptable than variety made from only soybean among semi-trained judges. Fried tempeh also was tested among adult (Aderibigbe et al. 2006) and children (Vaidehi et al. 1990) and resulted in high acceptability.

**Nutritional composition**

The crude protein composition was revealed by proximate analyses as the major portion (41.55%) of the dry matter in the uncooked tempeh samples. After stir-frying, the crude protein composition was slightly higher ($p = 0.015$) than uncooked tempeh samples. In comparison with fresh variety, a 38% increase of crude fat ($p < 0.001$) and a 29% decrease of carbohydrate ($p < 0.001$) compositions were found in stir-fried tempeh samples. Additionally, the contents of zinc and iron in stir-fried tempeh samples were reduced by 15% and 16% with $p$ values $< 0.001$ and $0.001$, respectively compared with uncooked ones.

A discrepancy of crude protein composition in tempeh samples can be occurred when their preparation carried out in different settings. For example, Murata et al. (1967) has described that the crude protein composition in tempeh prepared in a controlled environment of laboratory in Indonesia and Japan ranged from 46.9 to 56.9%. However, tempeh samples which were collected from local markets in Indonesia, their crude protein composition only ranged between 26.9 to 28.4% (Efriwati et al. 2013). One causal possibility is that the soybean as a main component of tempeh can be mixed with other grains which contain less protein like maize or rice to produce better flavor and to decrease its cost production. The increase of crude protein composition after stir-frying could be due to formation of new compounds which have a similarity with protein properties during the cooking process (DeMan 1999). These new compounds could have an influence in the protein measurement. The decrease of moisture in tempeh samples was also an explanation for an increase of crude protein composition after stir-frying (Ersoy and Özeren 2009; Bordin et al., 2013). The increase of crude protein composition after frying has been described also in various fishes compared with their raw varieties (Amin Ismail et al. 2004; Ghelichpour et al. 2012;
Zhang et al. 2013). In this study, since the protein increase was slight, it might be also only due to operational technique reason. Several related studies showed that frying was able to almost fully retain crude protein composition in the foods (Bognar 1998; Fillion et al. 1998). On the other hand, different authors reported the decrease of crude protein composition (Steiner-Asiedu et al. 1991) and net protein utilization (Henry 1998) after frying.

Often, carbohydrates are formed by simple sugars (glucose, fructose, maltose, lactose), complex sugars (maltose, sucrose) or in the form of complex carbohydrates such as starch. When food is cooked, the high temperature can cause starches to break down into monosaccharides. This breakdown provides the reactants for the Maillard reaction (non-enzymatic browning) to take place. Frying with oil results in higher temperature than boiling point of water. The frying oil is formed by long chains of hydrocarbons with strong covalent bonds which would not break easily. Thus, to break these bonds, higher temperature is needed. In this study, the carbohydrate composition in stir-fried tempeh samples was found lower than the uncooked tempeh samples. The decrease of carbohydrate could be due to conversion of starch into simple sugars (Damodaran et al. 2007; Palazoğlu et al. 2010). These simple sugars then would diffuse from food to oil during frying process (Inocent et al. 2011). Since tempeh is a fermented product, the storage prior cooking is able to contribute to the decrease of carbohydrate composition. The action of enzymes (α-D galactosidase, α-D glucosidase, and amylase) produced by the Rhizopus oligosporus during fermentation utilizes these reducing sugars for energy and growth (Egounlety et al. 2003).

Stir-frying increased the crude fat composition in tempeh samples significantly compared with uncooked variety in this study. The increase of crude fat could be a result of increased free fatty acids originated both from the frying oil and the tempeh itself (Sudarmadji et al. 1978). The frying oil can be absorbed by the food, causing an increase of calorie in it (Fillion et al. 1998). However, the assumption that all fried foods always contain higher fat and energy content than their uncooked ones might be an overestimation. Types of meats like pork, chicken and mackerel have been described to have lower fat composition than their raw varieties after frying with oil (Bognar 1998; Henry 1998). Foods derived from animals absorbs less oil than plant-origin foods during frying. It is because of oil is absorbed into a plant tissue which is filled with air in plant-origin foods. In contrast to animal-origin foods, intercellular space is occupied by fluid (Ghidurus et al. 2010; Fillion et al. 1998). In general, the amount of oil absorbed
by foods increases with longer period of frying. Ghidurus et al. (2010) has suggested that mushroom, peeled eggplant, onion, tomatoes, and flesh pineapple are foods with the highest oil uptake. Zinc and iron levels of uncooked tempeh were found low to fulfill recommended daily allowance. Only if a large quantity (ca 1 kg) of tempeh is consumed, it would compensate the RDA for zinc and iron (Institute of Medicine (US) Panel on Micronutrients 2001). Furthermore, the zinc and iron values were even reduced due to stir-frying process. The reduction of mineral content during frying can be explained by the migration of the metal ions into the oil. The migration of minerals from food to frying oil is due to binding with other insoluble compounds or catalyzing mineral oxidation (Boskou et al. 2010). Another possible explanation was that the proportion of minerals could be lower than uncooked variety due to absorption of the oil and lead to increasing weight of fried food. Due to the low level of minerals in tempeh, its consumption on daily diet should be accompanied by adequate food diversity.

**Total phenolic content**

Uncooked tempeh fermented for three days showed the highest amount of total phenolic content among the others, but its value was not significantly different from the uncooked tempeh fermented for two days. They described higher total phenolic content than both stir-fried tempeh samples. The total phenolic content in stir-fried tempeh fermented for two days were comparable with the samples of three-day fermentation. There were about 34% and 28% reduction ($p < 0.001$ for both samples) of total phenolic content after stir-frying for tempeh samples with three-day fermentation and two-day fermentation respectively. Stir-frying with sunflower oil had significantly decreased the content of total phenolic. This reduction can be caused by polyphenols disintegration or leached out during traditional cooking. Most of these bioactive compounds are known as unstable substances in high temperature treatment. The current result shows compatibility with the outcomes that are obtained after frying process of tomatoes, and onions (Crozier et al. 1997; Price et al. 1997).

