J Food Sci Nutr
Vol 12, p 209～216 (2007)
DOI: 10.3746/jfn.2007.12.4.209

J Food Science and Nutrition

Physicochemical and Functional Properties of Germinated *Glycine max* Merr Soybeans

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Abstract

To investigate the applicability as the functional food materials of germinated *Glycine max* Merr soybeans, its biochemical characteristics and its abilities to inhibit platelet aggregation and hydrolyze alcohol were examined. With the progression of germination time, crude protein content gradually increased, and on the 5th day of germination it was 30.19%. However, crude fat content tended to decrease, and on the 5th day of germination it was 14.30%. Total amino acid content was highest on the 3rd day of germination at 80,875 mg%. The free amino acid content doubled from day 0 of germination (1,273.35 mg%) until the 5th day of germination (2,742.99 mg%). Fatty acid analysis revealed that linoleic acid was highest among all the samples, ranging from 53.55～56.00%. Linolenic acid content slightly increased as the germination period was prolonged. The ability to inhibit platelet aggregation increased according to the germination period and then decreased again on the 5th day of germination; it was somewhat higher in the ethanol fraction. In measuring ADH, we found that the activity of the ethanol fraction increased with increasing days of germination. In the case of the water fraction, the activity decreased as germination was prolonged, and the ADH activity of the water fraction was higher than that of the ethanol fraction. Based on the above results, we deemed that the *Glycine max* Merr soybeans germinated for 2～3 days were most pertinent for use as functional food materials.

Key words: *Glycine max* Merr, germination, chemical constituents, platelet aggregation, ADH activity

INTRODUCTION

Glycine max Merr is an annual plant belonging to the leguminosae species, in which 2～3 black beans are contained in a pod. It is also referred to as *Rhynchosia Molubilis*. Consistent with the common usage of soybean, *Rhynchosia Molubilis* has been used as *Glycine Semen Germinatum*, *Glycine Semen Preparatum*, etc., and widely used as a medicinal herb (1). Recently, due to the extraction of glycitein from the skin of *Glycine max* Merr, which has a potent anti-oxidant effect, various products exploiting the functional properties of *Glycine max* Merr are in development. The most common use *Glycine max* Merr in international as well as Korean foods is as beans, including the skin that contains isoflavones, anthocyanins, etc. (2). Isoflavones are physiologically active components in beans of key interest, and *Glycine max* Merr contains more than other soybeans. Among them, genistein shows anti-oxidant effects by removing toxic reactive oxygen, and it interferes with the production of stress proteins such as heat shock protein (HSP), which allows cancer cells to evade immune system attack, as well as glucose related proteins (GRPs); hence, it is reported to mediate anti-cancer effects on breast, rectal, and prostate cancers (3,4).

Germinated beans, or bean sprouts, are a traditional Korean food, and because the growth period is short, cultivating them is relatively easy and inexpensive; thus, they are a widely popular food (5). Particularly, during germination and growth, the nutrient composition is greatly altered, with lipid reduced, fiber increased, vitamins C and A greatly increased, digestibility increased, and gas generating factors as well as the activation of trypsin inhibitors are decreased (6). In bean sprouts, ascorbic acid, more widely known as vitamin C, is concentrated in the root of the sprout and exerts the function of degrading alcohol, and thus, it ameliorates hangovers. It also suppresses the increase of lactic acid, and hence, sprouts are known to accelerate recovery from influenza and fatigue. Recently, its popularity is on the rise not only in the East, but also in the West (7). *Glycine Semen Germinatum* is dried immature bean sprouts (1.5～2

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cm), which have been described in the Donguibogam (8) as having a sweet taste and as non-toxic. *Glycine Semen Germinatum* is an essential component for the production of Woo Whang Chong Shim Won, and it is effective for edema, muscle aches, and hypotension. It also settles the stomach, is used as a tonic medicine and antitoxic agent; asparagine, xanthine, hypoxanthine, and vitamin C are among its important components (9). The germination process has long been investigated as a means to elucidate complex biochemical mechanisms, and recently, based on the fact that germination alters the composition of chemical components together with nutrients and functional value, efforts have been made to increase the value of this food (10).

