J Food Sci Nutr
Vol 10, p 6~10 (2005)

J Food Science and Nutrition

Effect of Cultivars, Cooking and Processing on the Trypsin Inhibitor Activity of Soybean

Penelope Felipe[1], Yoon Hyung Yang[1], Jung Hee Lee[1], Dai-Eun Sok[2], Hyoung Chin Kim[3], Won Kee Yoon[3], Hwan Mook Kim[3] and Mee Ree Kim[1†]

[1]Department of Food and Nutrition,
[2]College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea
[3]Bio-Evaluation Center, Korea Research Institute of Bioscience and Biotechnology, Deajeon 305-333, Korea

Abstract

The trypsin inhibitor activity (TIA) of various soybean cultivars was evaluated by measuring the inhibition of trypsin activity using N-benzoyl-DL-arginine-*p*-nitro-anilide (BAPNA) as the substrate. The TIA values of eleven white shelled soybean cultivars including a glyphosate-tolerant soybean (16.58 to 17.90 mg/g) were not significantly different among cultivars. Black shelled soybeans had higher TIA values, ranging from 40.09 to 52.11 mg/g, compared to white shelled soybeans ($p < 0.05$). When the TIA of commercially processed soybean foods were determined, no TIA was detected in soysauce, tofu and soybean paste. During conventional moist heating, the IT_{50} (Time required to reach 50% inhibition of TIA) values were decreased as heating temperature and cooking pressure increased. The IT_{50} values of moist heating were estimated to be 91.68, 37.71 and 19.50 min at 60, 80 and 100°C, respectively. The IT_{50} value of microwave cooking was 4.75 min at medium heat, while that of the pressure cooking at 120°C was only 2.62 min. Moreover, there was a negative relationship between temperature and IT_{50} values ($R=0.92$, $p < 0.01$). The TIA of soybean sprouts was completely inactivated after heating at 100°C for 5 min, although fresh soybean sprouts showed one fifth of the TIA value of white shelled soybeans. Based on our results, pressure cooking is the most effective cooking method to reduce TIA in soybeans.

Key words: soybean, trypsin inhibitory activity, cultivars, cooking, processing

INTRODUCTION

Soybean (*Glycine* max), a cheap source of dietary protein, contains about 35 percent of calories as protein. However, the utilization of available proteins is much less than that calculated from the chemical compositions, since various antinutritional substances, such as trypsin inhibitors, chymotrypsin inhibitors and lectins, reduce the digestion of proteins in animals, particularly monogastrics (1-5). Trypsin inhibitors may actually cause an increase in the secretion of digestive enzymes, including trypsin, chymotrypsin and elastase by inducing hypertrophy and hyperplasia of the pancreas. This led to the hypothesis that the growth depression caused by trypsin inhibitors was the consequence of an endogenous loss of amino acids in the form of enzymes being secreted by a hyperactive pancreas (1,6). As a consequence, antinutrients obstruct an optimal exploitation of the nutrients present in a food and decrease the nutritive value of soybeans. Therefore, the antinutritive effect of trypsin inhibitors in unheated soybean products has been the subject of much research (6). The destruction of trypsin inhibitors and consequent elimination of hypertrophic pancreas effects is an important step in the processing of raw soybeans into products with excellent protein quality (7).

In Korea, recently, the amount of soybean consumption has increased, while the proportion of domestically produced soybean decreased year after year (8). Most of soybeans have been imported from abroad (9) and used for production of soybean-based foods such as tofu and soybean paste, which are known to have health promoting effects (10-13). Nonetheless, there are a few reports concerning antinutrients such as trypsin inhibitor activity present in some varieties of domestic and imported soybean cultivars. The objective of this study was to examine the trypsin inhibitor activity among cultivars or processed soybean foods, and to evaluate the effect of cooking methods on the trypsin inhibitor activity of soybeans.

†Corresponding author. E-mail: mrkim@cnu.ac.kr
Phone: +82-42-821-6837, Fax: +82-42-822-8283

MATERIALS AND METHODS

Materials

Benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) and purified trypsin were purchased form Sigma Chemical Co. (MO, USA), and Tris base from Research Organics, Inc. (Ohio, USA). All the chemicals used were of the highest quality.

