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The Influence of Germination on Macronutrient Composition and Isoflavones Profile in Boiled Fermented Grobogan Soybean (Tempe)

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Abstract: High temperature treatment and fermentation are two major preparation methods applied by consumers to make soybean more edible. Germination is a simple way to induce certain bioactive compounds in soybeans. Objective: This study aimed to determine the impact of germination on macronutrient compositions and isoflavones' level in boiled tempe. Method: *Grobogan* soybean samples were sprouted at room temperature before the fermentation. The samples were fermented using 0.2% and 0.4% inoculum contained *Rhizopus oligosporus* before the boiling process. Fat, protein, and carbohydrate compositions were estimated by the soxhlet extraction, the Kjeldahl method, and spectrophotometric respectively. Genistein and daidzein contents in the samples were analysed by high-performance liquid chromatography (HPLC). Result: In comparison with ungerminated *Grobogan* tempe samples, based on dry weight, the germinated *Grobogan* tempe samples described a significant reduction in protein (24-hour germination period, $p=0.002$) and carbohydrate (24-hour and 48-hour germination periods, $p<0.01$) compositions. All germinated tempe samples had significantly higher on fat composition ($p<0.01$). Daidzein content was significantly higher in the sample with a 48-hour germination period and 0.4% starter ($p=0.008$) but genistein content was significantly lower ($p<0.01$) in the samples with a 24-hour germination period than the similar cultivar control sample. Conclusion: In a nutshell, boiled tempe made from germinated *Grobogan* soybean did not result in higher isoflavones level and might reduce macronutrient content.

Keywords: *germination, macronutrient, isoflavones, grobogan soybean*

1. Introduction

Fermentation of foods is a widespread phenomenon, with about one-third of present foods being fermented (Nout and Kiers, 2005). Tempe is a

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solid-state fermented food made from soybean inoculated with a fungus, usually *Rhizopus oligosporus*. However, the inoculation can also use other strains such as *Rhizopus oryzae, R. arhizus, R. stolonifer and R. microspores* (Astuti et al., 2000). Fermentation might improve the sensory, digestibility, and nutritional quality. Besides, it can reduce antigenic soy-proteins, which results in hypoallergenic soy food products (Frias et al., 2008).

Isoflavones are the most recognized subclasses of polyphenolic compounds, possessing antioxidant properties (Brouns, 2002). The aglycone family includes daidzein, genistein, and glycitein, while conjugated β-glucosides are daidzin, genestin, glycitin. Soybean isoflavones exist primarily as conjugated glucoside forms that are physiologically inactive. Aglycone is considered an active form. However, the production of the enzyme β-glucosidase by microorganisms used as starters and bacteria on the human intestine might reverse this process (Haron et al., 2009).

Dehulling, soaking, and boiling are common conventional preparation methods to make soybeans tender and to create a suitable environment for fermentation. Even though traditional cooking methods are not always disadvantageous for human health but they do affect the nutritional composition of foods. In soybean, nutrient and phenolic content might be lower after soaking and boiling (Haron and Raob, 2014). Conventional cooking methods, except steaming, can destroy nutritional and health-promoting compounds because of the high temperature involved (Yuan et al., 2009). Boiling is known as a domestic method that can reduce water-soluble compounds in foods because they leach into surrounding water because of cell lysis (Gliszczyńska-Swigłoet al., 2006).

Germination is the emergence of the radicle through the seed coat under favorable conditions for growth and development (Manz et al., 2005). The process of germinating might induce a modification of specific biologically active components in seeds. It was suggested that the addition of defatted soybean germ (hypocotyl) and cotyledon in a laboratory scale could be used to

increase the concentration of isoflavones in tempe (Nakajima et al., 2005). Germination and osmopriming also enhanced isoflavones content in Korean fermented unsalted soybean paste (Jeong et al., 2008). Regarding macronutrient composition in soybeans, crude protein and fat contents were increased, while the starch content was decreased, by the germination process (Kayembe and Jansen van Rensburg, 2013). Germination might be a potential option to improve the content of isoflavones in tempe. Therefore, this study sought to analyze the influence of germination on macronutrient composition, genistein, and daidzein levels in boiled tempe.

2. Material and Research Methods

2.1 Reagents and chemicals

Isoflavone standards namely genistein (≥98%), daidzein (≥98%) and n-hexane for HPLC (≥95%) were purchased from Sigma-Aldrich chemicals (St Louis, MO, USA). All other reagents used were analytical reagent grade.