In this study, to investigate the applicability of germinated *Glycine max* Merr soybeans as a functional food material, which has been used as a component of herbal medicine, we examined its biochemical characteristics during germination, as well as the ability of its extracts to inhibit platelet aggregation and degrade alcohol.

MATERIALS AND METHODS

Materials

The *Glycine max* Merr soybeans used in the experiments were produced in Korea in 2003. It was washed and sterilized with 3% NaClO solution for 2 minutes, soaked in 25°C water for 2 hours, and germinated for 5 days at 25°C using an automatic bean sprout cultivator (KSP-1000, KM Control Electronic Co.) (11). In order to use the *Glycine max* Merr in the examination of its biochemical and functional characteristics, it was freeze-dried and ground to a size smaller than 20 mesh.

Extraction method

The extract was used to evaluate the ability to suppress platelet aggregation and the ability to degrade alcohol. Thirty grams of the freeze-dried ground material was added to 5 volumes of ethanol, extracted using a reflux extractor for 2 hr three times at 80°C and filtered. The filtrate was then pressure-concentrated to a constant volume of 10 mL and fractionated with alcohol. Water was then added to the residue, extracted by the same method, and then fractionated with water.

Determination of growth characteristics in the germinated *Glycine max* Merr soybeans

The *Glycine max* Merr soybeans were harvested according to its germination period (0 day, 1 day, 2 days, 3 days, 4 days, and 5 days), and used to determine weight, length, and thickness. The length and thickness were measured by randomly harvesting 30 *Glycine max* Merr soybeans each. The measurements were performed with calipers (Mitutoyo Co., Japan), and average values were obtained.

Determination of protein and crude lipids

The crude protein and crude fat of the samples were quantitated according to the AOAC method (12). Briefly, the crude protein was extracted by the Kjeldahl method, and the crude fat was extracted by the Soxhlet extraction method using diethyl ether. Each sample was measured 3 times repeatedly, and the mean values are presented.

Determination of amino acid content

The total amino acid contents were analyzed by hydrolyzing the material with 6 N-HCl, filtering with a 0.45 μm membrane filter, and then analyzing by an automatic amino acid analyzer (13). For the free amino acid content, 75% ethanol was added to the sample and extracted. The protein was precipitated by adding 25% trichloroacetic acid (TCA) solution and then centrifuged. From this filtrate, color and lipid-soluble substances were removed by using diethyl ether, dissolving in the loading buffer solution (0.2 N sodium citrate, pH 2.2), filtering through a 0.45 μm membrane filter, and then analyzing by an amino acid autoanalyzer (14).

Determination of fatty acids

To analyze the fatty acids, the crude fat was extracted by diethyl ether and hydrolyzed with 1 N KOH/EtOH solution according to the method of Metcalf et al. (15). Then, BF_3-methanol was added and methyl esterization was carried out, with subsequent GC analysis. The analysis conditions are shown in Table 1.

Determination of inhibition of platelet aggregation

The ability to inhibit platelet aggregation was measured using an aggregometer (Chrono-log Model No. 490, Havertown, PA, USA) (16) and expressed as sample

Table 1. GC conditions for fatty acid analysis

Items	Conditions
Instrument	Shimadzu GC-17A
Detector	Flame ionization detector
Column	HP-5 (30 m × 0.32 mm × 0.25 μm film thickness) column
Column temp.	180°C (1 min) →200°C (2°C/5 min) →230°C (2°C/2 min)
Injector, detector temp.	250°C, 220°C
Split ratio	20:1