Soybean samples

Eleven soybean cultivars cultivated in Korea (2003), and two imported cultivars were analyzed as reported in Table 1. Among thirteen cultivars, HS2906 is a glyphosate-tolerant soybean which contains the CP4 EPSPS gene, and the other cultivars are not GMOs. Soybean seeds with uniform size, absolutely sound and undamaged were used for analysis. Processed soybean products (Table 2) were purchased from a local market in Daejeon, Korea.

Processing methods

Soaking : All soybean samples were immersed in distilled water (5°C) for 6 hrs, and then cooked according to the following methods.

Ordinary cooking : Soybeans were cooked in water (seed:water, 1:5) at 60, 80 or 100°C for 5, 15, 30, 45, 60 or 120 min.

Pressure cooking : Soybeans were autoclaved in water (seed:water, 1:5) with a pressure greater than or equal to 1 kgf/m^2 for 1, 2, 3 or 5 min.

Microwave cooking : Soybeans were cooked in a microwave oven (MR-202, LG Co., Ltd., Korea) at 2,450 MHz in water (seed:water, 1:5) using medium heat for 1, 2, 3 or 5 min.

Sample extraction

Treated soybean was homogenized and then extracted with 50 mL of 0.01 N NaOH (pH 8.9) for 3 hrs. The suspension was centrifuged for 20 min at 3,000 rpm, 4°C, and for another 1 hr at 15,000 rpm, 4°C. Finally, the resulting supernatant was used for the assay.

Table 2. Trypsin inhibitory activities (TIA) of processed soybean products

Soy products	Company, location	TIA (mg/g)
Tofu	Donghwa Foods Co., Busan	ND[1)]
Tofu	Dusol Co., Chungnamyesan	ND
Soybean paste	Haechandel Co., Daejeon	ND
Soybean paste	Jinmi Foods, Co., Ltd., Daejeon	ND
Soysauce	Sampyo Foods Co., Ichon	ND
Soysauce	Taesang Co., Ltd., Chonan	ND
Soymilk	Namyang Co., Ltd., Chonan	ND
Soymilk	Chung Food Co., Ltd., Chongju	ND

[1)]ND: Not detected.

Trypsin inhibition assay

Trypsin inhibition by soybean trypsin inhibitor was assayed by the method of Kakade et al. (14). An aliquot of the sample extract was suspended in 0.05 M Tris buffer (containing $CaCl_2$, pH 8.2) and then mixed with a known volume of trypsin solution, and the mixture was incubated several minutes to allow the trypsin-inhibiting factors to react with trypsin. An aliquot of BAPNA was then added to the suspension, so that the non-inhibited trypsin catalyzed the hydrolysis of BAPNA, forming *p*-nitroanaline. After 10 min reaction, the hydrolysis was halted by lowering the mixture pH with acetic acid, thereby denaturing the enzyme. The increase of absorbance was monitored at 410 nm using a spectrophotometer (Ultrospec 4300 pro, Amersham Bioscience, Uppsala, Sweden), and trypsin inhibition was estimated from the difference in the degree of BAPNA hydrolysis between the sample solution and the uninhibited trypsin

Table 1. Trypsin inhibitory activities (TIA) of different cultivars of soybeans

Cultivars	Characteristics	Crop year	Origin	TIA (mg/g)
HS2906	Glyphosate-tolerant (white shelled, WS)	2003	USA	16.75±0.15[a1)]
WS82	Conventional (WS)	〃	USA	17.90±0.53[a]
Hwangum kong	Conventional (WS)	〃	NewGene Co., Ltd., Korea	17.79±0.80[a]
Pungsan kong	Conventional (WS)	〃	〃	16.59±0.79[a]
Ouyu kong	Conventional (WS)	〃	〃	17.02±0.92[a]
Sinpaldal kong	Conventional (WS)	〃	RDA, Korea	17.54±0.85[a]
Hwangum kong	Conventional (WS)	〃	〃	17.02±0.40[a]
Daewon kong	Conventional (WS)	〃	〃	16.93±0.40[a]
Taekwang kong	Conventional (WS)	〃	〃	17.11±1.05[a]
Pungsan kong	Conventional (WS)	〃	〃	17.28±0.10[a]
Somyung kong	Conventional (WS)	〃	〃	16.67±1.06[a]
Yakkong	Conventional (black shelled, BS)	〃	NongHyup, Korea	40.09±2.76[b]
Seoritae	Conventional (BS)	〃	〃	52.11±3.62[b]

