Vietnam Journal of Science and Technology ***61*** *(4) (2023) 580-589*

doi:10.15625/2525-2518/17218

Hydrolysis of glycoside phytoestrogens of the extract from soy germ: A comprehensive study

Le Minh Chau[1, 2, *], Do Thi Hoa Vien[2], Ho Phu Ha[3]

[1]*Faculty of Food Technology, University of Economics - Technology for Industries, 456 Minh Khai Street, Hai Ba Trung, Ha Noi, Viet Nam*

[2]*Department of Biotechnology, School of Biotechnology and Food Technology, Ha Noi University of Science and Technology, Ha Noi, Viet Nam*

[3]*Department of Food Technology, School of Biotechnology and Food Technology, Ha Noi University of Science and Technology, Ha Noi, Viet Nam*

[*]Email: *lmchau.uneti@moet.edu.vn*

Received: 13 June 2022; Accepted for publication: 23 September 2022

Abstract. Phytoestrogens which mimic mammalian estrogens structurally and functionally are phenolic compounds. The aglycone phytoestrogens in soy germ including daidzein, genistein, and glycitein are known to have higher biological activity than other derivative forms. This study aimed to assess the potential of pure β-glucosidase to hydrolyze glucoside phytoestrogens from soy germ to increase aglycones content. The optimal conditions for the hydrolysis of aglycones from soy germ evaporated extract included a β-glucosidase content of 4 units/g of defatted soy germ flour and an incubation time of 5.5 hours. Under these conditions, the hydrolysis of β-glycosides, malonyl glycosides, and acetyl glycosides were more than 90 %, 60 %, and 60 %, respectively. The phytoestrogen aglycones content was 31.36 ± 0.06 µmol/g (14.97 ± 0.03 µmol/g daidzein, 7.58 ± 0.05 µmol/g glycitein, and 8.81 ± 0.02 µmol/g genistein), 5 times higher than that of the defatted soy germ flour.

Keywords: soy germ, phytoestrogen, isoflavone, aglycone.

Classification numbers: 1.2.1, 1.3.2

1. INTRODUCTION

Phytoestrogens which are derived from “Phyto” and “estrogen” are phenolic compounds including isoflavones, stilbene, coumestan, and lignan. The main phytoestrogens in the form of isoflavones are mainly found in the bean family. The amount of isoflavones in soy germ is about five to six times higher than that in cotyledons and higher than seed coat and whole seeds [1]. Soy isoflavones include aglycones (daidzein, genistein, glycitein), glycoside forms (daidzin, genistin, glycitin), acetyl glycoside forms (acetyl daidzin, acetyl genistin, acetyl glycitin), and the malonyl glycoside forms (malonyl daidzin, malonyl genistin, and malonyl glycitin) [2]. The β-glucoside conjugates, acetyl glycosides, and malonyl glycosides are inactive forms of isoflavones. The aglycone forms account for only about 2 - 5 % of total isoflavones but are

rapidly absorbed because of their hydrophilic capacity and lower molecular weight, showing higher biological activity than other derivatives in digestion [3, 4]. Moreover, isoflavones, primarily genistein and daidzein, show their phytoestrogenic activity with many health benefits including chemopreventive activity against breast and prostate cancer, and the ability to modify carcinogenesis, e.g. initiation, promotion, and cancer progression [5]. The conversion of isoflavone glycosides to aglycones can be achieved using chemical agents such as HCl [6] or base, or biological agents including enzyme β-glucosidase (cellobiase) [7], galactosidase [8], cellulase [9], and microorganisms capable of producing β-1–4 glycoside binding enzyme [10]. The most common and effective agent is the enzyme β-glucosidase. The main sources of enzyme β-glucosidases are widely distributed in plants (almonds), filamentous fungi, mammals, and microorganisms, for example, *Aspergillus niger, Phanerochaete chrysosporium, Agrobacterium* sp., and *Thermotoga martima.* To deconjugate isoflavone glycoside to biologically active aglycones, the β-glucosidic linkage between β-glycoside and aglycones must be broken. Previous studies on soy germs focused only on analyzing the content of β-glycosides and their aglycones and not on other conjugated forms such as malonyl glycosides and acetyl glycosides because their concentrations in some samples were too low to be determined [7, 11], or due to the point that β-glucosidase only hydrolyzed β-glycosides [12, 13]. In this study, all 12 isoflavones of soy germ were analyzed and the effect of β-glucosidase on all glycosides was determined to increase aglycones in the extract.