concentration required to inhibit platelet aggregation by 50% (IC_{50}). First, for the separation of the platelets, rats were anesthetized with diethyl ether, the abdomens were opened, and the blood was drawn with a syringe containing an anti-coagulation ACD solution (12.5 g of trisodium citrate dihydrate, 7.5 g of citric acid monohydrate, 10 g of glucose). Then, the samples were centrifuged at room temperature, the upper platelet rich plasma (PRP) was obtained, and centrifuged again. To the precipitated platelets, washing buffer (11.9 mM $NaHCO_3$, 0.33 mM NaH_2PO_4, 163.3 mM NaCl, 2.8 mM KCl, 1.1 mM $MgCl_2$, 11.2 mM α-D-glucose, 2.0 mM EDTA, 0.35% bovine serum albumin, pH 7.4) was added. The washed platelets obtained by centrifugation were resuspended with the suspension buffer (11.9 mM $NaHCO_3$, 0.33 mM NaH_2PO_4, 16.3 mM NaCl, 2.8 mM KCl, 1.1 mM $MgCl_2$, 11.1 mM α-D-glucose, pH 7.4), and the number of platelets was adjusted to 5×10^8/mL by a hemacytometer (Superior, Germany). In the experiments for platelet aggregation, 470 μL of separated platelet suspension was adjusted to 37°C, inserted into the hole of the aggregometer, and preincubated at 1,000 rpm for 5 minutes. Then, 10 μL of $CaCl_2$ solution (final concentration 1.0 mM) was added and reacted for 2 minutes. Next, 10 μL of water (control) was added and reacted for 2 minutes. Finally, the inducer of platelet aggregation ADP (final concentration 10 μM) was added and reacted for 5 minutes, and the transmittance was measured. The rate of the inhibition of platelet aggregation (%) was calculated as follows.

$$\text{Inhibition of platelet aggregation } \% = \frac{A-B}{A}\times 100$$

A: change in transparency of control, %
B: change in transparency of sample, %

Determination of ADH (alcohol dehydrogenase) activity

ADH activity was determined by measuring the light absorbance of NADH formed at 340 nm using a UV-spectrophotometer. Briefly, 0.2 mL of alcohol, 0.4 mL of NAD, and 0.1 mL of extract were added to a test tube, which was adjusted to a total volume of 5 mL with 0.05 M Tris buffer. It was then incubated for 10 minutes in a 25°C water bath, and 0.04 mL of ADH was added and reacted for 35 minutes. The control group was treated identically, except 0.04 mL of 0.05 M Tris buffer was added instead of ADH. Immediately after the reaction, the tests tubes were transferred to ice, and the ADH activity was measured using a spectrophotometer at 340 nm (17,18).

RESULTS AND DISCUSSION

Change of growth characteristics

The changes in length, weight, and thickness during the germination period of *Glycine max* Merr soybeans are shown in Table 2. As the germination period became longer, the lengths of the hypocotyls and cotyledons gradually increased, where the increases were evident around 4 days after germination. The weight also increased as the germination period was prolonged, and on the 5th day of germination it was approximately 0.73 g. The thickness during the germination period was 5.21 ~5.31 mm, but a large change was not detected. Nam (19) showed that increases in weight were primarily associated with increases in water content, and thus, weight showed a similar trend to the increase of water content.

Changes in crude protein and crude lipid

Bean sprouts are a good source of vegetable protein (20). In this study, the crude protein content (Table 3) increased as sprouting continued, which is consistent with other research showing increased crude protein and nitrogen content (21). The crude protein content of the bean sprouts germinated for 5 days was approximately 30.19%, which was somewhat lower than that reported by Koh et al. (22) where the crude protein content of *Glycine max* Merr soybeans were 39.06%. The crude fat content of the bean sprouts did not change substantially up to 3 days after germination (Table 3), but around the 4th day it showed a decreasing tendency; on the 5th day of germination it was 14.30%. Kim (23) reported

Table 2. Changes in growth characteristics of *Glycine max* Merr sprouts germinated for 5 days