[1)]Any two means in same column followed by the same letters are not significantly different ($p<0.05$) by Duncan's multiple range test.

solution. One trypsin unit is defined as an increase equal to 0.01 absorbance units at 410 nm after 10 min in 2 mL reaction volume (15). Total trypsin inhibitory activity (TIA), originally expressed as trypsin units inhibited, was converted to trypsin inhibitor units with the relationship that 1 g of pure trypsin has an activity of 1.9 trypsin units (16). All assays were carried out in room temperatures ranging from 23~25°C.

Statistical analysis

Each analysis of the trypsin inhibitor activity was repeated in triplicate for each cultivar or treatment. All data were presented as mean±standard error (SE). All statistical analyses were performed using an SPSS program for window. Statistical assessments were performed using ANOVA for the initial demonstration of significance at $p<0.05$, followed by post-hoc Duncan's multiple-range test (17).

RESULTS AND DISCUSSION

Soybean contains trypsin inhibitors which are naturally occurring antinutrient factors. There have been attempts to evaluate the difference in TIA values between soybean varieties, and ways to reduce TIA values. Therefore, there is a need to develop cultivars with lower TIA values and to develop processing methods to reduce TIA values.

Differences in cultivars

The trypsin inhibitory activities of thirteen cultivars including white or black shelled soybeans are shown in Table 1. The TIA for white shelled soybeans ranged from 16.6 to 17.9 mg/g on a dry weight basis. There were no significant differences among the nine white shelled soybean cultivars cultivated in Korea. Moreover, the trypsin inhibitory activities of the nine white shelled nine soybean cultivars cultivated in Korea were similar to previously reported values ranging from 16.70~27.20 mg/g (18). The TIA values (16.75 mg/g) of the glyphosate-tolerant soybean, HS2906, was not significantly different, when compared to that of conventional soybeans, which is consistent with previous reports that there was no significant difference between glyphosate-tolerant soybeans and non-genetically modified counterparts (15,19,20). Furthermore, the TIA value of cultivar of HS2906 soybean was in a similar range as conventional soybean (18). Interestingly, black shelled soybeans had significantly higher TIA values than white shelled soybeans, ranging from 40.00 to 52.10 mg/g.

Differences in processed soybean products

Processed soybean products such as tofu, soybean paste, soysauce, soybean sprouts and soymilk are commonly consumed in Korea. The trypsin inhibitory activities of processed soybean products are shown in Table 2. Tofu did not exhibit any trypsin inhibitor activity in two different commercial products. This might be attributed to the processing, which consists of soymilk preparation and coagulation of proteins with a salt such as calcium sulfate, followed by cooling. However, levels of TIA in other oriental tofu produced in a Western country was higher at 0.6 mg/g than that of Korean products (21). In addition, trypsin inhibitor activity was not detected in soybean-based fermented foods such as soybean paste and soysauce (Table 2). It is well known that soybean paste is a product prepared by the fermentation of soaked and steamed soybeans, and soysauce is a product of enzymatic or acidic hydrolysis of a mixture of fermented soybeans. During processing such as heating and fermentation, trypsin inhibitors in these fermented soybean products might be destroyed. However, the TIA value (0.3 mg/g) of soysauce from Western country was a little higher, compared to that of Korean products (21). It is surmized that TIA in processed foods might vary depending on the soybean cultivars and environmental factors such as soaking, pH and temperature. Boiled soybean sprouts are commonly consumed by Koreans. In even 5 min-boiled sprouts, the trypsin inhibitory activity was not detected, indicating that trypsin inhibitors present in soybean sprouts are heat labile. The TIA of raw soybean sprout was 3.33 mg/g (Table 3), which was lower, compared to that of the raw soybean seed. Therefore, it is surmized that the trypsin inhibitors in raw seed may be metabolized by unknown biochemical pathway during sprouting.

