

## Antioxidative Capacity of Soyfoods and Soy Active Compounds

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**Key words:** soybean, ORAC, isoflavone, peroxy radical scavenging, soy saponin

Soyfood isoflavones and soyasaponins are effective compounds in terms of their health-promoting properties. Their chemical structure plays an important role in their antioxidative activity. Thus, six isoflavones and four soyasaponins that are targeted in soyfood were evaluated for their peroxy radical scavenging capacities by the hydrophilic-oxygen radical absorbance capacity (H-ORAC) method. The antioxidant capacity of non-fermented and fermented soyfoods was also determined by the same method. The results revealed that isoflavones showed higher peroxy radical scavenging capacities than soyasaponin, with their activities found to depend on their chemical structure. The aglycone isoflavones promoted higher H-ORAC values than glycoside and malonyl glycoside isoflavones, respectively. On the other hand, DDMP saponin promoted a higher H-ORAC value than its derived compound, group B saponin, and the aglycone soyasaponin. In the case of soyfoods, fermented soyfoods had higher antioxidative capacity than the non-fermented ones, especially the long-term fermented products. Soybean-koji miso presented the highest H-ORAC value, followed by natto, soy sauce, and tempeh. Moreover, lightness ( $L^*$ ) of miso and soy sauce showed a negative correlation with H-ORAC value probably due to browning substances which might derive from the amino-carbonyl reaction. Considering the high antioxidant capacity of fermented soyfoods, it might relate to aglycone isoflavones which promote strong radical scavenging capacity. Thus, fermented soyfoods, especially miso and natto, could be considered as health-promoting foods.

### INTRODUCTION

A peroxy radical was reported as a cause of non-communicable diseases, such as cardiovascular disease, cancer, and neurological disease [Pizzino *et al.*, 2017]. As its excess *in vivo* and lifestyle diseases, such as arteriosclerosis, are related, food that includes antioxidative components is strongly recommended to be consumed for these diseases prevention. Soybean is a familiar plant-based protein food component to people in Japan and Southeast Asia which additionally promotes the antioxidative effect due to its functional ingredients, such as isoflavones [Han *et al.*, 2009; Kim *et al.*, 2022], saponins [Yoshiki *et al.*, 2001; Yoshiki & Okubo, 1995], tocopherols [Carrera & Seguin, 2016], anthocyanins [Kähkönen & Heinonen, 2003; Zilic *et al.*, 2019], and proanthocyanins [Xu *et al.*, 2017]. However, rather than consuming soybeans as fresh, almost all of them are consumed as processed products. These soyfoods, *e.g.* miso [Matsuo & Hitomi, 2007], soy sauce [Long *et al.*, 2000], natto [Ping *et al.*, 2012], and tempeh [Chang *et al.*, 2009; Kameda *et al.*, 2018], have also been reported as a source of natural antioxidants. The thermal

processes, as well as pressurization and fermentation are used to obtain soyfoods. They could induce degradation and/or conversion of soybean native active ingredients and, in consequence, affect their functionalities [Chitisankul *et al.*, 2015; 2021; Khosravi & Razavi, 2021; Ping *et al.*, 2012].

The antioxidant properties of foods and food ingredients have been measured by various methods, including the hydrophilic-oxygen radical absorbance capacity (H-ORAC) assay [Ou *et al.*, 2001], which is an *in vitro* method for determining the antioxidative capacity by measuring peroxy radical-scavenging activity of water-soluble substances [Zhong & Shahidi, 2015]. Isoflavones [Han *et al.*, 2009; Kim *et al.*, 2022], 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) saponins [Yoshiki *et al.*, 2001; Yoshiki & Okubo, 1995], peptides [Sanjukta & Rai, 2016; Tonolo *et al.*, 2020], and browning substances [Ando *et al.*, 2003] are the main water-soluble antioxidants which could be extracted by the aqueous ethanol solution from soybeans and processed soybean foods. The isoflavones contained in soybeans undergo demalonylation and further aglycone conversion by cooking and processing [Toda *et al.*, 2000].