Growth character	Part	Germination period (day)					
		0	1	2	3	4	5
Length (mm)	Whole	6.50±0.47	18.23±1.45	40.99±2.60	68.15±7.54	133.35±9.11	196.33±8.87
	Seed leaf	−	10.64±0.91	11.03±0.52	11.13±0.84	11.50±0.69	12.71±0.55
	Root	−	7.59±1.11	29.97±2.28	57.03±7.41	121.86±9.99	183.62±8.67
Weight (g)	Whole	0.13±0.22	0.23±0.04	0.30±0.04	0.34±0.04	0.53±0.07	0.73±0.06
	Seed leaf	−	0.22±0.04	0.24±0.03	0.22±0.03	0.25±0.03	0.28±0.03
	Root	−	0.01±0.00	0.06±0.01	0.16±0.02	0.28±0.04	0.45±0.03
Thickness (mm)	Seed leaf	5.03±0.33	5.21±0.39	5.25±0.33	5.25±0.63	5.21±0.37	5.31±0.22
	Root	−	1.09±0.14	1.97±0.25	2.00±0.37	1.96±0.17	2.02±0.13

Table 3. Changes in crude protein and crude lipid contents of *Glycine max* Merr sprouts germinated for 5 days

Germination period (day)	Crude protein (dry base, %)	Crude fat (dry base, %)
0	25.68±0.67	18.90±0.23
1	26.16±0.80	18.75±1.57
2	27.70±0.89	18.86±0.83
3	27.51±0.44	18.63±0.30
4	29.44±0.59	17.45±0.43
5	30.19±0.19	14.30±0.94

that in comparing the proximate composition of various Korean varieties of beans and sprouts, crude protein content increased and crude fat content decreased. Also, Yang and Kim (24) reported that as bean sprouts grew, the change in total nitrogen in the seed leaf area decreased, showing a pattern of catabolic metabolism, and in the hypocotyl parts, a synthetic metabolism pattern was detected. In addition, Shin (25) reported that in studies of lipid metabolism during germination, the dry weight of the cotyledons and crude fat concentration both decreased, whereas the dry weight of the hypocotyl parts increased, and crude fat content was constant without change; thus, the crude fat content, dependent on the growth of the bean sprouts, decreased.

Changes in amino acids

In the results for total amino acid content, as measured by hydrolyzing the bean sprouts (Table 4), a total of 19 types of amino acids were detected, and the maximum content (80,875 mg%) was detected on the 3rd day of germination. Regardless of the germination days, glutamic acid was detected to be the highest, and aspartic acid, leucine, and arginine were generally detected as high. Threonine, serine, glutamic acid, proline, glycine, alanine, cystine, methionine, isoleucine, leucine, tyrosine, lysine, and arginine all increased with germination, and their contents were highest on the 2nd and 3rd days of germination; thereafter, they showed a decreasing tendency. Glutamic acid, which is the major component for the savory taste of bean sprouts (26), and aspartic acid and arginine have been reported as useful substances for alcohol detoxification (27,28). In addition, arginine was recently reported as a major component producing NO, a factor controlling the tumor suppressor P53, and an inducer of hypoxia and HIF-I α, which induces the necrosis of mutant cells and is reported to be a superior substance physiologically and pathologically, preventing the production of free radicals that accompany hepatic injury, and thus, protects the liver (7).

In measuring the free amino acids contained in the bean sprouts of *Glycine max* Merr (Table 5), a total of 15 types of amino acids were analyzed. On 0 day of germination, the levels were in the order of arginine (693.58 mg%), glutamic acid (219.71 mg%), and aspartic acid (113.63 mg%), and on the 5th day of germination, the order was arginine (547.35 mg%), glutamic acid (503.10 mg%), and histidine (246.69 mg%). As the days of germination progressed, the free amino acids tended to increase, with the exceptions of aspartic acid, proline, tyrosine, and arginine. With regard to total content, when compared with 0 day of germination (904.12

Table 4. Changes in total amino acids of *Glycine max* Merr sprouts germinated for 5 days (Unit: mg%, dry base)