Effect of cooking on the trypsin inhibitory activity

Various conventional cooking methods such as moist heating, pressure cooking, and microwave cooking have been used in order to diminish the deleterious effects of antinutritional factors present in soybeans, and thereby to increase the nutritional quality of soybean. As shown in Fig. 1, TIA values decreased according to time and temperature; therefore, heat treatment reduced the antinutrient content. When trypsin inhibitors were subjected to heat denaturation, the decreasing rate of TIA varied

Table 3. Trypsin inhibitory activities (TIA) of soybean sprouts[1] during boiling

Boiling time (min)	TIA (mg/g)
0	3.33±0.70
5	ND[2]
10	ND
15	ND

[1]Soybean sprouts were purchased from Pulmuone Co., Cheongju.
[2]ND: Not detected.

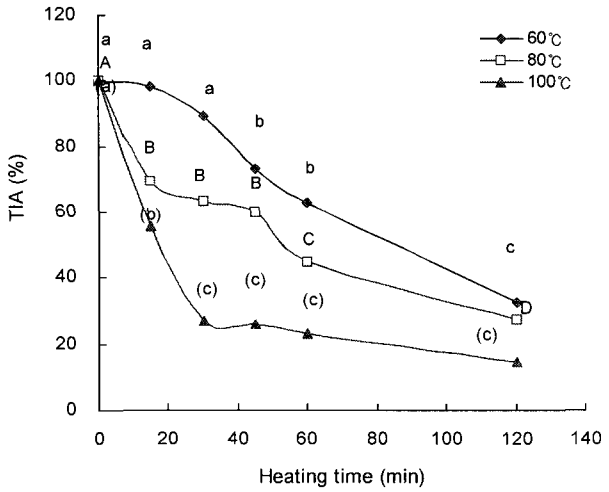

Fig. 1. TIA (%) of white shelled soybeans after moist heating at different temperatures.
[a,A,(a)]Values having the same letters in the same treatment or plot are not significantly different at $p<0.05$.

according to temperature and time of exposure. A significant reduction of TIA value was observed after 45 min at 60°C heating, whereas at 80°C and 100°C, significant changes were observed after heating for only 15 min ($p<0.01$). TIA value was lowered to 19% (3.7 mg/g) when heated at 100°C for 120 min, indicating that most trypsin inhibitors are heat labile, although some are heat resistant. When the decreasing patterns of TIA during heating at different temperatures (Fig. 1) were examined, TIA was observed to decrease rapidly during the initial 15 min of heating, and then were linearly related with heating time (R^2=0.96 at 60°C, R^2=0.99 at 80°C and R^2=0.96 at 100°C). These trends were observed to be similar to the report on Jinpum soybeans (22). When the time required to reach 50% inhibition (IT_{50}) of TIA was calculated based on the slope of different heating conditions, IT_{50} values were estimated to be 91.68, 37.71 and 19.50 min at 60, 80 and 100°C, respectively (Table 4). From these results, the IT_{50} values were observed to linearly decrease as the temperature increased. Moreover, there was a significant relationship between temperature and IT_{50} values (R^2=0.92, $p<0.01$). The activation energy (Ea) was found to be 21.02 kcal/mole. Among conventional cooking methods, pressure cooking

Table 4. Time required to reach fifty percent inhibition (IT_{50}) of TIA according to cooking methods

Cooking methods	IT_{50} (min)
Moist heating, 60°C	91.68
Moist heating, 80°C	37.71
Moist heating, 100°C	19.50
Moist pressure heating, 120°C (with higher 1 kgf/m^2)	2.62
Moist microwave cooking	4.75

(Fig. 2) was found to most effectively inhibit TIA with an IT_{50} value of 2.62 min, and the TIA was reduced to 15% of control after only 5 min. The heat lability of trypsin inhibitor might be explained by the notion that the disulfide bond interchange between trypsin inhibitor and other proteins was responsible for the increased thermal inactivation (23). Also the presence of moisture or other agents such as carbohydrates may catalyze the heat inactivation of soybeans (24).