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Submitted: 14 August 2021

Accepted: 8 February 2022

Published on-line: 4 March 2022

Their antioxidative activity was evaluated by the low-density lipoprotein (LDL) oxidation method and it had been shown that aglycone isoflavones promote higher antioxidative activity than glycosides [Lee *et al.*, 2005]. On the other hand, the antioxidative activity of isoflavones was evaluated by the ABTS method [Ruiz-Larrea *et al.*, 1997] and the liposome oxidation method [Arora *et al.*, 1998], which showed no significant differences between the properties of glycoside isoflavones and their aglycones. In addition, DDMP saponins were reported to promote antioxidative capacity [Yoshiki *et al.*, 2001; Yoshiki & Okubo, 1995]. But it also was reported that DDMP saponin can be degraded by heating, fermenting, and aging [Chitisankul *et al.*, 2015; Omizu *et al.*, 2011]. However, there is no report about an evaluation of the antioxidant capacity of individual isoflavones, soyasaponins, and their derived compounds by the same methodology including several processed soybeans. Therefore, this research aimed to evaluate the peroxy radical scavenging capacities of isoflavones, soyasaponin, and several soyfood samples by the H-ORAC assay.

## MATERIAL AND METHODS

### Samples and reagents

Soyfoods were purchased from food supermarkets in Morioka (Iwate, Japan). The samples included non-fermented soyfoods (NFS) and fermented soyfoods (FS) as shown in Table 1. The 11 samples of six kinds of NFS included steamed soybean, young soybean, soymilk, soy beverage, tofu, and fried tofu. The nine samples of four kinds of FS included natto, tempeh, miso, and soy sauce.

All standard reagents were of HPLC grade. They included six isoflavone standard reagents: malonyldaidzin, malonylgenistin, daidzin, genistin, daidzein, and genistein (Wako Pure Chemical Industries, Ltd., Osaka, Japan); and soyasaponin standard reagents: soyasapogenol A and soyasapogenol B (Koshiro Company Ltd., Osaka, Japan). The 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH) (Wako Pure Chemical Industries, Ltd., Osaka, Japan), fluorescein sodium salt (Sigma Aldrich, Tokyo, Japan), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) were analytical grade reagents.

### Isoflavone and saponin preparation

Six isoflavones and soyasaponins, soyasapogenol A and soyasapogenol B, were dissolved and mixed well in a 70% (v/v) aqueous ethanol solution by vortexing or ultrasonication with the final concentration of 500  $\mu\text{M}$  or 1 mM solution, respectively. DDMP saponin  $\beta\text{g}$  was extracted and purified from soybean hypocotyl [Chitisankul *et al.*, 2015]. Purified DDMP saponin  $\beta\text{g}$  and group B saponin Bb were analyzed using HPLC [Chitisankul *et al.*, 2019], and the purity of the purified saponin was 95% or higher. The purified DDMP saponin solution was composed of 852.1  $\mu\text{M}$  saponin  $\beta\text{g}$  and the group B saponin solution was composed of 1118  $\mu\text{M}$  saponin Bb.

### Sample preparation

Liquid soyfoods were directly extracted by 10-fold volume (w/v) of a 70% (v/v) aqueous ethanol solution.

TABLE 1. Code, name, and description of soyfoods including non-fermented (NF) and fermented (F) products.

Code	Sample name	Sample description
NF1	Steamed soybean	Steamed whole seeds
NF2	Edamame	Heated and frozen green (young) soybean
NF3	Soymilk	Regular type soymilk
NF4	Soymilk w/o LOX	Soymilk from lipoxygenase enzyme deficient soybean
NF5	Modified soymilk	Blended soymilk
NF6	Soybean beverage	Beverage from whole soybean (including okara)
NF7	Black soybean beverage	Beverage from whole black soybean (including okara)
NF8	Momen tofu	Regular type tofu
NF9	Silken tofu	Very soft tofu
NF10	Abura-age	Twice-fried tofu, deep fried thin sliced tofu
NF11	Nama-age	Deep fried tofu
F1	Natto	Regular type natto
F2	Black soybean natto	Black soybean natto
F3	Tempe	Fermented soybean with <i>Rhizopus</i> spp.
F4	Rice miso, sweet – white	Rice-koji miso: sweet-white type
F5	Rice miso, light yellow	Rice-koji miso: light yellow type
F6	Barley miso	Barley-koji miso
F7	Soy miso	Soybean-koji miso
F8	Dark soy sauce	Dark soy sauce
F9	Light soy sauce	Light soy sauce