2. MATERIALS AND METHODS

2.1. Materials

Soy germ with a size of 0.1 mm was supplied by Vinanusoy Viet Nam Co., Ltd (commercially available) and β-glucosidase from almonds with a content of 4 U/g was purchased from Sigma-Aldrich with a purity of 98 % (EC: 3.2.1.21). Phytoestrogens standards (Wako Pure Chemical Industries Ltd.) were used for HPLC analysis. Other reagents were of analytical grade and were purchased from Fisher Scientific (USA).

2.2. Methods

Soy germ was defatted using 95 % n-hexane at a solid/liquid ratio of 1:5 and was shaken at 180 rpm for 5 hours. The defatted soy germ with a moisture content of 5.79 ± 0.09 % was packed in a dark glass grinder and stored at – 4 ^{o}C until further analysis. The content of total isoflavones in the defatted soy germ was 1748.01 ± 8.25 mg/100g (40.80 ± 0.17 µmol/g).

Preparation of total phytoestrogen extract from soy germ

The total phytoestrogen extraction was conducted as follows: defatted soy germ flours were added with 65 % ethanol, at pH 9, and the solid/liquid ratio was 1:12; extraction time was 90 minutes. The liquid extract was separated from insoluble fractions by filtration. The extraction was then evaporated under vacuum at 45 °C [14, 15].

Method of β-glucosidase enzyme activity analysis

β-glucosidase activity was determined according to Ghose [16] with some improvements by measuring the hydrolysis rate of 15 mmol of cellobiose. One unit of enzyme activity was defined as the amount of β-glucosidase that releases 1 µmol of glucose per minute. Glucose was measured using a D-glucose Assay Kit (GOPOD Format) - Megazyme.

Hydrolysis of soy germ extract by enzyme β-glucosidase

The enzyme β-glucosidase hydrolyzed glycoside isoflavones to aglycone forms. The extract of total phytoestrogens from 2 g of defatted soy germ flour was adjusted to pH 5 using 0.02 N HCl and kept at room temperature for 1 hour. Then, this cloudy suspension was centrifuged at 6000 rpm, at 4 ^{o}C for 10 minutes to remove insoluble matter [9].

The enzyme β-glucosidase was added at various concentrations (1, 2, 3, 4, 5 U/g of defatted soy germ) in sodium phosphate buffer (pH 5.0) until the volume of each sample was 10 mL, followed by incubation at 37 ^{o}C for 4.0; 4.5; 5.0; 5.5; 6.0 hours.

The enzyme hydrolysis conversion (%) was defined as follows:

$$Enzymatic\ hydrolysis\ conversion\ (\%) = \frac{M_0 - M_1}{M_0}.100\%$$

M_0: the content of glycoside forms before enzymatic hydrolysis (µmol/g); M_1: the content of glycoside form after enzymatic hydrolysis (µmol/g)

HPLC analysis

The HPLC analysis method was developed by the National Institute of Nutrition according to the Song method with some improvements [17].

Each test sample (0.5 g or 2 mL) was added with 10 mL of acetonitrile and 2 mL of 0.1 M HCl, then added with 4 mL of deionized water for the flour sample and 2 mL of deionized water for the liquid sample with constant shaking on an orbital shaker at ambient temperature. Samples were sonicated for 10 minutes and shaken for two hours at temperatures below 25 °C. Each mixture was centrifuged at 5000 rpm for 30 minutes, then 1 mL of the extract was placed in a test tube and blown dry with N_2. Standards were dissolved in a test tube with 1 mL of methanol and sonicated for 10 minutes. Samples were shaken for 1 minute and then filtered through a 0.45 µm PTFE membrane before being injected into the HPLC system.