**The ferric reducing ability of plasma assay**

Uncooked three-day fermented tempeh samples demonstrated higher reducing power compared with all the tempeh samples. However, this value was lower than the positive control, butylated hydroxyl toluene (BHT). Stir-frying significantly decreased the reducing power only in the case of the uncooked tempeh with a three-day fermentation
period. The reducing power of the stir-fried tempeh samples and uncooked fermented for two days were not significantly different. The FRAP assay revealed approximately 19% and 11% reduction with \( p = 0.016 \) and \( p = 0.489 \) for tempeh samples with three-day and two-day fermentation period respectively. Stir-fried tempeh samples were found lower in the reducing power and this might be due to the degradation of reducing agents like flavonoids as a result of the high temperature application (Segev et al. 2012).

**Thiobarbituric acid reactive substances assay**

In contrast to FRAP assay, TBARS assay indicated that stir-frying produces an increase AO activity of tempeh. Lower MDA level was found in the stir-fried tempeh samples than uncooked varieties. Stir-frying resulted in approximately 29% and 26% reduction of MDA levels in two-day fermentation and three-day fermentation varieties respectively. The highest inhibition of TBARS formation was seen in the positive control, BHT. The TBARS value of BHT was comparable with three-day fermentation stir-fried tempeh samples but higher than all other tempeh varieties. High temperature treatment or cooking process might produce novel AO chemical species which could improve AO activity. These AO chemical species could be transitional substances that are from polyphenols oxidation process (Miglio et al. 2008). Based on the fact that AOs do not act alone, they are presumed to undergo synergism with others (Kim et al. 2006). Similarly, Niki et al. (2000) has suggested that the resultant of AO activities is due to the product of their antagonistic or synergistic behavior.

**The phytic acid content**

Stir-fried tempeh samples fermented for two and three days showed approximately 38% and 41% lower PA concentration respectively than uncooked samples with \( p < 0.001 \) for both comparisons. There was no significant difference between uncooked two-day fermentation tempeh samples compared with uncooked three-day fermentation tempeh varieties. The alleviation of PA was observed in the tempeh after stir-frying process and it was confirming the earlier study of Sutardi et al. (1985a). This reduction could be due to heat instability of PA and phytase activity during the storage of tempeh samples at 4ºC for 24 hours prior to their frying (Sutardi et al. 1985a). In this study, comparable PA concentrations were found in both uncooked tempeh samples compared with raw soybean (Table 8).
**Tempeh provision**

The result in the sensory test, stir-fried tempeh demonstrated higher acceptability than other cooking methods among healthy volunteers. However, a fried-food is known as a type of mucus forming foods (Pelton 2002; Zand *et al.* 1999). Since chough is a common symptom of active TB patients, fried food supplementation might deteriorate their respiratory system. As a second option, steamed tempeh demonstrated low acceptability in a preliminary test among active TB patients. Therefore, the third cooking method option, boiling was chosen along with a commercial instant flavor to prepare tempeh. Boiling is also simple in preparation especially for flavor improvement and it has softer consistency. Based on sensory test result, taste and after taste indices of boiling was comparable with steaming and stir-frying respectively. Boiling was recommended by the medical committee and nutritionists of the hospital for tempeh preparation. Additionally, boiled tempeh has been served in the meal for hospitalized active TB patients. An Indonesia local cultivar soybean ‘Grobogan’ has been chosen to avoid controversies and negative believes regarding genetically modified organism (GMO) in the community. The edible weight of boiled tempeh was 166.5 g with 24.99 ± 0.17 g crude soy protein and 228.74 ± 0.47 kcal for daily consumption.

Characteristics of the selected patients assessed at baseline on socioeconomic and demographic information, showed no significant difference between intervention and control group. However, age had a dynamic to be higher in control group than intervention group. In the both groups, the number of positive sputum smear was higher than smear-negative. Additionally, Javanese and Madurese were the main ethnic groups among the participants in the both groups. The monthly income of both groups was predominantly low and middle categories. By the time it is written, both categories earned less than approximately $218 per month. Most of the full time employees were private informal sector workers which means they did not work officially in well-established companies. The level of education of participants from both groups was only about 7% who had bachelor degree. The main education was up to only primary school. This information reflected that the participants’ socio-economic status as well as their education level were predominantly low.

**Body weight change**

After the intervention period for two months, patients in the intervention group gained 1.36 kg more in body weight than control group. This marginally increase of weight
gain in this study was found to be relatively lower than previous macronutrient intervention studies which ranged from 1.7 to 2.6 kg (Paton et al. 2004; PrayGod et al. 2012; Jahnavi et al. 2010). Various settings used in these related studies might explain the range of the difference. A possible explanation is that the total calorie in daily supplementation applied in this study (228.74 ± 0.47 kcal) was lower than other studies that ranged from 600 to 881.93 kcal. Additionally, the body weight change in this study was not a result from the improvement of caloric intake (2113.16 ± 429.41 vs. 1972.12 ± 530.04 kcal, p=0.10) or protein intake (77.52 ± 26.63 vs. 71.42 ± 21.06 gr/day, p=0.16) which were consumed by the patients in intervention and control groups respectively.

Wasting in active TB is not directly caused by inflammatory cytokines, rather loss of body fat can result in prolonged inflammation (van Crevel et al. 2002). Since TB standard therapy only replenish mostly fat mass, nutritional supplement with high protein quality is recommended to improve nutritional status and further can depress inflammation (Sanchez et al. 2011). Pro-inflammatory cytokines like TNF-α and IL 6 have been known to play an important role in muscle protein synthesis (van Hall 2012). Therefore, when pro-inflammatory cytokines can be suppressed, weight gain could be more effective due to repletion of both fat and lean mass during TB standard therapy.

A study showed that supplementation of soy isoflavones in patients with chronic kidney disease reduced levels of CRP, IL-6 and TNF-α (Fanti et al. 2006). More recent study involving 1,005 adult women in China, showed that consuming of soy foods significantly diminished levels of IL-6, TNF-α, and soluble TNF receptors (Wu et al. 2012). Soy nut consumption for eight weeks by postmenopausal women with the metabolic syndrome decreased other inflammatory markers also such as NO, serum E selectin, IL -18, and CRP. In that study, soy nut supplementation showed more reduction of inflammatory markers than the soy extracts powder consumption (Azadbakht et al. 2007).