Use of microwave ovens for cooking is becoming popular, due to its reduction of cooking time. Penetration and heating of food by microwaves are instantaneous, while conventional cooking methods transfer thermal energy from product surface towards its center 10 to 20 times more slowly. Microwave frequencies of 2,450 and 915 MHz are officially recognized internationally in the food industry. Due to higher surface or "skin" effect, the frequency of 2,450 MHz is commonly employed in microwave ovens (25). Fig. 2 shows the decrease in the TIA of soybeans moist-cooked at medium heat in a 2,450 MHz microwave oven. TIA was decreased to about 50% of control after 5 min. These results were similar to the reports that the trypsin inhibitor activity in soybean grain at 49.7% moisture was not completely inactivated even after 8 min microwave treatment. However, the effects of microwave cooking are notably dependent on the techniques and conditions, including time, temperature, moisture content and pH (26). In the case of dry microwave-roasted products, the trypsin inhibitor content decreased to 85.33~92.34% after 3 min cooking (25). After 6 min heating at 8.6% moisture in microwave oven, the trypsin inhibitor activity in soybean grain was not detected, while a longer time was required to inactivate the trypsin inhibitor at higher moisture content,

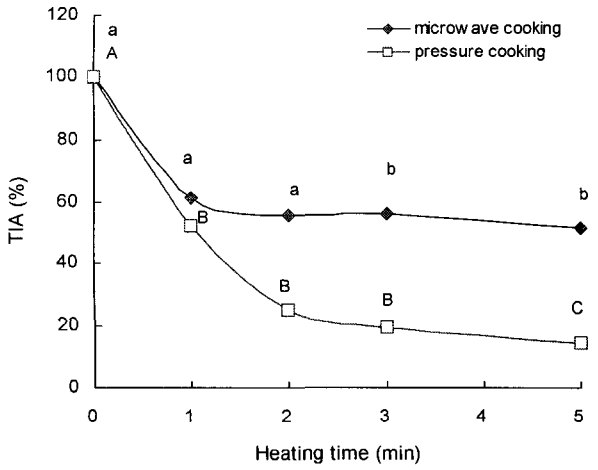

Fig. 2. Effect of moist heating at 120°C with pressure cooking or microwave cooking on the TIA of white shelled soybeans.
[a,A]Values having the same letters in the same treatment or plot are not significantly different at $p<0.05$.

24.3% (27). Hafez et al. (28) reported that the efficiency of microwave treatment on TIA decreases with the increase of moisture content above 30%.

Based on present results, the most effective cooking method to reduce TIA would be a pressure cooking.

ACKNOWLEDGEMENTS

This research was supported by Ministry of Science & Technology (MOST), Korean government (Project No. M1-0337-02-0001-03-B10-02-000-00).