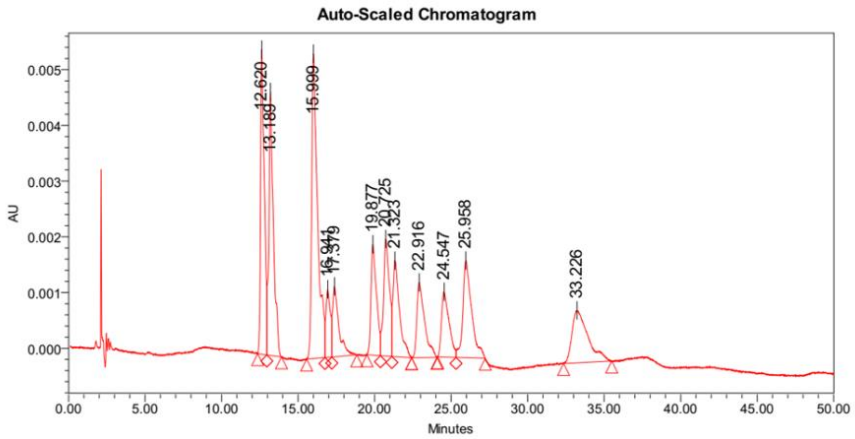

Figure 1. Calibration curves of standard.

Phytoestrogens were analyzed by Alliance System, Waters, USA equipped with a Zorbax SB-C18 (5 µm × 4.6 mm × 150 mm). The HPLC conditions were set at a column temperature of 35 ^{o}C, detection wavelength of 260 nm, mobile phases A - 0.1 % acetic acid and B - acetic acid/acetonitrile 20/80, flow rate of 1.0 mL/min. The detection was carried out under linear gradient elution with mobile phase percentage changing from A 88 %, B 12 % to A 60 %, B 40 % and was completed at A 88 %, B 12 %. The quantification of each phytoestrogen was performed by integrating the chromatographic peak areas into the calibration curves (Figure 1).

Statistical analysis: All measurements were conducted in triplicate and were statistically analyzed using the analysis of variance (ANOVA). Duncan's multiple range test using the SPSS software program version 25 (SPSS Inc., Chicago, IL, USA) were performed. The significance of the difference was defined at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Quantification of phytoestrogens in soy germ extract

Phytoestrogens content from defatted soy germ flour, and the soy germ extract before enzymatic hydrolysis (after evaporation and pH adjustment) were analyzed by the HPLC method, as shown in Table 1.

Table 1. Content of phytoestrogens.

Phytoestrogens	Content of phytoestrogens (µmol/g)	
	Soy germ defatted flour	Soy germ extract before enzymatic hydrolysis
Daidzin	7.18 ± 0.03	9.57 ± 0.03
Glycitin	3.86 ± 0.03	5.14 ± 0.03
Genistin	3.95 ± 0.02	5.63 ± 0.02
Malonyl daidzin	0.95 ± 0.01	1.32 ± 0.01
Malonyl glycitin	0.57 ± 0.00	1.16 ± 0.01
Malonyl genistin	5.81 ± 0.10	5.77 ± 0.02
Acetyl daidzin	7.74 ± 0.01	7.77 ± 0.02
Acetyl glycitin	0.29 ± 0.01	0.39 ± 0.01
Acetyl genistin	4.73 ± 0.02	4.77 ± 0.02
Daidzein	1.79 ± 0.03	1.92 ± 0.02
Glycitein	2.43 ± 0.01	2.72 ± 0.01
Genistein	1.52 ± 0.00	1.74 ± 0.01
Total β-glycosides	14.99 ± 0.07	20.35 ± 0.08
Total malonyl glycosides	7.32 ± 0.10	8.25 ± 0.03
Total acetyl glycosides	12.76 ± 0.03	12.93 ± 0.01
Total glycosides	35.07 ± 0.17	41.52 ± 0.10
Total aglycones	5.73 ± 0.02	6.38 ± 0.01
Total phytoestrogens	**40.80 ± 0.17**	**47.90 ± 0.11**

The content of total phytoestrogens in defatted soy germ flour was 1748.01 ± 8.25 mg/100 g (40.80 ± 0.17 µmol/g, ending with 35.07 ± 0.17 µmol/g total glycosides and 5.73 ± 0.02 µmol/g total aglycones).