The sample solution was homogenized (15,500 rpm, 1 min, Polytron homogenizer, Jakarta, Indonesia) and left to stand for 1 h with every 10 min vortex mixing. The supernatant was obtained by centrifugation at 27,000 $\times g$  and 15°C for 15 min (Kubota 7780 centrifuge, Tokyo, Japan) as a crude extract, and then filtered with a 0.45  $\mu\text{m}$  membrane filter. For all tofu samples, the outer part was cut off with a ceramic knife, then the samples were fined in a mortar. Steamed soybean, edamame, natto, tempeh, and miso were crushed with a mallet and then fined in a mortar. Each solid soyfood sample was extracted in the same manner as liquid samples. The crude extracts were kept in the opaque bottles at -20°C until analysis.

### Color measurement

For miso and soy sauce, CIE- $L^*$  (lightness),  $a^*$  (green to red color),  $b^*$  (blue to yellow color) were measured using a colorimeter (color meter Z-300A, Nippon Denshoku Industries Co., Ltd., Tokyo, Japan). Miso was packed in a Petri dish, the lid was put on, and the reflectance was measured. In addition, soy sauce was placed in a glass cell and permeation was measured. Miso was triplicate measured per sample, and soy sauce was measured once per sample.

### Peroxyl radical scavenging capability evaluation

Hydrophilic-oxygen radical absorbance capacity (H-ORAC) assay was used to measure peroxyl radical scavenging capability [Ou *et al.*, 2001]. The standard and sample solution were diluted with phosphate buffer (75 mM, pH 7.0), then the Trolox standard solution of 6.25  $\mu$ M to 100  $\mu$ M was used as a standard curve. The H-ORAC values of isoflavone and soyasaponin were reported as mol Trolox equivalent (TE)/mol and those of the processed soybean food as mmol Trolox equivalent (TE)/100 g sample. A fluorescence plate reader (Bio-Tek FL600, Bio-Tek Instruments, Inc., Winooski, VT, USA) was used for measurements.

### Statistical analysis

Analysis of variance (ANOVA) of the experimental data was performed and the least significant difference was evaluated by Tukey's test at a 95% confidence interval. The correlation coefficient (*r*) of the experimental data was analyzed. All analyses were repeated in triplicate.

## RESULTS AND DISCUSSION

### Peroxyl radical scavenging capacity of isoflavones and soyasaponins

The peroxyl radical scavenging capacity of isoflavones and soyasaponins had been shown as H-ORAC values (mol TE/mol) in Figure 1. Isoflavones presented higher antioxidative capacity than soyasaponins. The chemical structure of active ingredients promoted different activities. For isoflavones, high H-ORAC values presented in the order of aglycone form then glycoside form, and malonyl glycoside form. Daidzein had the highest antioxidant ability, with the H-ORAC value reaching  $9.94 \pm 0.45$  mol TE/mol, followed by genistein, genistin, daidzin, and malonyl glycoside isoflavone, respectively. The result was supported by a previous report which revealed that the antioxidative activity of aglycones (daidzein, genistein) was higher than that of glycosides (daidzin, genistin) depending on the method of measuring the lag time of LDL oxidation (lag time assay) [Lee *et al.*, 2005]. Recently, Kim *et al.* [2022] also found that the ABTS<sup>•+</sup> scavenging activity and ferric-reducing antioxidant power (FRAP) of soy isoflavones as aglycone were higher than those of their glycosides, and malonyl glycoside derivatives had the lowest