Total amino acid	Germination period (day)					
	0	1	2	3	4	5
Phosphoserine	71.20	47.00	28.90	51.90	57.90	86.40
Taurine	135.60	128.60	139.20	161.70	124.40	179.10
Aspartic acid	5,197.70	5,434.10	6,336.60	7,053.10	8,657.80	10,825.30
Threonine	2,342.60	2,455.70	2,729.20	3,021.60	2,941.10	2,889.90
Serine	3,742.20	3,864.90	4,555.60	4,324.90	4,249.30	4,176.70
Glutamic acid	13,458.10	13,844.00	15,605.80	15,500.20	14,777.30	13,001.10
Proline	4,510.80	4,030.40	6,122.60	6,930.00	6,166.90	5,833.40
Glycine	2,914.70	2,917.80	3,182.10	3,191.00	3,103.60	3,010.30
Alanine	3,207.70	3,158.50	3,636.80	3,586.60	3,590.30	3,577.40
Valine	3,418.60	3,544.20	3,960.70	4,019.30	4,064.10	4,302.10
Cystine	863.40	908.40	1,073.20	1,073.30	1,016.30	999.50
Methionine	760.70	733.90	869.30	813.50	848.60	704.10
Isoleucine	3,142.00	3,138.90	3,618.80	3,813.10	3,726.30	3,759.40
Leucine	5,196.40	5,338.30	6,013.40	6,235.30	6,122.00	6,008.70
Tyrosine	2,079.80	2,263.10	2,437.90	2,434.20	2,345.30	2,296.70
Phenylalanine	3,531.40	3,631.50	4,114.50	4,160.20	4,171.50	4,266.30
Lysine	4,413.40	4,632.60	5,144.20	5,142.90	4,997.80	4,960.10
Histidine	1,642.70	1,715.00	1,937.20	1,998.40	2,086.10	2,157.90
Arginine	6,441.70	6,713.40	7,066.40	7,364.20	6,934.10	6,703.50
Total	67,070.70	68,500.30	78,572.40	80,875.40	79,980.70	79,737.90

Table 5. Changes in free amino acids of *Glycine max* Merr sprouts germinated for 5 days (Unit: mg%, dry base)

Free amino acid	Germination period (day)					
	0	1	2	3	4	5
Aspartic acid	44.40	71.34	98.85	107.87	113.63	134.34
Threonine	11.45	18.83	51.80	51.56	81.86	151.80
Serine	25.28	41.57	135.55	184.44	190.45	277.38
Glutamic acid	219.71	321.34	379.53	399.22	442.66	503.10
Proline	17.66	19.67	-	12.64	-	-
Glycine	10.01	3.77	5.13	10.55	13.05	35.94
Alanine	49.16	59.74	115.33	114.70	162.69	145.86
Valine	24.53	36.03	48.27	64.13	54.85	76.35
Cystine	-	1.45	4.18	6.37	9.62	9.94
Methionine	1.67	1.31	1.78	1.02	2.28	5.23
Isoleucine	8.69	14.11	29.83	41.01	78.73	127.27
Leucine	7.93	14.40	29.72	33.39	41.67	56.52
Tyrosine	11.73	13.90	27.41	19.48	17.19	13.43
Phenylalanine	14.84	18.35	46.16	57.91	97.42	127.55
Lysine	8.33	15.00	33.26	52.91	51.77	57.69
Histidine	55.15	54.86	91.34	138.63	227.50	246.69
Arginine	393.58	410.89	405.50	502.23	547.35	562.81
Total	904.12	1,116.56	1,503.64	1,798.06	2,132.72	2,531.90

mg%), the total content on the 5th day of germination (2,531.90 mg%) was detected to be approximately 2.8 times higher. Yang and Kim (24) reported that although beans are composed of mostly protein nitrogen, during bean sprout growth, amino acids, peptides, and other water soluble protein nitrogen rapidly decrease, whereas non-protein nitrogen rapidly increases, and such a tendency was shown to be more evident when sprouts were germinated at high temperatures. Therefore, to elevate the nutritional value of bean sprouts, it is desirable to germinate them at the lowest temperature possible, and to shorten the days of germination.