It is not known yet whether tempeh consumption longer than two months in active TB patients will be beneficial. Paton et al. (2004) indicated that nutritional intervention in TB patients can increase body weight gain up to three months only. Longer than three months, the body weight change was not significantly different anymore compared with control. On the contrary, Martins et al. (2009) showed that the daily meal supplementation for six-month period still resulted in marginally higher proportion of weight gain than nutritional advice. However, high calorie supplementation after early stage of standard TB therapy was found mainly in fat mass instead of lean mass (Paton
et al. 2004). A possible explanation of this ‘catch up’ fat phenomenon is due to a suppressed thermogenesis which can increase a risk for metabolic or cardiovascular disease in the future (Dulloo et al. 2002; Crescenzo et al. 2003). Additionally, high protein composition in soy products can result in appetite reduction due to satiety and therefore lessen the food intake (Leidy et al. 2015; Neacsu et al. 2014). Satiety of the patients might be a cause in unfruitful supplementation to improve calorie and protein intake during intervention in this study. Daily tempeh consumption among TB patients only replaced a part of their regular intake instead of as a food supplement.

**Handgrip strength**

In this study, the intervention group gained 3.06 kg more in handgrip strength than the control group after supplementation period. Several related studies among TB patients showed between 1.5 to 2.3 kg improvement in handgrip strength gain after nutritional supplementations for 6 weeks to 3 months (Jahnavi et al. 2010; Paton et al. 2004; PrayGod et al. 2012). Higher result in the lean mass strength could be resulted from the soy protein which was used in the intervention. Without nutritional intervention, repletion of the body mass during first weeks of standard TB therapy has been found mainly fat deposition instead of lean mass (Sanchez et al. 2011). This condition could be due to several reasons. Under systemic inflammatory conditions, inflammation status of muscle cells can cause negative proteins turnover (Nicastro et al. 2012). On the other hand, soybean is considered as a source of soy protein which provides complete essential amino acids for muscle development (Asif et al. 2013). Additionally, an in vitro study found that soy isoflavones could prevent muscle atrophy induced by TNFα in myotubes (Hirasaka et al. 2013). Other explanation could be related with insulin tolerance which has been proposed as a potential marker and risk factor for active *M. tuberculosis* infection (Mao et al. 2011). Insulin has been known to its role to increase the rate of protein synthesis in muscle, adipose tissue, liver, and other tissues. Preliminary evidences from animal and human studies suggest that various fermented soybeans produce better improvement in insulin resistance and insulin secretion than non-fermented products (Kwon et al. 2010). For example, a water-soluble extract of fermented soybean (Touchi) was found to decrease fasting blood glucose and HbA1C in mild type 2 diabetes patients (Fujita et al. 2001). Another possibility is that patients in the intervention group could have better muscle protein synthesis because they followed the instruction to consume
tempeh three times a day, while the control group received no information. Thus, patients in the intervention group had more even dietary protein distribution which produce greater muscle protein synthesis than control group (Mamerow et al. 2014). Other positive effects of soy protein and isoflavones related with physical function are prevention of antioxidants depletion induced by physical activity (Brown et al. 2004) and their anti-fatigue properties (Liu et al. 2014).

Since most of the productive participants in this study had informal jobs with low to middle incomes, they needed to resume their works as soon as possible. Studies have shown the positive correlation between income and dietary diversity (Ruel 2003; Rashid et al. 2006). Lack of income will affect the diversity of nutritious foods consumed by the patients for faster recovery. Faster convalesce in physical ability would help the patients to earn their income earlier during standard therapy period. Additionally, physical function improvement affects their travelling ability to obtain the drug and result in an increase of adherence (PrayGod et al. 2011). An increase of compliance to the TB standard therapy may decrease amount of drop out, drug resistance, and relapse in the future.

**Six-minute walk test outcomes**

In this study, the outcome of handgrip strength change was supported by the result of 6MWT. After tempeh supplementation, the distance gain of 6MWT by patients in the intervention group was 23.93 m farther than control group. Ralph et al. (2013) reported 30.7 m farther distance change of 6MWT than the control group after two-month intervention of arginine. On the contrary, the same study described that vitamin D supplementation results in 15.1 m shorter distance change of 6MWT than the control group. The 6MWT in this study is meant to provide adjunctive information since physical function is multidimensional aspects. Therefore, the discrepancy in p value between outcome of handgrip strength change (<0.001) and 6MWT change (0.02) might be due to the different nature of both tools. The handgrip strength test reflects a maximal power of lower arm muscles while 6MWT depicts sub maximal power of leg muscles. Additionally, handgrip strength is a proxy indicator for muscle function (Ramlagan et al. 2014) while 6MWT replicates daily activities (Solway et al. 2001) and reflects cardiovascular fitness (Adedoyin et al. 2010).
Table 9: Summary of randomized controlled trials of macronutrient supplementation for adult active pulmonary TB patients with standard therapy.