The phytoestrogens content of soy germ extract before enzymatic hydrolysis was 47.90 ± 0.11 µmol/g (41.52 ± 0.10 µmol/g total glycosides and 6.38 ± 0.01 µmol/g total aglycones), which was higher than that in defatted soy germ flour due to evaporation.

3.2. Hydrolysis of glycosides in soy germ extract by enzyme β-glucosidase

Hydrolysis of glycosides by enzymatic processes using β-glucosidase from almond (EC: 3.2.1.21) to increase aglycones - their bioavailability in soy products was conducted (Figure 2). The effect of enzyme concentration on glycoside conversion is shown in Table 2.

Table 2. Effect of enzyme concentration on glycoside conversion.

Number	Hydrolysis conversion (%)	Enzyme concentration (U/g)				
		1	2	3	4	5
1	Daidzin	76.33	87.40	91.69	94.76	90.67
2	Glycitin	52.42	77.57	85.58	90.68	85.00
3	Genistin	64.40	83.72	87.74	92.31	86.29
4	Malonyl daidzin	49.84	57.65	57.83	57.84	58.34
5	Malonyl glycitin	68.02	69.42	65.34	66.02	67.53
6	Malonyl genistin	64.29	65.73	64.01	64.18	64.21
7	Acetyl daidzin	66.77	69.32	69.64	70.03	70.01
8	Acetyl glycitin	52.53	53.90	53.09	50.02	53.32
9	Acetyl genistin	61.30	62.33	54.08	54.32	54.54
	Total β-glycosides	66.70	83.78	88.98	93.00	87.95
	Total malonyl glycosides	62.58	65.01	63.24	63.46	63.78
	Total acetyl glycosides	64.17	66.08	63.02	63.24	63.42

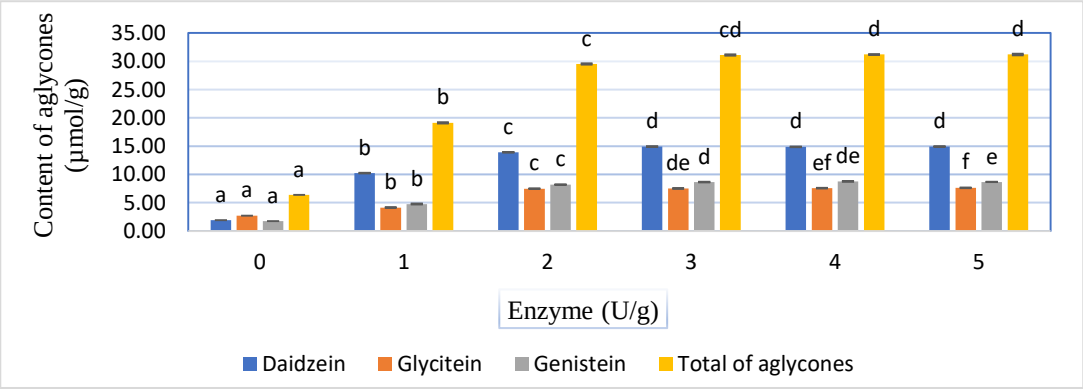

Figure 2. Effect of enzyme concentration on aglycones content.

Finally, after the enzymatic hydrolysis, an enzyme concentration of 4 U/g and an incubation time of 4 hours were selected, and the total aglycone under this condition was 31.21 ± 0.05 (µmol/g), including 14.88 ± 0.04 µmol/g daidzein; 7.58 ± 0.04 µmol/g glycitein, and 8.75 ± 0.05 µmol/g genistein. The effect of incubation time on glycosideconversion is shown in Table 3.

Table 3. Effect of incubation time on glycoside conversion.