2.2 Preparation of the samples

Grobogan soybean samples were sprouted in a domestic setting at room temperature for tempe preparation for 24 and 48 hours. The germinated soybean samples were boiled and fermented using 0.2% and 0.4% inoculum contained *Rhizopus oligosporus* for tempe preparation. Fermentation of soybean was prepared using a conventional method with two times boiling process before the inoculation. Before chemical composition analyses, the tempe samples were boiled in the pre-heated fresh tap water at 100^0C for 30 minutes. Soxhlet method was used for fat composition evaluation and the crude protein composition was determined by the Kjedahl method. For carbohydrate composition analyses, samples were assessed by the Anthrone method. The proximate analyses were expressed as percentages and the values were converted to a dry matter basis. Moisture percentages were subtracted from

100% and the results were converted to decimal. Then, the percentage of each guaranteed analysis was divided by that decimal. Genistein and daidzein levels in the boiled tempe samples were measured using high-performance liquid chromatography (HPLC).

2.3 Statistical analyses

Results were expressed as means and standard deviations. The means comparison of more than two groups was analysed by the univariate analysis of variance followed by Dunnett T3 post-hoc test. Tukey HSD post-hoc test was used to test carbohydrate and fat composition based on the dry weight. The level of significance among the different samples was set at $p<0.05$. All statistical analyses were performed using the SPSS software package.

3. Results and Discussion

3.1 Macronutrient composition

Proximate analyses in different boiled tempe samples are depicted in Table 1, while Table 2 summarizes the macronutrient compositions based on the dry weight. On the dry weight composition, proximate analyses showed that the protein composition in boiled tempe samples with a 48-hour germination period and higher inoculum concentration (0.4%) was significantly lower than the control samples without germination procedure ($p=0.008$). The reduction was about 4.6% during germination compared to the *Grobogan* cultivar control tempe samples. This outcome was in line with a similar study that also reported the reduction of protein in soybean (37.37 ± 2.07 vs. $17.99 \pm 3.33\%$, $p<0.05$) with the 48-hour germination period (Megat Rusydi et al., 2011). Veluppillai et al. (2009) described that the reduction of total protein composition during the germination process was simultaneous with the augmentation of amino acids due to an escalation of proteolytic activity. The decline of protein composition might be due to proteolysis exceed protein synthesis in the growing sprouts (Rodríguez et al., 2008). Another suggested explanation is that nitrogenous

matters (amino acids or short peptides) might be leached out due to pouring water during the germination procedure of soybean (Chen and Chang, 2015). However, other studies showed increases crude protein composition in soybean after germination (Kaushik et al., 2010; Shi et al., 2010). Nonogakiet et. al. (2010) proposed an explanation that protein synthesis happens in the imbibition process to restore cellular damage during the dry state of seeds.

On the other hand, the fat composition of the tempe samples based on dry weight with all germination periods was significantly higher than the control samples in the same cultivar. The tendency of increased fat composition in soybean due to germination was also consistent with a previous study (Kayembe and Jansen van Rensburg, 2013). A similar phenomenon has been observed among different plant seeds, such as ground beans (Echendu et al., 2009). The fat increment was likely due to a discrepancy in the dehulling process applied to soybean sprouts and non-germinated soybean. Higher hull portion removal of soybean sprouts could cause a higher proportion of cotyledon. Therefore, the fat composition might increase proportionately since it is one of the components that exist in a cotyledon (Ghavidel and Prakash, 2007). On the contrary, related studies reported the decrease of fat composition during germination due to the use of fat as energy during the germination process (Kaushik et al., 2010; Megat Rusydi et al., 2011).

In regards to carbohydrate composition based on dry weight; germination significantly reduced it in all tempe samples compared to the same cultivar control samples ($p<0.01$). This outcome was supported by other related studies (Kaushik et al., 2010; Shi et al., 2010; Megat Rusydi et al., 2011; Kayembe and Jansen van Rensburg, 2013). The decline of carbohydrate composition could be caused by starch breakdown in cotyledon into smaller molecules such as glucose and fructose to provide energy for cell division during maturation process (Vidal-Valverde et al., 2002; Nonogaki et al., 2010). Additionally, the carbohydrate breakdown could be related to α-amylase (Ohtsubo et al., 2005) or β-amylase (Suda et al., 1986).

3.2 Daidzein and genistein contents

Table 3 shows isoflavones' (daidzein and genistein) levels in different types of boiled tempe samples. Longer period of germination and higher inoculum concentration indicated higher in daidzein contents than the control samples from the same cultivar soybean. The increase of daidzein during soybean germination is consistent with that of Shi et al. (2010), who observed a significant increase of daidzein, especially after a 5-day germination period. However, different results were found on the genistein contents in tempe samples with the germination process. In comparison with the control sample of the same cultivar soybean, the germination procedure resulted in a significantly lower genistein content. Shorter periods of germination described lower genistein contents. Zhu et al. (2005) described a reduction in both daidzein and genistein contents in soybean seeds after germination. The changes of daidzein and genistein content during germination could vary and it might be due to different cultivars of soybeans used in studies (Jeong et al., 2008). However, an early phase of germination in soybeans could produce a higher total isoflavones content than longer periods of germination (Zhu et al., 2005; Shi et al., 2010).