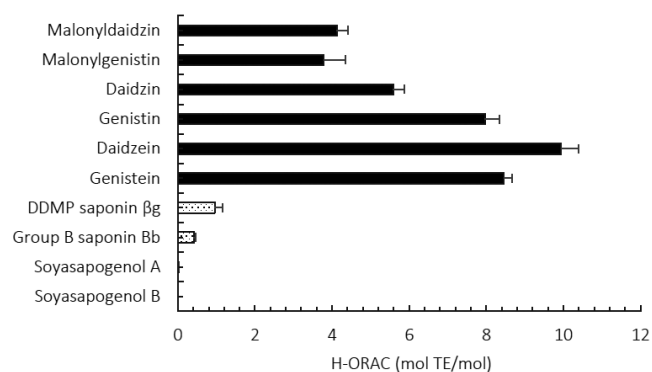


FIGURE 1. Hydrophilic-oxygen radical absorbance capacity (H-ORAC) of isoflavones and saponins.

activity. Isoflavones are generally found as glycoside forms in natural sources, but their bioavailability is lower compared to aglycones [Kim *et al.*, 2022; Setchell *et al.*, 2002]. However, the glycoside isoflavone would be converted to aglycone by gut microflora [Bultosa, 2016].

On the other hand, high H-ORAC values of soyasaponins are presented in the order of DDMP glycoside (DDMP saponin β) then glycoside (group B saponin Bb), and aglycone (soyasapogenol A, soyasapogenol B) (Figure 1). According to this statement, it clearly shows that the changing chemical structure affects the antioxidant activity of the compound. The degradation of DDMP saponin resulted in antioxidant activity reduction, which was agreeable with other reports [Yoshiki *et al.*, 2001; Yoshiki & Okubo, 1995]. The reports also revealed that the DDMP site is involved in superoxide scavenging ability [Yoshiki *et al.*, 2001; Yoshiki & Okubo, 1995], thus group B saponin Bb which lacks the DDMP site will not present scavenging activity. Following the evaluation of soyasaponin peroxyl radical scavenging activity in this research, DDMP saponin β presented strong peroxyl radical scavenging activity while group B saponin Bb presented low peroxyl radical scavenging activity. These results suggest that DDMP saponin β can scavenge both peroxyl radicals and superoxide. Although, group B saponin Bb cannot scavenge superoxide [Yoshiki *et al.*, 2001; Yoshiki & Okubo, 1995], it can eliminate peroxyl radical. On the other hand, the aglycone saponins: soyasapogenol A and soyasapogenol B, showed no scavenging activity against peroxyl radicals (Figure 1). From the above findings, it was speculated that the peroxyl radical scavenging ability was related to the DDMP site and sugar chain portion of soyasaponin. Nevertheless, DDMP saponin could be naturally found in fresh soybean or low processed soybean products which might vary depending on soybean variety and processing treatment [Chitisankul *et al.*, 2019, 2021].

### Peroxyl radical scavenging capacity of non-fermented soyfoods

The nutraceutical property due to the antioxidative capacity of 11 non-fermented soyfoods (NFS) differed and depended on the type of products (Figure 2), namely to their processing treatments. The NFS products could be categorized into three major groups: (1) steamed soybean (NF1) and frozen boiled edamame (NF2) as low-processed products, (2) soymilk or soy beverages (NF3-NF7), and (3) tofu (NF8-NF11). Only heat processes were applied to obtain the products of the first group whilst several treatments such soaking, extraction, and heating were required to produce other NFS. Among all NFSs, the steamed soybean showed the highest H-ORAC value ( $2.35 \pm 0.31$  mmol TE/100 g). However, H-ORAC of the second low-processed product – edamame (young soybean seed), was much lower. It could be assumed that the difference was due to the content of the main antioxidative compounds, isoflavones, and soyasaponins. Indeed, it was reported that contents of daidzein and genistein in soybean were at 0.25–1.23 mg/g and 0.33–1.17 mg/g, respectively [Wu *et al.*, 2004]. On the other hand, edamame contained daidzein and genistein in the amounts of 0.11–0.55 mg/g and 0.16–0.62 mg/g, respectively [Wu *et al.*, 2004]. Moreover, as reported in the previous