Changes in fatty acids

In analyzing the fatty acids of *Glycine max* Merr soybeans, we found that linoleic acid, which has shown to be greatly effective in the reduction of cholesterol (29), was the fatty acid found in highest concentrations in all samples, ranging from 53.55～56.00%. Also, as the germination period was prolonged, it was confirmed that linolenic acid content slightly increased (Table 6). Lee and Chung (30) have reported that the composition ranges of fatty acids in traditional bean sprout foods range form 11.1～14.3% for palmitic acid, 2.4～3.7% for stearic acid, 14.6～32.4% for oleic acid, 44.8～58.9% for linoleic acid, and 5.6～10.7% for linolenic acid. Furthermore, the composition of fatty acids in bean sprouts and the seed leaves of bean sprouts were in the order of linoleic acid, oleic acid, and palmitic acid (31), which is consistent with our results. On the other hand, the unsaturated fatty acid and saturated fatty acid contents were 80.94～81.34% and 17.87～18.82%, respectively, where the content of unsaturated fatty acid was significantly higher, and the P/S ratio was within the range of 4.31～4.55. The P/S ratio is an important value for evaluating the fatty acid content of the diet,

Table 6. Changes in fatty acids of *Glycine max* Merr sprouts germinated for 5 days (Unit: %)

Fatty acid	Germination period (day)					
	0	1	2	3	4	5
Palmitic acid	10.88	10.87	11.07	11.42	11.44	11.57
Stearic acid	3.26	3.36	3.51	3.52	3.43	3.65
Oleic acid	20.08	19.33	19.55	19.47	20.64	18.74
Linoleic acid	54.67	56.00	55.02	54.84	53.55	55.10
Linolenic acid	6.59	5.92	6.37	6.71	7.11	7.18
Arachidic acid	2.56	2.45	2.67	2.64	2.46	2.56
Behenic acid	1.29	1.19	1.16	1.14	1.12	1.04
Unk[1)]	0.67	0.88	0.66	0.26	0.24	0.15
Saturated fatty acid (S)	17.99	17.87	18.40	18.72	18.45	18.82
Polyunsaturated fatty acid (P)	81.34	81.25	80.94	81.02	81.31	81.03
P/S ratio	4.52	4.55	4.40	4.33	4.41	4.31

[1)]Unknown compound.

and Lee and chung (32) have reported that the P/S ratio of bean sprouts is 4.37.

Inhibition of platelet aggregation

In beans, 12 types of isoflavones, including the non-glucosides daidzein, genistein, and glycitein; the glucosides daidzin and genistin; and the malonyl and acetyl forms of glycitin, have been detected, and it is reported that *Glycine max* Merr soybeans contains a substantial amount of isoflavones (33,34). It has been reported that by increasing the activity of antioxidant enzymes, such flavonoids suppress the oxidation of lipids and LDL, and interfere in the aggregation of platelets, thus preventing arteriosclerosis, hypertension, and coronary heart diseases (35,36).

The ability of *Glycine max* Merr soybeans to inhibit platelet aggregation, according to the germination period, is shown in Fig. 1. The results indicate that the ethanol fraction's ability to inhibit aggregation was generally superior to that of the water fraction, and tended to increase as germination time increased. Since inhibition of platelet aggregation is thought to be related to isoflavone content, this result is well-supported in a report by Kim et al. (37) that compared the concentrations of isoflavones contained in bean sprouts and beans, where total isoflavone content of the bean sprouts was, on average, 1.5 times higher as compared to the beans. Furthermore, a report by Wang and Murphy (38) showed that during soybean germination and sprouting, total isoflavone content increased in proportion to the germination period. On the other hand, Kim et al. (39) reported that the isoflavone composition of bean sprouts, and distribution according to the parts, were different according to germination time, and generally, the isoflavone content of the bean sprouts germinated for 7 days was 419.3～2,035.1 μg/g, with a very large deviation. Taken together, the ability of bean sprouts to inhibit platelet aggregation is considered to be affected by isoflavone content, and it found that in bean sprouts germinated for 1～4 days, platelet inhibition ability was most superior.

