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Effect of Cultivars and Cooking Methods on the Trypsin Inhibitor Activities of Potatoes

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Abstract The trypsin inhibitor activities (TIA) of various potato cultivars were evaluated by measuring the inhibition of trypsin inhibitor activity using N-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) as substrate. The TIA values of 5 potato cultivars (1.99 to 2.88 mg/g) were significantly different among cultivars ($p<0.05$). When the TIA values of commercially processed potatoes were determined, no TIA was detected. During cooking, the IT_{50} (time required to reach 50% inhibition of TIA) values were decreased as heating temperature and time increased. The IT_{50} of moist heating was estimated to be 0.34 min at 100°C, whereas for deep-fat frying the IT_{50} was 0.13 min at 180°C and 5.28 min for oven baking at 100°C. The IT_{50} value of microwave cooking was 0.194 min at medium heat, and which was similar to that of pressure cooking at 120°C (0.185 min). Moreover, there was a negative relationship between temperature (≥80°C) and IT_{50} values ($R^2=0.99$, $p<0.01$). The TIA of potato was completely inactivated by moist heating at 100°C within 5 min, whereas the pressure cooking at 120°C and deep-fat frying at 180°C within 60 and 30 sec, respectively. Based on our results, deep-fat frying is the most effective cooking method to reduce TIA in potatoes.

Keywords: potato, trypsin inhibitor activity, cultivar, cooking

Introduction

Protease inhibitors are widely distributed in plants, animals, and microorganisms (1). Especially, protease inhibitors in soybean and potato have been well studied, because these plants are consumed world widely as important food sources (1-3). Plant protease inhibitors are accumulated in storage organ such as seed or tuber during maturation. The function of protease inhibitors was known to exert to defense mechanism against insect biting (2-4). Protease inhibitors represent 20-50% of water soluble proteins in potato tubers (5). The trypsin inhibitors cause to increase to secret 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 (6,7). As a consequence, antinutrients obstruct an optimal exploitation of the nutrients present in a food and decrease the nutritive value of potato. Therefore, the antinutritive effect of trypsin inhibitors in unheated potato has been the subject of much research (8). The destruction of trypsin inhibitors and consequent elimination of hypertrophic pancreas effects is an important step in the processing of raw potato into products with excellent protein quality (9).

Potatoes (*Solanum tuberosum*) are one of the major four crops produced in world-wide (10). In Korea, recently, the amount of potato consumption has increased (11). Recently, 5 domestic potato cultivars were cultivated by National Institute of Highland Agriculture (NIHA) in Korea and actively distributed to farmers. Nonetheless, there are a few reports concerning antinutrients such as trypsin inhibitor activity present in these cultivars. The objective of this study was to examine the trypsin inhibitor activity among cultivars, and to evaluate the effect of cooking methods on the trypsin inhibitor activity of potatoes.

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Materials and Methods

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

Potato samples Five potato varieties cultivated in Korea were collected, and their characteristics were reported in Table 1. Sound and undamaged potato tubers with uniform size were used in present study. Processed potato products (Table 2) were purchased from a local market in Daejeon, Korea.

Heat processing methods Potatoes tubers were washed, peeled, and dried with clean linen. The potatoes were cut into $1\times1\times1$ cm^3 pieces and mixed thoroughly, and each 20 g was put into a plastic bag. To evaluate the extent of inactivation of trypsin inhibitors by heat processing, the 'Superior' potato samples were treated with according to the following cooking methods, moist-heating, pressure, and microwave cooking. For moist-heating method, potatoes were heated in water (tuber : water, 1 : 5) for 15, 30, 60,