Number	Hydrolysis conversion (%)	Incubation time (h)				
		4.0	4.5	5.0	5.5	6.0
1	Daidzin	94.76	94.85	94.84	94.96	95.62
2	Glycitin	90.68	90.85	91.38	91.49	92.26
3	Genistin	92.31	92.54	92.67	92.72	93.42
4	Malonyl daidzin	57.04	57.79	58.90	59.59	59.38
5	Malonyl glycitin	66.02	65.29	64.43	66.11	64.60
6	Malonyl genistin	51.79	53.13	53.44	56.63	56.10
7	Acetyl daidzin	70.83	71.37	70.80	70.40	69.98
8	Acetyl glycitin	50.02	51.65	52.10	53.19	47.67
9	Acetyl genistin	51.04	54.63	53.51	53.80	48.19
	Total β-glycosides	93.00	93.15	93.32	93.42	94.12
	Total malonyl glycosides	63.46	63.44	63.46	63.36	62.93
	Total acetyl glycosides	63.24	64.18	63.44	63.35	62.66

As can be seen in Table 3, when increasing the incubation time from 4 to 6 hours, the hydrolysis conversion of total β-glycosides slightly increased, but the hydrolysis conversion of malonyl glycosides and acetyl glycosides did not increase.

The effect of incubation time on the content of each aglycone and total aglycones is shown in Figure 3.

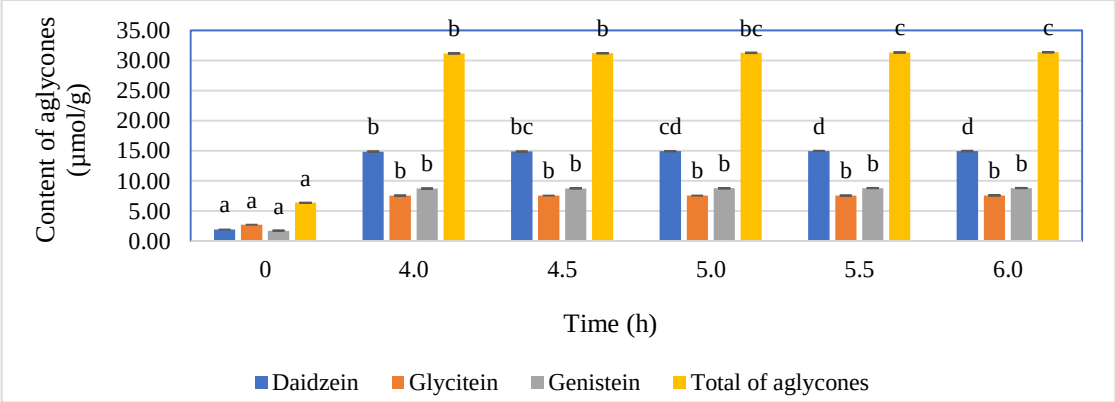

Figure 3. Effect of incubation time on the aglycones content.

The content of total aglycones at the time of incubation for 5.5 hours was also higher than that after 4 hours of incubation ($p < 0.05$), which was 31.36 ± 0.06 μmol/g (including 14.97 ± 0.03 μmol/g daidzein, 7.58 ± 0.05 μmol/g glycitein, and 8.81 ± 0.02 μmol/g genistein).

3.3. Discussion

3.3.1. Content of phytoestrogens from soy germ

The isoflavone content of soy germs from Vinanusoy was also consistent with isoflavones content in the varieties from Thailand and Korea in previous reports, for example, the isoflavones content of Chieng Mai-60 soy germ was 30.60 μmol/g [18], the isoflavones content of Korean soy germ varieties ranged from 1110.9 to 3131.1 mg/100g [19]. The aglycones concentration in the soy germ sample was 8.9 %, which was quite higher than the average concentration in previous reports (2 - 5 %). Studies showed that the glycoside forms were found in higher concentrations in soy germ. The composition of glycosides in the sample was also very different from that of Korean embryos. This result was due to genetic differences in varieties, cultivars, breeding conditions, seasons, climate, and locations [18, 20, 21]. The amount of glycosides composition was in the following order: β-glycosides > acetyl glycosides > malonyl glycosides. In contrast, Korean varieties were in order: malonyl glycosides > β-glycosides > acetyl glycosides [22]. This was advantageous because β-glycosides were conjugated and could be biotransferred to aglycones with the highest conversion rate (more than 90 %). Some Korean varieties lacked glycitein and its derivatives [19], therefore they could not bring full knowledge of these substances, but in our study, the defatted soy germ flour sample had full 12 components of isoflavones. Instead of only three aglycones, the development of a database to investigate the levels of all 12 dietary isoflavones is necessary.