<table>
<thead>
<tr>
<th>Study</th>
<th>Location &amp; duration of intervention</th>
<th>Study population</th>
<th>Nutritional intervention</th>
<th>Major findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paton et al., 2004</td>
<td>Singapore 6 weeks or up to BMI of patients = 20</td>
<td>Outpatients (18 to 69 year-old) with evidence of active tuberculosis (symptoms with positive sputum smear or a positive chest X-ray), BMI &lt; 20. The patients were assigned into nutritional supplement group (n = 19) and control group (n = 17).</td>
<td>Intervention group received 600 – 900 kcal/day oral nutritional supplements (6.25 g protein, 20.2 g carbohydrate, 4.92 g fat, 150 kcal/100 mL) with nutritional advice and control group received nutritional advice only.</td>
<td>Increase in body weight (2.57 ± 1.78 v 0.84 ± 0.89 kg, p = 0.001), total lean mass (1.17 ± 0.93 v 0.04 ± 1.26 kg, p = 0.006), and handgrip strength (2.79 ± 3.11 v 0.65 ± 4.48 kg, p = 0.016) at week 6.</td>
</tr>
<tr>
<td>Perez Guzman et al., 2005</td>
<td>Mexico City, Mexico 8 weeks</td>
<td>Newly diagnosed, hospitalized, and HIV - patients (17 to 60 year-old) with positive sputum culture &amp; smear. The patients were assigned into intervention group (n=10) and control group (n=11).</td>
<td>800 mg/d cholesterol (cholesterol-rich food: butter, beef liver, egg yolk, and milk derivatives) for experimental group and 250 mg/d cholesterol as a placebo.</td>
<td>Lower percentage of patients with positive sputum culture at week 2 (80% v 9%, p = 0.0019). The bacillary population decreased faster (p = 0.0002). Sputum production decreased faster (p &lt; 0.05).</td>
</tr>
<tr>
<td>Martins et al., 2009</td>
<td>Dili, Timor Leste. 32 weeks</td>
<td>Outpatients (≥ 18 year-old) newly diagnosed pulmonary tuberculosis with positive or negative sputum smear tests. The patients were assigned into experiment group (n = 136) and control group (n = 129).</td>
<td>Daily meal (weeks 1-8) and food package (weeks 9-32) with frequency 5-6 times per week (1800 kJ or 430.2 kcal, 18.4 g protein, 55.6 carbohydrates) and nutritional advice was a placebo.</td>
<td>Modestly higher weight gain at the end of treatment at week 8 (5.2% v 3.5% improvement, p = 0.04) and week 32 (10.1% v 7.5% improvement, p = 0.04).</td>
</tr>
<tr>
<td>Study</td>
<td>Location</td>
<td>Duration</td>
<td>Description</td>
<td>Intervention</td>
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<td>-------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Jahnvi &amp; Sudha., 2010</td>
<td>Anganwadis, India</td>
<td>3 months</td>
<td>Outpatients aged 18–65 years. Symptoms (+) with a positive sputum smear, or one culture positive specimen from an extra pulmonary site, BMI &lt; 20</td>
<td>Sweet balls made from wheat flour, caramel, groundnuts and vegetable ghee, providing 6 g protein and 600 kcal energy, and 100 g of sprouted grams and nuts every day.</td>
</tr>
<tr>
<td>Sudarsanam et al., 2011</td>
<td>Vellore, Tamil Nadu, India</td>
<td>6 Months</td>
<td>Newly diagnosed patients (&gt; 12 year-old) with +/- sputum but clinical and radiological evidence or culture – and/or biopsy-proven extra pulmonary TB with and without human immunodeficiency virus (HIV). The patients were assigned into supplement group (n = 51) and control group (n = 52).</td>
<td>Supplement consisted 25 g roasted wheat, 15 g roasted groundnut, 15 g roasted Bengal gram and 15 g jiggery (930 kcal and 31.5 g protein) with a micronutrients tablet and control group did not receive anything.</td>
</tr>
<tr>
<td>PrayGod et al., 2011</td>
<td>Mwanza, Tanzania</td>
<td>60 days</td>
<td>Sputum positive pulmonary TB with HIV co-infected patients. The patients were assigned into supplement group (n = 152) and control group (n = 153).</td>
<td>Intervention was 3690 kJ or 881.93 kcal biscuits with micronutrients and 27 g protein and control group received a 615 kJ biscuit with micronutrients, and 4.5 g protein.</td>
</tr>
</tbody>
</table>
**Strength and limitation of the study**

The strength of this study includes randomized and controlled study design which allows two comparisons: before and after as well as intervention and control groups. Though several conditions could not be controlled, randomization of the patients could show homogenous population in both groups. The provision of the food during the intervention period utilized traditional and local resources. Tempeh was not only low cost and nutritious but also easy to prepare without any sophisticated utensils. Therefore, tempeh provision can be applicable as an alternative functional food especially for areas with low income population that are suffering from food insecurity. In regards to food based approach study, the preparation of the materials and cooking method during the intervention were standardized. Besides, the novelty of this study was the application of lower calorie food supplement than previous studies in adult active TB patients.

On the other hand, due to time and logistic constraints, this study had several limitations like unknown population of HIV (+) co-infection and TB multi-drug resistant patients. The standard procedure of the hospital at the time of the study was that the HIV test carried out only for suspected TB patients and not for all newly diagnosed TB patients. This procedure was also applied for sputum culture examination. Daily activities of the participant could not be monitored in both groups since several patients resumed their works which might vary in their starting points during intervention period. Often, the patients started to resume their work in the second month of standard TB therapy. Since this study was based on minimum number the participants, utilization of larger quantity of subjects might provide more convincing evidence.

A placebo concept could not be applied since the patients see and taste the food. The compliance was assessed only based on the patient and/or a family member reports instead of direct observation. Though the patients in control group were instructed not to eat tempeh for two months, but tempeh was common food and easy to be bought in the local markets. One suspect adverse event was reported but it could not be confirmed further whether it was due to supplementation or TB standard therapy. A family member reported vomiting during supplementation but the patient refused to meet and dropped out from the study and TB treatment course. The original taste of tempeh without flavor was bland so that a commercial flavor was applied to improve its acceptability. However, bored of the taste was still the most common feedbacks from the patients during intervention period.
5 Conclusion and future recommendation

Tempeh is a nutritious food that contains antioxidants and bioactive components like isoflavones. The present study concludes that the fermentation with *R. oligosporus* can enhance total polyphenols and also AO activity of soybean. However, high temperature which is involved in cooking process could change chemical properties in tempeh. Additionally, due to low content of minerals in tempeh, consumers still need the diversity in foods.

Daily consumption of 166.5 g boiled tempeh along with standard therapy for two months among pulmonary active TB resulted in improvements on changes of body weight and physical function which was measured by handgrip strength and 6MWT. These beneficial effects did not come from additional protein and caloric intakes; further study is required to explore its underlying mechanism.