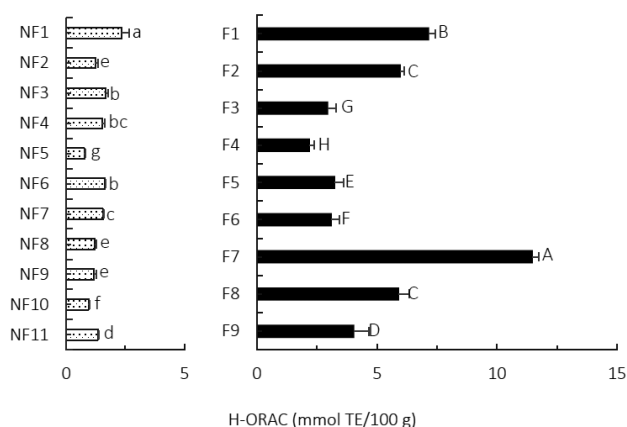


FIGURE 2. Hydrophilic-oxygen radical absorbance capacity (H-ORAC) of non-fermented (NF) and fermented (F) soyfoods. Details on the samples NF1–N11 and F1–F9 are provided in Table 1. Different letters present significant differences between samples ( $p < 0.05$ ), lowercase letter for non-fermented soyfoods and uppercase letter for fermented soyfoods.

research, edamame contained much less soyasaponins than mature soybeans, and more active DDMP saponins were degraded to group B saponin during heating [Chitisankul *et al.*, 2021]. Thus, the different antioxidant capacities of these low-processed samples might not be related to the food process but varied depending on the maturity of soybean.

For soymilk and soy beverages, four of five samples showed non-significant differences in H-ORAC values and only H-ORAC of modified soymilk (NF5) was significantly ( $p < 0.05$ ) lower (Figure 2). Soy solid content of soymilk and soy beverages might play a significant role in their functional properties. On the report of product labeling, solid contents of regular (non-modified) soymilk (NF3), lipoxygenase-deficient soymilk (prepared from a soybean variety ‘Kinusayaka’ deficient in all of 3 lipoxygenase isozymes) (NF 4) and soy beverage (NF6) were 9 to 14 g/100 g or more, whereas the modified soymilk (NF5) contained about 7 g/100 g. Therefore, it could be considered that soy solid content might play an important role in antioxidative capacity in soymilk and soy beverages. In turn, black soybean beverage (NF7) was expected to be a rich antioxidative compound source. The previous study revealed that black soybean, in addition to isoflavones and saponins, contained significant amounts of anthocyanins and proanthocyanidins [Xu *et al.*, 2017; Zilic *et al.*, 2019] and showed significantly higher antioxidative capacity than regular soybean [Chitisankul *et al.*, 2019]. However, anthocyanins are unstable water-soluble components that could be degraded by enzymatic reaction and thermal treatment [Slavu(Ursu) *et al.*, 2020]. The thermal treatment is generally required to preserve and process food for safety purposes. The traditional soymilk process might require thermal treatment at 99°C while steam-infusion treatment might facilitate a higher temperature of about 99–154°C [Johnson *et al.*, 1981]. In the last group of NFS samples, tofu products showed the lowest peroxyl radical scavenging capacity, especially abura-age, twice-fried tofu (NF10) (Figure 2). There was no significant difference in H-ORAC between momen tofu (NF8) and silken tofu (NF9), with their H-ORAC values being lower than that of nama-age, fried tofu (NF11).

In summary, there was a significant difference in H-ORAC values among each group of NFS soyfoods. It was considered that the slight difference found was mainly due to the difference in the isoflavone and saponin contents and the respective compositions caused by the difference in the food processing treatment such as thermal process, extraction, and coagulation. This indicates that the bioactive compounds may change upon food processing and that each food product requires a different treatment process. The chemical structures of active compounds such as isoflavones and soyasaponins have an important role in nutraceutical property in soyfood as mentioned above. Malonyl glycoside, which is the main component of soybean isoflavone, is unstable to heat and derived from malonyl glycoside to glycoside and further to aglycone upon thermal treatment [Kasuga *et al.*, 2006; Toda *et al.*, 2000]. DDMP saponin can be degraded to group B saponin by thermal treatment and group E saponins by lipoxygenase-induced radical reaction during grinding [Chitisankul *et al.*, 2015]. It was considered that the non-fermented soybean foods had different antioxidative capacities due to their isoflavone and DDMP saponin compositions, depending on the processing treatment during the manufacturing process.