REFERENCES

1. Liener IE. 1994. Implications of antinutritional components in soybean foods. *Crit Rev Food Sci Nutr* 34: 31-67.
2. D'Mello JPF. 1995. Antinutritional substances in legume seeds. In *Tropical legumes in animal nutrition.* D'Mello JPF, Devendra C, eds. CAB International, Edinburgh, UK. p 135-172.
3. Siddhuraju P, Becker K, Makkar HPS. 2000. Studies on the nutritional composition and antinutritional factors in three different germplasm seed materials of an underutilised tropical legume, *Mucuna pruriens* var. *Utilis. J Agric Food Chem* 48: 6048-6060.
4. Makkar HPS, Becker K. 1998. Plant toxins and detoxification methods to improve feed quality of tropical seeds. Review. Pre-conference symposium II on management of feed resources and animal waste for sustainable animal production in Asia-Pacific Region beyond 2000; 8th Conference on Animal Production June, Seoul National University, Seoul, Korea. p 467-480.
5. Makkar HPS, Becker K, Sporer F, Wink M. 1997. Studies on nutritive potential and toxic constituents of different provinces of *Jatropha curcas. J Agric Food Chem* 45: 3152-3157.
6. Rackis JJ. 1965. Physiological properties of soybean trypsin inhibitors and their relationship to pancreatic hypertophy and growth inhibition of rats. *Fed Proc* 24: 1488-149.
7. Anderson RL, Rackis JJ, Tallent WH. 1979. Biologically active substances in soy products. In *Soy protein and human nutrition.* Wilke HL, Hokins DT, Waggle HD, eds. Academic Press, New York, NY. p 209-233.
8. Lee S. 2000. Country paper: Republic of Korea. Report of the APO seminar on processing and utilization of legumes. October 9-14, 2000. Asian productivity organization, Tokyo, Japan. p 165-175.
9. United Soybeans Board. Koreanmarket approaches 1.5 million bushels of US soybeans. Available from: http:// www.ag.uiuc.edu. Accessed Jan. 10, 2005.
10. Sirtori CR, Risks 2001. Benefits of soy phytoestrogens in cardiovascular diseases, cancer, climacteric symptoms and osteoporosis. *Drug Safety* 24: 665-682.
11. Anthony MS, Clarkson TB, Hughes CL, Morgan TM, Burke GL. 1996. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal Rhesus monkeys. *J Nutr* 126: 43-50.
12. Arjmandi BH, Getlinger MJ, Goyal NV, Alekel L, Hasler CM, Juma S, Drum ML, Hollis BW, Kukreja SC. 1998. Role of soy protein with normal or reduced isoflavone content in reversing bone loss induced by ovarian hormone deficiency in rats. *Am J Clin Nutr* 68: 1358S-1363S.
13. Lee CH, Yang L, Xu JZ, Yeung SYV, Huang Y. 2005. Relative antioxidant activity of soybean isoflavones and their glycosides. *Food Chem* 90: 735-741.
14. Kakade ML, Rackis JJ, McGhee JE, Puski G. 1974. Determination of trypsin inhibitor activity of soy products. In *A collarobative analysis of improved procedure.* American Association of Cereal Chemists, Minnesota, USA.
15. Padgette SR, Taylor NB, Nida DL, Bailey MR. 1996. The composition of glyhosate-tolerant soybean seeds is equal to that of conventional soybeans. *J Nutr* 126: 702-717.
16. Anderson LR, Wolf WJ. 1995. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. *J Nutr* 125: 581-588.
17. Steel RGD, Torrie JH. 1960. Analysis of variance I: The one-way classification. In *Principles and procedures of statistics.* McGraw-Hill, New York. p 99-132.
18. Hafez YS. 1983. Nutrient composition of different varieties and strains of soybean. *Nutr Rep Int* 28: 1197-1206.
19. Novak WK, Haslberger AG. 2000. Substancial equivalence of antinutrients and inherent plant toxins and genetically modified novel foods. *Food and Chem Toxic* 38: 473-483.
20. Taylor NB, Fuchs RL, MacDonald J, Shariff AR, Padgette SR. 1999. Compositional analysis of glyphosate-tolerant soybeans treated with glyphosate. *J Agric Food Chem* 47: 4469-4473.
21. Doell BH, Ebden CJ, Smith CA. 1981. Trypsin inhibitor and activity or conventional foods which are part of the British diet and some soya products. *Qual Plant-Plant Foods Hum Nutr* 31: 139-150.
22. Kim YM, Kim YW. 1988. Changes of enzyme activity, trypsin inhibitor, tannin and phytic acid during heat treatment of soybean. *Kor J Food Sci Technol* 30: 1012-1017.
23. Friedman M, Gumbmann MR, Grosjean OK. 1984. Nutritional improvement of soy flour. *J Nutr* 114: 2241-2246.
24. Oste RE, Brandon DL, Bates AH, Friedman M. 1990. Effect of mailliard reaction of the Kunitz soybean trypsin inhibitor on its interaction with monoclonal antibodies. *J Agric Food Chem* 38: 258-261.
25. Zilic SM, Bozovic IN, Mladenovic-Drinic SD, Savic S, Bekric VL. 2002. Effects of microwave radiation on content of soybean soluble proteins, trypsin inhibitor and urease activity. *Food Sci Biotechnol* 11: 590-594.
26. Nestares T, Barrionuevo M, Urbano G, Lopez-Fias M. 1996. Effect of processing methods on the calcium, phosphorus and phytic acid contents and nutritive utilization of chick pea (*Cicer arietinum* L.). *J Agric Food Chem* 47: 2807-2812.
27. Rodda ED, Hill PR, Harshberger KE. 1984. Microwave-roasted soybeans. Transactions of ASAE. p 282-286.
28. Hafez YS, Mohamed AL, Hearath W. 1984. Nutrient composition of different strains of weinged bean seeds and tubers. *Nutr Rep Int* 29: 253-261.

(Received January 18, 2005; Accepted February 21, 2005)