3.3.2. Hydrolysis of glycoside phytoestrogens in soy germ

The effects of enzyme concentration on glycoside conversion

It could be seen that all conjugated β-glycosides, malony glycosides, and acetyl glycosides were converted into aglycones. For β-glycoside forms, when 1.0 U/g β-glucosidase was added, only 76.33 % of daidzin, 52.42 % of glycitin, and 64.40 % of genistin were converted after 4 hours, which might be the result of a low rate of enzymatic hydrolysis. With increasing enzyme concentration to 4 U/g, the conversions were both significantly increased ($p < 0.05$) and reached the maximum value, the conversions of β-glycosides were 94.76 %, 90.68 %, and 92.31 % for daidzin, genistin, and glycitin, respectively. At an enzyme concentration of 5 U/g, the hydrolysis conversion decreased. This could be explained by the fact that the final product, glucose molecules, had a direct inhibitory effect on β-glucosidase activity. Kinetic studies of glucose inhibition were an area of debate, although the actual inhibition mechanism was largely dependent on the sources of enzymes [23]. The second reason for the decrease in hydrolysis conversion was that when glucose and other soluble molecules dissolved during hydrolysis, they were bound to water molecules, leaving the enzymatic system lacking water to carry out the hydrolysis reaction [24]. The hydrolysis conversion of malonyl glycosides and acetyl glycosides under the enzyme β-glucosidase was more than 60 %, which was observed in some reports on the hydrolysis of isoflavone glycosides in soy milk by β-glucosidase [8, 25] with the metabolism of malonyl and acetyl forms reaching about 50 % and 60 % of the baseline [25]. A similar result was also reported by Ismail *et al*. [26] and it was possible that the enzyme and substrate possessed specific complementary geometric shapes that fit exactly into one another, β-glucosidase acted on isoflavone glycosides as a "Lock and Key" model (Fisher 1894) [8]. The effect of enzyme concentration on the content of aglycones and total aglycones is shown in Figure 1.

The effect of incubation time on glycoside conversion

In general, when the enzyme concentration was highest, the rate of enzymatic reactions increased rapidly during the first 30 minutes of the reaction. It was difficult to determine exactly the optimum incubation time of enzyme-catalyzed reactions with two or more substrates or

products. Most isoflavoneβ-glycosides were hydrolyzed after 4 hours except for malonyl and acetyl glycosides. The incubation time depended on many factors, such as the total isoflavone glycoside content and the enzyme concentration. In contrast, in the study of Pham *et al*., after 240 minutes of hydrolysis by β-glucosidase, about 93 % of the total isoflavone glycosides of soy milk were hydrolyzed. Malonyl and acetyl glycosides were still present at 240 minutes, although at a very low concentration [8]. In this experiment, the incubation time was 5.5 hours, which is longer than the time of 5 hours for glycosides of Chiang Mai-60 soybean embryo to be hydrolyzed to aglycones by β-glucosidase G0395 [7], and shorter than the optimal time of 7.5 hours for the hydrolysis of soybean embryos using the response surface methodology with the Plackett-Burman model [11].

In summary, the biotransformation conditions of isoflavone glycosides in soy germ extract were 4 U/g enzyme β-glucosidase and 5.5 hours. After the hydrolysis, the content of aglycones was 31.36 ± 0.06 µmol/g (14.97 ± 0.03 µmol/g daidzein, 7.58 ± 0.05 µmol/g glycitein, 8.81 ± 0.02 µmol/g genistein). The ratio of aglycones/total isoflavones (% mol) in the extract was 77.62 % (including 36.69 % daidzein, 18.57 % glycitein, and 21.60 % genistein) which was 5 times higher than that of the defatted soy germ flour.

4. CONCLUSIONS

Soy germ is a remarkable source of bioactive phytochemicals due to its isoflavone aglycones. The conversion of glycosides to aglycones by β-glucosidase could increase the production of aglycones. It could be seen that all conjugated β-glycosides, malony glycosides, and acetyl glycosides were converted into aglycones. The hydrolysis conversion of conjugated β-glycosides was highest at more than 90 %, followed by malonyl glycosides and acetyl glycosides at about 60 %. The conditions for hydrolysis of glycosides to aglycones were 4 U/g enzyme β-glucosidase and 5.5 hours. The content of aglycones (µmol/g) in the extract was 5 times higher than that of the defatted soy germ flour.