#### Peroxl radical scavenging capacity of fermented soybean food

Fermented soyfoods (FS) could be categorized into four groups; natto (F1 and F2), tempeh (F3), miso (F4–F7), and soy sauce (F8 and F9). Among all FS samples, soy miso showed the highest H-ORAC value followed by natto, soy sauce, other types of miso (F5 and F6), and tempeh, respectively (Figure 2). In the comparison of NF and FS samples, all FS products had higher H-ORAC values than NFS samples. The H-ORAC value of FS samples ranged from  $2.21 \pm 0.19$  to  $11.53 \pm 0.41$  mmol TE/100 g while whole soybean seed (NF1) had 2.35 mmol TE/100 g.

High antioxidative capacity of black soybean natto was expected because, as mentioned above, the seed coat of black soybean is rich in anthocyanins. Moreover, the previous research revealed that the H-ORAC value of black soybean was  $5,870 \pm 115$   $\mu$ mol TE/100 g while that of soybean was  $4,369 \pm 418$   $\mu$ mol TE/100 g [Chitisankul *et al.*, 2019]. However, the previous research also reported that isoflavone content of soybean was higher than that of black soybean [Chitisankul *et al.*, 2019]. Thus, it could be considered that isoflavones make a significant contribution to the peroxyl radical scavenging capacity instead of anthocyanins in natto. In addition, the H-ORAC value of natto was 3-fold higher compared to steamed soybean. Therefore, it clearly showed that the fermented process of natto could induce nutraceutical properties as enhancing antioxidant capacity. The bio-transformation of isoflavones plays an important role to enhance those functionalities. The consistent reports explained the glycoside conjugates of isoflavones could be converted to isoflavone aglycones by  $\beta$ -glucosidase of *Bacillus subtilis* during fermentation [Dajanta *et al.*, 2009; Khosravi & Razavi, 2021; Ping *et al.*, 2012]. Hence, the enhanced antioxidative capacity of regular soybean natto compared to black soybean natto might be due to three reasons: regular soybean had a higher isoflavone content, aglycone isoflavones presented

stronger antioxidative capacity than anthocyanins, and degradation of water-soluble anthocyanins during natto production resulted in functionality losses. Moreover, it was found that natto had a higher antioxidant capacity than steamed soybeans (Figure 2). The browning substances derived from the amino-carbonyl reaction [Ando *et al.*, 2003] and antioxidant peptides released from proteins during fermentation [Sanjukta & Rai, 2016; Tonolo *et al.*, 2020] could additionally contribute to the antioxidant potential of natto.