For future study, tempeh provision might be applied also for positive sputum smear TB with HIV (+) co-infection. However, preparation of the food should be considered carefully since higher severity of the disease is more likely followed by lower general state and appetite of the patients. Various cooking preparations of tempeh without compromising nutritional content should be addressed to minimize boredom of the taste during supplementation period. Flavored tempeh extract or dried powder with environmental friendly packaging might be an alternative especially for storage and distribution during intervention period. More studies are required to develop an efficacious food supplement for active TB patients. Functional foods that rich of antioxidants and bioactive compounds such as flavonoids, omega 3, and curcumin might be options for TB supplementation studies. If the dietary intervention is preferred more than two months, the diversity of food supplementation should be considered. The patients should be encouraged to have a healthy balance diet instead of depending on nutritional supplements.

Based on these findings, inflammatory cytokines, total antioxidants status, and insulin resistance indices are to be explored to provide comprehension regarding underlying mechanisms. Body composition assessment can give an information whether body mass repletion is predominantly from lean mass or fat mass. In regards to body composition studies, longer period of dietary intervention might be needed to determine exact duration that gives the most benefit for the TB patients. For instance, the dietary intervention is only needed as long as it gives positive effect on lean mass repletion. On the other hand, longer period of food supplementation especially with excessive calorie can lead to mainly fat mass deposition rather than lean mass.
Though TB standard therapy is six months, two months or less intervention period is suitable especially when it is carried out among TB outpatients. The reason is that after two-month intensive phase, most of active TB patients already resume their works which may involve various physical activities. Different types of works can cause different levels of energy expenses and fatigue which produce higher variance in outcomes.

Handgrip strength and 6MWT are appropriate tools to measure physical function among active TB patients. However, since physical activity consist complex aspects, a combination of several tools can be used in a study to give a comprehensive view of interpretation. Other objective assessments of physical function like FEV$_1$ and timed stand test have been used among active pulmonary TB patients.
6 Summary

Malnutrition due to wasting is a common feature in active pulmonary tuberculosis (TB) patients. Weight loss and decreased muscle mass are commonly associated with fatigue and physical inactivity. Reduction in lean mass due to wasting can cause physical function impairment in people with active tuberculosis. Standard TB treatment alone improves nutritional status, but it might not be sufficient to achieve adequate nutritional recovery especially in food insecure areas. The adjunctive nutritional provision which is locally available along with standard anti-TB therapy, has potential as an alternative approach, for faster recovery in weight gain and physical function. Quicker recovery of physical function results in these patients returning to work earlier. Eventually, the patients will be able to provide varying nutritious foods independently to maintain their health status after the recovery. Tempeh is recognized as a nutritious and affordable food which is commonly consumed in many households in Indonesia. The dietary intervention study using tempeh was carried out in the Lung hospital, Surabaya, Indonesia. Newly diagnosed active pulmonary TB patients participated in the study between November 2013 and February 2015.

At the end, the objective of the study became twofold because of several analyses which had been done to determine the properties of tempeh prior to the dietary intervention study. The first objective was meant to prepare tempeh as a suitable food supplement in the dietary intervention study. Its purpose was to analyze the effect of stir-frying on acceptability, chemical contents and antioxidant activities of tempeh. Second objective was, to determine the efficacy of tempeh supplementation on changes in body weight and physical ability in newly diagnosed active pulmonary TB patients with standard therapy.

Sensory evaluation was conducted by administering a questionnaire along with tempeh samples. Fermentation of soybean was carried out in the solid state using raw soybeans (Glycine max) and was inoculated with a starter containing Rhizopus oligosporus. The total phenolic content of uncooked and stir-fried tempeh samples was examined using Folin-Ciocalteu’s method and the phytic acid (PA) level was analyzed by high-performance liquid chromatography (HPLC). Antioxidant activities of tempeh samples were measured by the thiobarbituric acid reactive substance (TBARS) and ferric ion reducing/antioxidant power (FRAP) methods. Sensory evaluation revealed that stir-fried tempeh was more acceptable than other traditional cooking methods among healthy volunteers. In comparison with uncooked tempeh samples, stir-frying
increased content of fat and protein, but reduced carbohydrate, iron, zinc, and phytic acid levels of tempeh. Stir-fried tempeh samples presented lower total phenolic content and FRAP assay result. However, stir-fried tempeh samples showed a higher inhibition of TBARS formation.

Body weight change has been proposed as one of the clinical parameters that provides prognosis of TB treatment outcome such as failures, relapses and mortality. In this study, daily 166.5 g boiled tempeh supplementation for two months resulted in a higher body weight change among active pulmonary TB patients in the intervention group (2.80 ± 2.33 kg) compared with the control group (1.44 ± 2.42 kg, \( p = 0.02 \)). Patients in the intervention group gained 1.36 kg more weight than those in the control group. This result was marginally higher than minimum body weight change value calculated using the G*power software before the intervention. A minimum body weight change of 1.1 kg was determined to make significant difference (\( p < 0.05 \)) with a minimum number of 64 participants.

Physical function change was assessed using handgrip strength and a six-minute walk test. Handgrip strength has been considered as a valid and reliable proxy measure of physical function. Static force from the dominant hand of patients was measured by squeezing a baseline dynamometer. After the supplementation period, patients in the intervention group showed a higher handgrip strength change (3.90 ± 4.50 kg) than in the control group (0.84 ± 4.83 kg, \( p < 0.001 \)). The gain in handgrip strength in the intervention group was 3.06 kg more than in the control group. In addition, handgrip strength value in the intervention group (28.94 ± 10.13 kg) was higher than in the control group (25.54 ± 9.04 kg, \( p = 0.046 \)) at month 2. Six-minute walk test (6MWT) has been known as an approach to evaluate submaximal levels of exertion and cardiovascular fitness. At the end of the study, the change in 6MWT distance in the intervention group (49.67 ± 58.49 m) was greater than in the control group (25.75 ± 56.85 m, \( p = 0.02 \)). The distance gain in 6MWT was 23.92 m higher in the intervention group than in the control group.