Acknowledgments. The authors are grateful to the National Institute of Nutrition for the support in the HPLC analysis.

CRediT authorship contribution statement. Le Minh Chau: Methodology, Investigation, Funding acquisition, Formal analysis. Do Thi Hoa Vien: Supervision. Ho Phu Ha: Supervision.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Yue X., Abdallah A. M., and Xu Z. - Distribution of Isoflavone and antioxidant activities of soybean cotyledon, coat, and germ, Journal of Food Processing and Preservation **34** (5) (2009) 795-806. https://doi.org/10.1111/j.1745-4549.2009.00395.x.
2. Wang H. and Murphy P.A. - Isoflavone content in commercial soybean foods, Journal of Agricultural and Food Chemistry **42** (8) (1994) 1666-1673. https://doi.org/10.1021/jf00044a016.
3. Izumi T., Piskula M. K., Osawa S., Obata A., Tobe K., Saito M., Kataoka S., Kubota Y., and Kikuchi M. - Soy aglycones are absorbed faster and in higher amounts than their

glucosides in humans, The Journal of Nutrition **130** (7) (2000) 1695-1699. https://doi.org/10.1093/jn/ 130.7.1695.

4. Le M. C., Do T. H. V., and Ho P. H. - Study on antioxidant activity of phytoestrogen extracts from soy germ, Vietnam Journal of Science and Technology **58** (2020) 270-279. https:// doi.org/10.15625/2525–2518/58/6A/15606.
5. Forslund L. C. and Andersson H. C. - Phytoestrogens in foods on the Nordic market, 2017, Nordic Council of Ministers.
6. Zheng B., Yegge J.A., Bailey D.T., and Sullivan J.L. - Patent US5679806A Process for the isolation and purification of isoflavones, Hauser Inc: United State of America, 1997.
7. Tipkanon S., Chompreeda P., Haruthaithanasan V., Suwonsichon T., Prinyawiwatkul W., and Xu Z. - Optimizing time and temperature of nezymatic conversion of isoflavone glucosides to aglycones in soy germ flour, Journal of Agricultural and Food Chemistry **58** (21) (2010) 11340-11345. https://doi.org/10.1021/jf103090p.
8. Pham T.T. and Shah N. - Hydrolysis of isoflavone glycosides in soy milk by β–galactosidase and β–glucosidase, Journal of Food Biochemistry **33** (1) (2009) 38-60. https://doi.org/ 10.1111/j.1745–4514.2008.00206.x.
9. Wang M. - An effective and green method for the extraction and purification of aglycone isoflavones from soybean, Food Science and Biotechnology **22** (3) (2013) 705-712. https:// doi.org/10.1007/s10068–013–0135–4.
10. Fujita A., Alencar S., and Park K. Y. - Conversion of isoflavone glucosides to aglycones by partially purified β–glucosidases from microbial and vegetable sources, Applied biochemistry and biotechnology **176** (2015) 1659-1672. https://doi.org/10.1007/s12010–015–1668–1.
11. Tipkanon S., Charoen R., Savedboworn W., and Moursy A.A. - Hydrolysis optimization of isoflavone conversion in soy germ (Chiang Mai 60) using response surface methodology (RSM), King Mongkut's University of Technology North Bangkok, Int. J. Appl. Sci. Technol. **8** (2) (2015) 127-135. https://doi.org/10.14416/j.ijast.2015.02.001.
12. Nemitz M. C., Fachel F. N. S., Beckenkamp A., Buffon A., Teixeira H. F., and Poser G. L. V. - Soybeans isoflavone aglycone–rich extracts: Optimization by different bioprocesses and production of a purified fraction with promising wound healing property, Industrial Crops and Products **105** (2017) 193-202. https://doi.org/10.1016/ j.indcrop.2017.04.065.
13. Hati S., Ningtyas D. W., Khanuja J. K., and Prakash S. - β–Glucosidase from almonds and yoghurt cultures in the biotransformation of isoflavones in soy milk, Food Bioscience **34** (2020) 100542. https://doi.org/10.1016/j.fbio.2020.100542.
14. Le M. C., Do T. H. V., and Ho P. H. - Optimization of phytoestrogens extraction from soy germ using response surface methodology, Journal of Military Science and Technology **74** (2021) 70-78 (Vietnamese).
15. Le M. C. and Do T. H. V. - Optimization of phytoestrogen extraction from soy germ, in Proceeding of The 13th SEATUC Symposium 2019.
16. Ghose - Measurement of cellulase activities, Pure and Applied Chemistry **59** (2) (1987) 257-268. https://doi.org/10.1351/pac198759020257.
17. Daud A., Sulistyarti H., Retnowati R., and Ginting E. - High Performance Liquid Chromatography (HPLC) Method for determination of isoflavones content in shade–