Although soy-miso (F7) showed the highest antioxidant activity, other kinds of miso (F4-F6) presented much lower peroxy radical scavenging capacity especially the sweet-white type of rice miso (F4) with H-ORAC value of  $2.21 \pm 0.19$  mmol TE/100 g (Figure 2). In the consent report, the antioxidant properties of different kinds of miso were evaluated by determining 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and superoxide scavenging activities [Matsuo & Hitomi, 2007]. The soy-miso presented the highest antioxidative capacity following dark-yellow rice miso, light yellow rice miso, and sweet-white rice miso. And barley miso showed similar property with dark-yellow rice miso. Moreover, it was reported that the DPPH radical scavenging ability and the superoxide scavenging activity were strongly positively correlated with the total isoflavone content and aglycone content, respectively [Matsuo & Hitomi, 2007]. During *Aspergillus* spp. fermentation in miso, the  $\beta$ -glucosidase was produced and activated to hydrolyze glycoside isoflavone to aglycone type isoflavones [Yamabe *et al.*, 2007; Yan *et al.*, 2016]. Furthermore, during the malting process in soy miso, aglycones (daidzein and genistein) could be converted to *o*-dihydroxy-isoflavones (ODI) while rice miso and barley miso do not contain ODI [Esaki *et al.*, 2001b]. It was reported that ODI also contribute to the antioxidant properties of miso, since the higher the amount of ODI, the higher the antioxidant capacity [Esaki *et al.*, 2001a]. Additionally, the lower lightness ( $L^*$ ) of the miso sample was revealed as related to higher antioxidative capacity. It was speculated that the browning substance derived from the amino-carbonyl reaction during fermentation and aging could contribute to the peroxy radical scavenging activity of miso. For soy sauce, the H-ORAC value of dark-colored soy sauce (F8) was higher than that of light-yellow soy sauce (F9) (Figure 2). Similarly to miso, soy sauce undergoes fermentation and aging which result in aglycone isoflavone production; but there was a low total isoflavone content in soy sauce [Toda *et al.*, 2000]. Although the aglycone isoflavones were formed during the soy sauce production, they were detected as remaining in soy sauce cake, a by-product [Esaki *et al.*, 2004]. However, a positive correlation was found between the ODI content of soy sauce and its antioxidative capacity, and it was reported that ODI also contributed to the antioxidative capacity of soy sauce [Esaki *et al.*, 2002]. According to this evaluation, it is considered that the peroxy radical scavenging activity of soy sauce, which was recognized by the ORAC value, is related to the browning substance derived from the amino-carbonyl reaction and ODI. Tempeh (F3) had a similar H-ORAC value as rice miso and barley miso, but lower than that of natto (Figure 2).  $\beta$ -Glucosidase produced by *Rhizopus filamentous* fungi, which is used for tempeh fermentation, hydrolyzes

TABLE 2. Color parameters of miso and soy sauces.

Soybean foods	$L^*$	$a^*$	$b^*$
Rice miso: sweet-white type	$65.33 \pm 0.03^a$	$4.35 \pm 0.05^d$	$35.37 \pm 0.03^c$
Rice miso: light yellow type	$48.82 \pm 0.04^b$	$9.33 \pm 0.08^a$	$41.96 \pm 0.02^a$
Barley miso	$44.86 \pm 0.08^c$	$6.86 \pm 0.01^b$	$35.68 \pm 0.08^b$
Soy miso	$10.90 \pm 0.09^d$	$5.73 \pm 0.26^c$	$8.24 \pm 0.26^d$
Dark soy sauce	14.16	29.92	23.38
Light soy sauce	43.64	36.73	72.23

Values for miso are expressed as mean  $\pm$  standard deviation ( $n=3$ ); for soy sauce value  $n=1$ . Different letters in column presented significant differences ( $p < 0.05$ );  $L^*$ , lightness;  $a^*$ , redness;  $b^*$ , yellowness.

isoflavone glycosides into aglycones [Kameda *et al.*, 2018]. It was suggested that the isoflavone aglycone produced during fermentation might contribute to the antioxidant properties of tempeh [Murakami *et al.*, 1984].

### Color of fermented soybean food vs. peroxy radical scavenging capacity

The color parameters of long-term fermented soyfood samples, miso, and soy sauce were measured and  $L^*$ ,  $a^*$ , and  $b^*$  values are shown in Table 2. The relationship of color parameters with antioxidative capacity was also evaluated. The  $L^*$  value of miso decreased in order of sweet-white rice miso, light yellow rice miso, barley miso, and soy miso, the darkest miso. The  $a^*$  value of light yellow rice miso was slightly higher than that of the other four miso samples. There was no significant difference in the  $b^*$  value among miso samples, except soy-miso which showed the lowest yellowness. On the other hand, dark soy sauce showed lower  $L^*$ ,  $a^*$ , and  $b^*$  values than light soy sauce. After  $L^*$ ,  $a^*$ ,  $b^*$  value measurement, the correlations between these values and peroxy radical scavenging capacity were found for miso and soy sauce. The results revealed that there were no significant correlations of H-ORAC values with  $a^*$  and  $b^*$  values. On the other hand, a strong negative correlation ( $r = -0.9747$ ,  $p = 0.0048$ ) was found between the  $L^*$  and H-ORAC values of miso samples and soy sauce, excluding soy miso (Figure 3). Therefore, the low  $L^*$  value, darkness