At baseline, patients had comparable body weight, handgrip strength and 6MWT values for both groups. Characteristics of selected patients at enrollment were homogenous in terms of age, gender, BMI, sputum smear, and socioeconomic status after being randomly assigned into two groups (intervention and control). All three indices (body weight, handgrip strength, and 6MWT) in the intervention group were found to have significantly increased (\( p < 0.001 \)) after the intervention period. However, in the control group, only two parameters (body weight and 6MWT) showed significant
improvement ($p < 0.001$) but there was no significant difference for handgrip strength ($p = 0.24$) after two months. During the intervention period, assessment of calorie and protein intakes using 24-hour dietary recall resulted in no significant difference.

In summary, tempeh is a nutritious food and contains bioactive components, but high cooking temperatures could change its chemicals properties and antioxidant activities. Tempeh was also found to lack mineral ingredients such as iron and zinc; therefore, consumers are still suggested to consume a diversity of foods. Daily consumption of 166.5 g boiled tempeh for two months among patients with active pulmonary TB on standard therapy showed a positive effect on weight gain and physical function change. In this study, these beneficial effects were not related directly with the amount of daily caloric and protein intakes which need further study.
Zusammenfassung


Eine Veränderung im Körpergewicht wurde als klinischer Parameter vorgeschlagen, der eine Prognose über Tuberkulose Behandlungsergebnisse wie z.B. Misserfolg, Rückfälle und Mortalität liefert. In der vorliegenden Studie führte eine tägliche Supplementation mit 166,5 g gekochtem Tempeh über den Zeitraum von zwei Monaten zu einer größeren Veränderung in Körpergewicht bei behandlungsbedürftigen Lungen- und Thorax-Tuberkulosepatienten in der Interventionsgruppe (2,80 ± 2,33 kg) im Vergleich zur Kontrollgruppe (1,44 ± 2,42 kg, p = 0,02). Patienten in der Interventionsgruppe nahmen 1,36 kg mehr zu als jene in der Kontrollgruppe. Dieses Ergebnis war geringfügig höher als der minimale Körpergewichtsveränderungswert, der vor der Intervention mittels G*Power Software berechnet wurde. Eine minimale Körpergewichtsveränderung von 1,1 kg wurde festgelegt, um einen signifikanten Unterschied (p < 0,05) bei einer minimalen Anzahl von 64 Patienten auszumachen.

Eine Veränderung der physischen Funktion wurde mittels Handgriffstärke und Sechs-Minuten-Geh-Test (6MWT) ermittelt. Handgriffstärke wird als eine valide und zuverlässige Messgröße für physische Funktionen erachtet. Statische Kraft der dominanten Hand der Patienten wurde durch Drücken eines Dynamometers gemessen. Nach der Supplementierungsphase wiesen Patienten in der Interventionsgruppe eine größere Veränderung an Handgriffstärke auf (3,90 ± 4,50 kg) als Patienten der Kontrollgruppe (0,84 ± 4,83 kg, p < 0,001). Die Zunahme an
Handgriffstärke lag um 3,06 kg höher als in der Kontrollgruppe. Des Weiteren war der Handgriffstärkewert in der Interventionsgruppe (28,94 ± 10,13 kg) nach dem zweiten Monat höher als der in der Kontrollgruppe (25,54 ± 9,04 kg, p = 0,046). 6MWT ist bekannt als eine Herangehensweise, um das submaximale Niveau an Belastung und kardiovaskulärer Leistungsfähigkeit zu bewerten. Am Ende der Studie war die Veränderung der Distanz beim 6MWT in der Interventionsgruppe (49,67 ± 58,49 m) größer als in der Kontrollgruppe (25,75 ± 56,85 m, p = 0,02). Der Distanzzuwachs beim 6MWT war in der Interventionsgruppe im Vergleich zur Kontrollgruppe 23,92 m höher. Zum Zeitpunkt der Baseline-Erhebung hatten Patienten beider Gruppen vergleichbare Werte bezüglich Körpergewicht, Handgriffstärke und 6MWT. Die ausgewählten Patienten wiesen zur Zeit der Anmeldung homogene Eigenschaften bezüglich Alter, Geschlecht, BMI, Abstrich und sozioökonomischem Status auf, nachdem sie randomisiert in zwei Gruppen (Intervention und Kontrolle) eingeteilt wurden. Alle drei Indizes (Körpergewicht, Handgriffstärke und 6MWT) sind in der Interventionsgruppe nach der Interventionsphase signifikant angestiegen (p < 0,001). In der Kontrollgruppe hingegen wiesen zwei Parameter (Körpergewicht und 6MWT) signifikante Verbesserungen auf (p < 0,001), jedoch ließ sich kein signifikanter Unterschied bei der Handgriffstärke (p = 0,24) nach zwei Monaten feststellen. Während der Interventionsphase ergab die Bewertung von Kalorien- und Proteinaufnahmen mittels eines 24-Stunden Ernährungsprotokolls keinen signifikanten Unterschied.

8 Acknowledgements

I would like to sincerely thank to Prof. Dr. med Michael Krawinkel for the opportunity to be doctoral student in his working group and his support during the study. I also thank to Prof. Dr. med Katja Becker for her willingness to be second reviewer. I wish to thank to Dr. Johannes Herrmann for his assistance in statistics analyses. All individuals are from Justus Liebig University Gießen.

I am also grateful to Prof. Dr. med. Hans Konrad Biesalski, Prof. Dr. Donatus Nohr, Dr. Ignasius Radix A.P.J, Dr. Ratna C. Purwestri and Sandeep Kumar Thamtam, Msc, all University of Hohenheim, for their support for the tempeh analyses.

I gratefully acknowledge support from for all participants and the enumerators of the study for their contributions. I am grateful to Kusdiantoro MD from Surabaya Lung Hospital, Indonesia for his clinical advices. Special thank goes to Indonesian Directorate General of Higher Education (DIKTI) for their financial support. I am also thankful to my dearest family: Jacoba, Nehesa, and Ezra for their lovingkindness.
9 References


Irawati A & Rozanna R (1994) Pemberian formula tempe pada penderita gizi buruk untuk mempercepat penyembuhan. *Penelitian Gizi dan Makanan (The Journal of Nutrition and Food Research)*, 0(0). Available at:


10 Appendix

10.1 Statement/Erklärung an Eides Statt

I declare: this dissertation submitted is a work of my own, written without any illegitimate help by any third party and only with materials indicated in the dissertation. I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me. At any time during the investigations carried out by me and described in the dissertation, I followed the principles of good scientific practice as defined in the “Statutes of the Justus Liebig University Giessen for the Safeguarding of Good Scientific Practice”.