tolerant soybean Dena I, IOP Conference Series: Materials Science and Engineering **546** (3) (2019) 032004. https://doi.org/10.1088/1757–899x/546/3/032004.

18. Tipkanon S., Chompreeda P., Haruthaithanasan V., Prinyawiwatkul W., No H. K., and Xu Z. - Isoflavone content in soy germ flours prepared from two drying methods, International Journal of Food Science & Technology **46** (11) (2011) 2240-2247. https://doi.org/10.1111/ j.1365–2621.2011.02742.x.
19. Lee K. S., Woo S. Y., Lee M. J., Kim H. Y., Ham H., Lee D. J., Choi S. W., and Seo W. D. - Isoflavones and soyasaponins in the germ of Korean soybean [Glycine max (L.) Merr.] cultivars and their compound–enhanced BMP–2–induced bone formation, Applied Biological Chemistry **63** (1) (2020) 26. https://doi.org/10.1186/s13765–020–00508–y.
20. Carrão–Panizzi M. C. and Bordingnon J. R. - Activity of beta–glucosidase and levels of isoflavone glucosides in soybean cultivars affected by the environment, Pesquisa Agropecuária Brasileira **35** (2000) 873-878. https://doi.org/10.1590/S0100–204X20000005 00002.
21. Carrão–Panizzi M. C., Berhow M., Mandarino J. M. G., and Oliveira M. C. N. D. - Environmental and genetic variation of isoflavone content of soybean seeds grown in Brazil, Pesquisa Agropecuária Brasileira **44** (2009) 1444-1451. https://doi.org/10.1590/ S0100–204X2009001100011.
22. Kim J. A., Hong S. B., Jung W. S., Yu C. Y., Ma K. H., Gwag J. G., and Chung I. M. - Comparison of isoflavones composition in seed, embryo, cotyledon and seed coat of cooked–with–rice and vegetable soybean (Glycine max L.) varieties, Food Chemistry **102** (3) (2007) 738-744. https://doi.org/10.1016/j.foodchem.2006.06.061.
23. Xiao Z., Zhang X., Gregg D. J., and Saddler J. N. - Effects of sugar inhibition on cellulases and β–glucosidase during enzymatic hydrolysis of softwood substrates, Applied Biochemistry and Biotechnology **115** (1) (2004) 1115-1126. https://doi.org/10.1385/ ABAB: 115:1–3:1115.
24. Puri D. J., Heaven S., and Banks C. J. - Improving the performance of enzymes in hydrolysis of high solids paper pulp derived from MSW, Biotechnology for biofuels **6** (2013) 107-107. https://doi.org/10.1186/1754–6834–6–107.
25. Tsangalis D., Ashton J. F., McGill A. E. J., and Shah N. P. - Enzymic transformation of isoflavone phytoestrogens in soymilk by β–glucosidase–producing Bifidobacteria, Journal of Food Science **67** (8) (2002) 3104-3113. https://doi.org/10.1111/j.1365–2621.2002. tb08866.x.
26. Ismail B. and Hayes K. - β–glycosidase activity toward different glycosidic forms of isoflavones, Journal of Agricultural and Food Chemistry **53** (12) (2005) 4918-4924. https:// doi.org/10.1021/jf0404694.