Table 1. Proximate analyses in different types of boiled tempe samples*

	Tempe			Percentage of				
Sample	Soybean cultivar	Germination (hour)	Inoculum (%)	Protein	Carbohydrate	Fat	Moisture	Ash
A	*Grobogan*	24	0.2	13.72 ± 0.24^{a}	3.18 ± 0.15^{a}	6.54 ± 0.23^{a}	71.68 ± 0.29^{c}	2.03 ± 0.10^{a}
B	*Grobogan*	24	0.4	14.51 ± 0.11^{a}	3.85 ± 0.80^{ab}	6.73 ± 0.11^{a}	70.49 ± 0.35^{bc}	1.80 ± 0.20^{a}
C	*Grobogan*	48	0.4	14.55 ± 0.15^{ab}	4.08 ± 0.61^{ab}	7.83 ± 0.17^{b}	68.37 ± 0.11^{a}	2.21 ± 0.13^{a}
D	*Grobogan*	-	0.2	15.19 ± 0.19^{b}	4.74 ± 0.40^{c}	6.43 ± 0.19^{a}	68.29 ± 0.11^{a}	2.14 ± 0.14^{a}
E	Unknown†	-	0.2	15.21 ± 0.19^{b}	3.64 ± 0.08^{a}	7.67 ± 0.14^{b}	69.18 ± 0.14^{b}	1.73 ± 0.16^{a}

*UNIANOVA followed by Dunnett T3 post-hoc test (n=3)
† Common imported soybean from a local market

Different superscript letters (a-c) in the same row indicate significant difference ($p<0.05$).

Table 2. Macronutrient composition dry weight basis in different types of boiled tempe samples (n=3)

Tempe cultivar	Percentage of			
	Protein*	Carbohydrate*	Fat**	Dry matter**
A	48.20 ± 0.09^{b}	10.97 ± 0.05^{a}	22.89 ± 0.75^{b}	28.22 ± 0.19^{a}
B	49.26 ± 0.94^{b}	12.91 ± 0.06^{c}	22.69 ± 0.47^{b}	29.44 ± 0.38^{ab}
C	45.41 ± 0.24^{a}	12.67 ± 0.25^{c}	24.76 ± 0.51^{c}	32.01 ± 0.47^{c}
D	47.58 ± 0.19^{b}	14.88 ± 0.03^{d}	20.63 ± 0.30^{a}	31.74 ± 0.09^{c}
E	49.12 ± 0.61^{b}	11.68 ± 0.09^{b}	24.58 ± 0.21^{c}	30.88 ± 0.07^{b}

*UNIANOVA followed by Tukey HSD post-hoc test

**UNIANOVA followed by Dunnett T3 post-hoc test

Different superscript letters (a-d) in the same row indicate significant difference ($p<0.05$).

Table 3. Daidzein and genistein contents in different types of boiled tempe samples*

Tempe sample				Isoflavones	
Soybean variety	Germination (hour)	Inoculum (%)		Daidzein (µg/gr)	Genistein (µg/gr)
Grobogan	24	0.2		249.78 ± 3.88^{e}	246.67 ± 1.94^{a}
Grobogan	24	0.4		247.09 ± 0.79^{e}	263.07 ± 1.14^{b}
Grobogan	48	0.2		211.94 ± 0.42^{b}	272.65 ± 0.36^{c}
Grobogan	48	0.4		273.90 ± 3.58^{d}	274.11 ± 3.09^{cd}
Grobogan	-	0.2		231.85 ± 0.85^{e}	285.82 ± 1.83^{d}
Unknown†	-	0.2		98.60 ± 0.68^{a}	266.75 ± 3.92^{bc}

*UNIANOVA followed by Dunnett T3 post-hoc test (n=3)

† Common imported soybean from a local market

Different superscript letters (a-d) in the same row indicate significant difference ($p<0.05$).

4. Conclusion, Implication, Limitation

The influence of germination on boiled tempe, made from cultivar *Grobogan*, resulted in the reduction of protein and carbohydrate compositions

and an increase in fat composition. In regards to isoflavones' contents, germination caused an increase in daidzein content and a decline in genistein content. Therefore, boiled tempe made from *Grobogan* soybean sprout might not always produce a nutritionally superior product. Different germination periods, inoculum concentrations, and soybean cultivars might produce different results on macronutrient composition and isoflavones profile.

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