Budhi Setiawan
11.2 Participant information
Tuberculosis (TB) is a major infectious disease in Indonesia. It can cause weight loss and lack of ability to do activities. People with TB need to take antibiotics for a long time, usually 6 months, to be cured. We want to find out if fermented soybean (tempeh) taken with the antibiotics might help patients with TB to get better faster. We would like to ask people with lung TB who take standard therapy to take part in this study.

Tempeh is an Indonesia's common food that you know. It should not cause harm when you are eating tempeh since it is widely consumed for a long period. It is very uncommon for Indonesian, but some people have allergy to this food. When you have complaints and symptoms during the study related with Tempeh, do not be hesitated to stop eating the food and report it to me. We will contact a doctor who will make a follow up examination and treatment needed. Some contact numbers will be given to you, in case if you have any question and complaint. We will make the food with good standard of hygienic to maintain its quality.

The body needs nutrition, such as carbohydrate, protein, vitamins and minerals to stay healthy. People who are suffered from infection, they have tendency to eat less due to their illness. Good quality of macronutrient will able to help them to maintain their nutritional needs especially during recovery period. Some experts think that good macronutrient intake may help people with TB haves faster body weight and physical function recovery. However, there is no clear answer to this yet. You can help us try to answer this important question, if you do not mind to be part of this study.

If you agree, we will ask you to take fermented soybean (tempeh) or without taking it for 4 months in addition to the usual TB antibiotics (which you take for 6 months). Some people (not all) will receive fermented soybean based on which group you will be assigned. A computer will do the allocation to two groups randomly. One group will get only antibiotics from the TB clinic and other group will get antibiotics along with fermented soybean. There will be evaluation how much food do you take in one day every month for four months. We will also ask you to do body weight measurement, a test for handgrip strength and a walking test for 6 minutes. It will be done three times, before we start the study, 2\textsuperscript{th} and 4\textsuperscript{th} month. We will do the measurement during your regular follow up visit in TB clinic.

Whether you agree or not to participate in this study, you will receive the standard TB antibiotics that the health center usually uses to treat people with tuberculosis. As part
of normal examination, the TB clinic will ask you to provide a sputum sample, have your height and weight measured, get a chest x ray and have blood taken and you will be asked questions about your health.

Taking part in this study is voluntary; it does not cost you any money. All information collected is confidential. Results will be given to your doctor if they can help the doctor to treat your illness. No personal identification will be revealed to persons outside the study. You do not have to participate if you do not want to, this will not affect your medical treatment. If you decide to participate, you can withdraw from this study at any time for any reason and still receive standard treatment for tuberculosis by the staff at the health Centre.

10.2 Participant consent form

I have read the patient information sheet and have had the details explained to me by the witness below. I understand that I will receive the usual treatment and tests for tuberculosis. I understand that I may also have extra fermented soybean (tempeh) depend in which group I have been assigned.

I understand that the extra food (tempeh) are being tested to find out if they will help people with TB to get better faster. They are very unlikely to be harmful, but could potentially cause allergy. I may not receive any direct benefit from this study, but the results will help answer questions about TB treatment, and therefore may be of help to other people in the future.

I understand that I have tuberculosis and I will be asked to come to TB Clinic for repeated body weight measurements, handgrip strength tests, 6-minute walk test before study, 2nd and 4th month and also monthly questionnaire for 4 months. I understand that this will take longer than a usual clinic appointment. I also understand that a research staff will read my medical records at the health Centre and some information about my health will be collected.

I understand that all information collected is confidential and no information will be available to anyone outside the study. Results will be given to my doctor if they can help the doctor to treat my illness. I understand that I do not have to participate in this study. I can withdraw from this study at any time for any reason and still receive standard treatment for tuberculosis by the staff at TB Clinic.
PARTICIPANT
I (print name) .............................................................................................................. agree to take part in this study.

Signed  Date .................................................................................................................... _____ / _____ / 20_____

WITNESS
I (print name) .................................................................................................................... have explained the study & information sheet

Signed  Date .................................................................................................................... _____ / _____ / 20_____

10.3 Serious adverse event (SAE) report form

<table>
<thead>
<tr>
<th>TITLE OF THE STUDY:</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRINCIPAL INVESTIGATOR:</td>
</tr>
<tr>
<td>Mobile Phone:</td>
</tr>
<tr>
<td>DATE OF ADVERSE EVENT:</td>
</tr>
<tr>
<td>LOCATION OF SAE:</td>
</tr>
<tr>
<td>WAS THIS AN UNEXPECTED ADVERSE EVENT?</td>
</tr>
<tr>
<td>BRIEF DESCRIPTION OF PARTICIPANT (Do NOT include identifiers.)</td>
</tr>
<tr>
<td>Diagnosis:</td>
</tr>
<tr>
<td>BRIEF DESCRIPTION OF THE NATURE OF THE ADVERSE EVENT:</td>
</tr>
<tr>
<td>CATEGORY OF THE SAE:</td>
</tr>
<tr>
<td>[ ] life-threatening</td>
</tr>
<tr>
<td>[ ] hospitalization</td>
</tr>
<tr>
<td>[ ] required intervention</td>
</tr>
<tr>
<td>[ ] other</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>OUTCOME OF THE SAE:</td>
</tr>
<tr>
<td>[ ] resolved</td>
</tr>
<tr>
<td>[ ] continued</td>
</tr>
<tr>
<td>[ ] death</td>
</tr>
<tr>
<td>[ ] don’t know</td>
</tr>
<tr>
<td>HAVE SIMILAR ADVERSE EVENTS OCCURRED ON THIS PROTOCOL?</td>
</tr>
<tr>
<td>If “Yes”, how many? ______</td>
</tr>
<tr>
<td>Please Describe:</td>
</tr>
<tr>
<td>What steps do you plan to take as a result of the adverse event reported above?</td>
</tr>
<tr>
<td>[ ] no action required</td>
</tr>
<tr>
<td>[ ] amend consent document</td>
</tr>
<tr>
<td>[ ] amend protocol</td>
</tr>
<tr>
<td>[ ] inform current subjects</td>
</tr>
<tr>
<td>[ ] terminate or suspend protocol</td>
</tr>
<tr>
<td>[ ] other, describe:</td>
</tr>
<tr>
<td>INVESTIGATOR’S SIGNATURE:</td>
</tr>
</tbody>
</table>
10.4 Data collection form