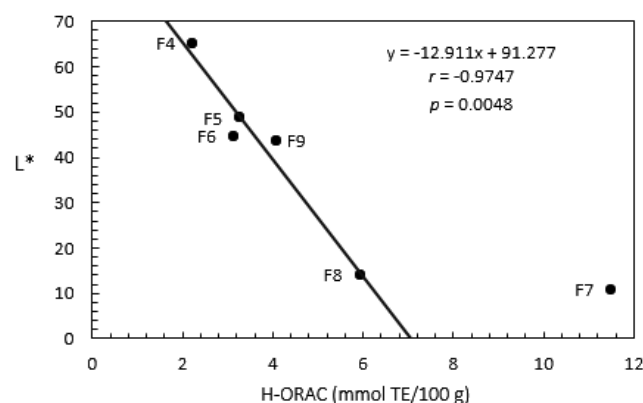


FIGURE 3. Correlation between lightness ( $L^*$ ) and hydrophilic-oxygen radical absorbance capacity (H-ORAC) of long-term fermented soyfoods; miso (F4-F7) and soy sauces (F8-F9). For code of samples see Table 1. The correlation coefficient ( $r$ ) and significance level ( $p$ ) were calculated excluding soy miso (F7).

of soy sauce or miso, might be an index of browning reagent contents, the amino-carbonyl reaction product which induced a higher H-ORAC value. The result was supported by other consistent reports. The correlations of  $L^*$  value with both DPPH radical and superoxide scavenging activities of various miso paste samples were negative [Matsuo & Hitomi, 2007]. In our study, soybean miso presented a significantly higher H-ORAC value than the expected H-ORAC value based on the  $L^*$  value (Figure 3). It could be explained that soybean miso had a higher ratio of soybean content than other kinds of miso, and the soybean miso production might be different too. Therefore, the strong peroxy radical scavenging capacity of soybean miso might be due to the high content of isoflavones, saponins, ODI [Esaki *et al.*, 2001a,b], and antioxidant peptides released from proteins [Sanjukta & Rai, 2016].

## CONCLUSIONS

Since the H-ORAC method is an *in vitro* method for measuring antioxidant capacity, its results might not directly reflect the antioxidative capacity *in vivo*, but it can be a simple primary screening tool for antioxidant capacity measurements in many soyfoods. Fermented soybean could promote higher antioxidant properties compared to non-fermented soyfood due to peroxy radical scavenging capacity. The chemical structure of bioactive compounds in soybean plays an important role in the antioxidant properties of soyfood products. The technological processes could cause transformations of those healthy functional compounds and thus enhance or diminish the antioxidative capacity. The experiment demonstrated that derived isoflavone as aglycone could promote higher antioxidative activity while derived soyasaponins, such as group A and group B saponins, showed lower activity than DDMP-saponin. According to the H-ORAC value of soyfood sample, fermented soyfood presented the highest antioxidant capacity which was due to a high aglycone isoflavone content. Furthermore, it was also considered that peptides and free amino acids produced by aging also contributed to the antioxidant capacity of fermented soybean foods. Among all processed soyfood samples, the soy-miso sample had the highest H-ORAC value followed by natto samples. Eventually, considering the serving size and antioxidant capacity, natto is the most effective product compared to miso or soy sauce having a high salt content. However, long-term fermented soyfood should still be in the focus of interest to researchers as health promoting food. There is a lack of data on their minor bioactive components such as antioxidant peptides and ODI. Thus, these minor components should be considered for further study.

## RESEARCH FUNDING

This research was partly supported by “JSPS RON-PAKU (Dissertation Ph.D.) Program 2012 (Grant ID No. THA – 11206)” and “Kasetsart University Research and Development Institute”.

## CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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