Changes in ADH activity

The effects of the ethanol and water fractions of the samples on ADH activity are shown in Fig. 2. In the case of the ethanol fraction, when the control group was at 0%, ADH activity was substantially stimulated from 22.19% at day 0 to 216.76% on the 5th day, and as germination was extended, the activity increased gradually. For the water fraction, in comparison with the control group, ADH activity was stimulated from 508.99% at day 0 to 304.94% on the 5th day. Nevertheless, the activity according to germination time decreased. In addition, compared to the ethanol fraction, the water fraction stimulated ADH activity more, which is in agreement with another report where a water fraction stimulated ADH activity more than other organic fractions (40). Considering reports among amino acids, aspartic acid is shown to increase the ratio of NAD^+/NADH, resulting in the stimulation of alcohol degradation by ADH. Asparagine binds to acetaldehyde and thus reduces its toxicity (41). Aspartic acid and other amino acids extracted from bean sprouts, puffer fish, etc. have the effect of protecting the liver from alcohol (42), and aspartic acid was found to be and important factor for stimulating the activity of ADH and other enzymes. In addition, in reports by Magonet et al. (43) and Park (41),

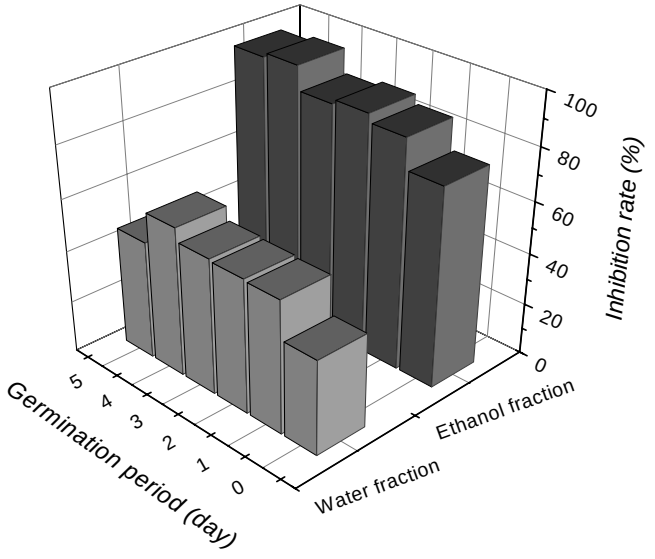

Fig. 1. Inhibition rate (%) of platelet aggregation for *Glycine max* Merr sprouts germinated for 5 days.

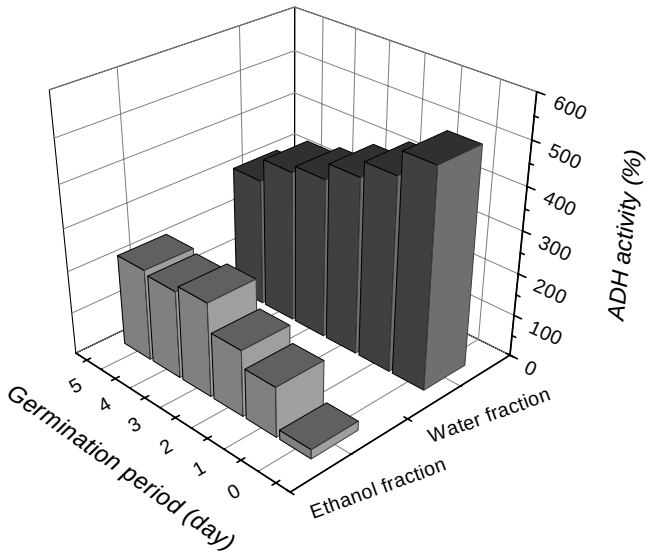

Fig. 2. ADH activity of *Glycine max* Merr sprouts germinated for 5 days.

Ca^{2+}, Zn^{2+}, asparagine, and aspartic acid were considered to be factors affecting the activity of ADH. Furthermore, in studies on bean sprouts, dropworts, radishes, and cabbage it was shown that bean sprouts contain 50 mg% Ca^{2+}, 1 mg% Zn^{2+}, 1,200 mg% asparagine, and 7,470 mg% aspartic acid; dropworts contain 71 mg% Ca^{2+} and 250 mg% asparagine; and radishes contain a small amount of Ca^{2+} and 450 mg% aspartic acid. Finally, in our study, the water fraction showed a higher activity than the ethanol fraction, which was considered to be due to the water fraction containing more factors such as Ca^{2+} and Zn^{2+}.

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(Received September 19, 2007; Accepted November 12, 2007)