Baseline data collection form

Section A: Inclusion and Exclusion Criteria

First name: __________________  Surname: __________________
Date: __ __/ __ __/ 20__ __
Data collector: __________________

1. Smear or chest X-ray positive pulmonary TB: Yes □ No □
2. Never treated for TB before: Yes □ No □
3. Agree to continue treatment in Surabaya for 6 months: Yes □ No □
4. Consent given: Yes □ No □
5. Age 18-45: Yes □ No □

If „No“ to any questions 1 to 5, PATIENT IS NOT ELIGIBLE

6. Pregnant? Yes □ No □
7. Lactation? Yes □ No □
8. Heavy smoker? Yes □ No □
   (more than 20 cigarettes per day)
9. Other diseases? Yes □ No □
   (HIV, Cardiovascular, Kidney, Thyroid)
10. Taking other medicine? Yes □ No □
    (for any other concomitant illness or study)

If „Yes“ to any questions 6 to 10, PATIENT IS NOT ELIGIBLE

Section B: Respondent Baseline Information

Enrolment Site: ____________________________
Date of birth: __ __/ __ __/ __ __ __ __
Age: _____________________________
Address: _____________________________
Phone (mobile & house): _____________________________
Sex: Female □ Male □
Ethnicity: _____________________________
Education: _____________________________
Employment: _____________________________
Height (m): _____________________________
Weight (kg): _____________________________
Investigator’s name: _____________________________

Signature: _____________________________
10.5 Informed consent for the patients

Place: Lung Hospital, Surabaya, Indonesia
Investigator: Budhi Setiawan, dr, Mkes

The effect of fermented soybean supplementation among active pulmonary tuberculosis patients with standard therapy in Indonesia

Declaration of Consent

The patient's name in block capitals

Born on .................................................. participant number ........................................

I am in a personal interview with the investigator

Name of the doctor

It has been elucidated fully and clearly regarding the tested treatment method and the comparison method and the essence, importance, implications and risks of the clinical trial. I have also read and understood the text of patient information, as well as the following printed privacy policy. I had the opportunity to speak with the investigator about the conduct of the trial. All my questions have been answered satisfactorily.

Opportunity for documentation of additional questions from the patient or any other aspects of the education conversation:

_________________________________________________________________________________

_________________________________________________________________________________

I had plenty of time to decide.

I understand that I can withdraw my consent to participate in the trial at any time and for any reason (oral or written) without cause any disadvantage for my medical treatment.
Research project: 147/13: *The effect of fermented soybean supplementation among active pulmonary tuberculosis patients with standard therapy in Indonesia.*

The Ethics Committee of the Justus-Liebig-University in Giessen/Germany on request of Prof. Dr. M. Krawinkel has discussed, evaluated and approved this project in its session of July 18th 2013 under ethical and scientific aspects. The responsible leader of this research project is Prof. Dr. M. Krawinkel, Giessen. The Ethics Committee raises no objection to this project, which may be an important scientific contribution.

For the Ethics Committee:

Prof. Dr. H. Tillmanns
MD., Chairman

Giessen, August 15th 2013
HEALTH RESEARCH ETHICS COMMITTEE
MEDICAL FACULTY OF
WIJAYA KUSUMA SURABAYA UNIVERSITY

“ETHICAL CLEARENCE”
No. 13/SLE/FK/UKWS/III/2013

HEALTH RESEARCH ETHICS COMMITTEE
MEDICAL FACULTY OF WIJAYA KUSUMA SURABAYA UNIVERSITY
HAVE STUDIED CAREFULLY THE PROPOSED STUDY DESIGN,
THEREFORE DECLARE THAT

RESEARCH ENTITLED:
“THE EFFECT OF FERMENTED SOYBEAN SUPPLEMENTATION AMONG
ACTIVE PULMONARY TUBERCULOSIS PATIENTS WITH STANDARD
THERAPY IN INDONESIA”

PRINCIPAL RESEARCHER:
Budi Setiawan, MD

THE RESEARCH SITE:
DOTS (Directly Observed Treatment, Short Course) outpatitnt department of
Surabaya Lung Hospital in Surabaya, East Java Province, Indonesia

DECLARE:
WORTHY OF ETHICS

Surabaya, 8 March 2013

The Dean Medical Faculty of
Wijaya Kusuma Surabaya University

Prof. Djanggan Sargowo, MD,PhD,
Sp.PD, Sp.JP(K), FIHIA, FACC,
FCAPC, FESC, FASCC

The Head Unit
Health Research Ethics Committee

Paulus Samuel Poli, MD,PhD,
10.8 Sample size calculation

![Sample size calculation using G*Power 3.1.5](image)

- **Test family**: Means: Difference between two independent means (two groups)
- **Statistical test**: Mean: Difference between two independent means (two groups)
- **Type of power analysis**: A priori: Compute required sample size - given α, power, and effect size
- **Input Parameters**:
  - Tail(s): Two
  - Effect size d: 0.500000
  - α err prob: 0.05
  - Power (1-β err prob): 0.8
  - Allocation ratio N2/N1: 1
- **Output Parameters**:
  - Noncentrality parameter: 2.8284271
  - Critical t: 1.9789056
  - df: 128
  - Sample size group 1: 64
  - Sample size group 2: 64
  - Total sample size: 128
  - Actual power: 0.8914290

- **Additional settings**:
  - n1 = n2
  - Mean group 1: 0
  - Mean group 2: 1.1
  - SD within each group: 2.2
  - SD group 1: 0.5
  - SD group 2: 0.5

- **Buttons**: Calculate and transfer to main window

---

85