120, 180, 240, and 300 sec at 80 and 100°C, or 5, 15, 30, 45, 60, and 120 min at 60°C. For pressure cooking, potatoes were autoclaved with a pressure greater than or equal to 1 kg_f/m^2 for 15, 30, 45, and 60 sec at 120°C. For microwave cooking, potatoes were heated in microwave oven (MR-208; LG Co., Ltd., Seoul, Korea) at a frequency of 2,450 MHz with medium power of 700 W for 15, 30, 45, and 60 sec. For deep-fat frying, potatoes were heated in soybean oil for 15, 30, 45, 60, 90, and 120 sec at 160, 170, 180, 190, and 200°C. For oven baking, potatoes ($1\times1\times1$ cm^3 pieces or whole with skin) were wrapped with aluminum foil and baked in the preheated electric oven (EHF 648/48Tt; Heller Co., Ltd., Germany) for 10, 20 30, 40, and 60 min at 200°C. The cooked potatoes were immediately immersed in ice water (<5°C) for 10 min, and then freeze-dried.

Sample extraction Raw or cooked potato powder was homogenized and then extracted with 50 mL of 0.01 N NaOH (pH 8.9) for 3 hr. The suspension was centrifuged for 20 min at 1,500×g, 4°C, and for another 1 hr at 25,000×g, 4°C. Finally, the resulting supernatant was used for the further assay.

Trypsin inhibition assay Trypsin inhibitior activity (TIA) in potato was determined by the method of Kakade *et al.* (12). 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 as a substrate was then added to the suspension, so that the un-inhibited trypsin catalyzed the hydrolysis of BAPNA, forming *p*-nitroanaline. After 10 min reaction, the hydrolysis was stopped by lowering pH of the reaction mixture with acetic acid, thereby the trypsin was denatured. The absorbance was measured at 410 nm using a spectrophotometer (Ultrospec 4300 pro; Amersham Bioscience Co., Ltd., Uppsala, Sweden), and TIA was estimated from the difference in the degree of BAPNA hydrolysis between the sample solution and the uninhibited trypsin 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 (13). Total TIA, originally expressed as trypsin units inhibited, were converted to trypsin inhibitor units with the relationship that 1 mg of pure trypsin has an activity of 1.9 trypsin units (14). All assays were carried out in room temperatures ranging 23 to 25°C.

Statistical analyses Each analysis of TIA 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 analysis of variance (ANOVA) for the initial demonstration of significance at $p<0.05$, followed by post-hoc Duncan's multiple-range test (15).

Results and Discussion

Differences in cultivars Potato contains trypsin inhibitors, which are naturally occurring antinutrient factors. There have been attempts to evaluate the differences in TIA values between potato varieties, and find 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.

The TIA of 5 potato varieties cultivated in NIHA, Korea were reported in Table 1. The TIA for potatoes ranged from 1.99 to 2.88 mg/g on a dry weight basis. These TIA values of potatoes are much smaller (about 1/10) than those of soybean (16). There were significant differences among the 5 potato varieties cultivated in Korea ($p<0.05$). Moreover, the TIA of the 5 potato varieties cultivated in Korea was similar to previously reported values (17).

Differences in processed potato products Processed potato products such as potato chips and French fried potatoes are commonly consumed in Korea. The trypsin inhibitory activities of processed potato products are shown in Table 2. All potato products did not exhibit any trypsin inhibitor activity in several different commercial products. This might be attributed to the processing, which consists of cutting and deep-fat frying at high temperature. During processing such as deep-fat frying at high temperature, trypsin inhibitors in commercial fried potato products might be destroyed.

Effect of cooking methods on the trypsin inhibitor activities Various cooking methods such as moist heating, pressure cooking, microwave cooking, deep-fat frying, and oven baking have been used in order to decrease the deleterious effects of anti-nutritional factors present in potatoes, and thereby to increase the nutritional quality of potato. Among 5 different cultivars, 'Superior' was used for cooking effect, because 'Superior' has been reported that it has good qualities for both cooking and chip processing (18).

Table 1. Trypsin inhibitor activities (TIAs) of different cultivars of potatoes[1)]

Cultivars	Use	Crop year	TIA (mg/g)
'Superior'	General food	NIHA[2)], Korea, 2003	2.083 ± 0.126^c
'Atlantic'	General food	NIHA, Korea, 2003	2.880 ± 0.043^a
'Shepody'	French fry	NIHA, Korea, 2003	2.565 ± 0.064^b
'Jopung'	General food	NIHA, Korea, 2003	1.985 ± 0.021^c
'Namsuh'	General food	NIHA, Korea, 2003	1.841 ± 0.074^d

[1)]Any 2 means in the same column followed by the same letters are not significantly different ($p<0.05$) by Duncan's multiple range test.
[2)]National Institute of Highland Agriculture.

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

Products	Company	TIA (mg/g)
French fried potato	KFC	ND[1)]
French fried potato	Lotteria	ND
French fried potato	McDonald	ND
French fried potato	Papais	ND
Poca chip	Orion Confectionery Co., Ltd.	ND
Saengsaeng gamja chip	HaiTai Confectionery Co., Ltd.	ND
Lay's	Pepsico Food Co., China	ND

[1)]Not detected.

As shown in Fig. 1, TIA values decreased according to time and temperature; therefore, heat treatment reduced the anti-nutrient content of potatoes. When trypsin inhibitors were subjected to heat denaturation, the decreasing rate of TIA varied according to temperature and time of exposure. A significant reduction of TIA value was observed after 15 min at 60°C by moist heating, whereas at 80 and 100°C, significant changes were observed after moist heating for only 30 sec ($p<0.05$). TIA value was reached to 13.9% (0.33 mg/g) when heated at 100°C for 2 min, indicating that all trypsin inhibitors are heat labile. In the case of oven baking at 200°C, the TIA value of whole potato was decreased to 5.3% after 10 min as shown in Fig. 2, whereas the TIA of potato pieces was reduced to 4.87% after 1 min. Most popular processing method of potato has been deep-fat frying. When the potatoes were subjected to deep-fat frying, the TIA values were observed to be remarkably decreased to 4.51% after 15 sec at 180°C as shown in Fig. 3, whereas at 160°C, TIA value was retained 5.0% for 15 sec (data were not shown). The effect of TIA inactivation by heat treatment was varied by food such as soybean, yam, sweet potato, and taro tubers. It was reported that soybean trypsin inhibitors are heat resistant (16,19). TIA values of sweet potato were retained 17 to 31% by cooking at 100°C for 30 min, while those of taro were 7 to 11% at the same condition (19). In addition the heat lability was appeared differently by cultivars of potatoes and taro tubers (20).

When the decreasing patterns of TIA during moist-heating at different temperatures (Fig. 1) were examined, TIA was observed to decrease rapidly during the initial 30 sec of heating at 80 and 100°C, or 15 min at 60°C, and then were linearly related with heating time (R^2=0.97 at 80°C, R^2=0.91 at 100°C, R^2=0.96 at 60°C). These trends were observed to be similar to other cooking methods as well as the reports on soybeans (16,20). 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 for moist-heating were estimated to be 45.4, 0.45, and 0.34 min at 60, 80, and 100°C, respectively, whereas IT_{50} values for deep-fat frying were 0.263, 0.131, and 0.130 min at 160, 180, and 200°C, respectively (Table 3). From these results, the IT_{50} values were observed to linearly decrease as the temperature increased. Moreover, there was a significant relationship between temperature (≥80°C) and IT_{50} values (R^2=0.99, $p<0.01$) for moist-heating. The activation energy (Ea) was found to be 4,045 kcal/mole for moist-heating and 4,932 kcal/mole for deep-fat frying. For oven baking, when we compared to the IT_{50} values of potato size to be cooked at the same temperature, the IT_{50} value of whole potato (about 200 g) was much longer than that of potato pieces (1×1×1 cm): The IT_{50} values was 5.28 min for whole potato whereas 0.526 min for potato pieces. It is obvious that the greater is the size of food to be cooked, the longer is the heating time to cook.

Pressure cooking was found to effectively inhibit TIA with an IT_{50} value of 0.194 min (Table 3), and the TIA was reduced to 13% of control after only 1 min (Fig. 3). 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 (21). Also the presence of moisture or other agents such as carbohydrates may catalyze the heat inactivation of potatoes similar to soybeans (22).

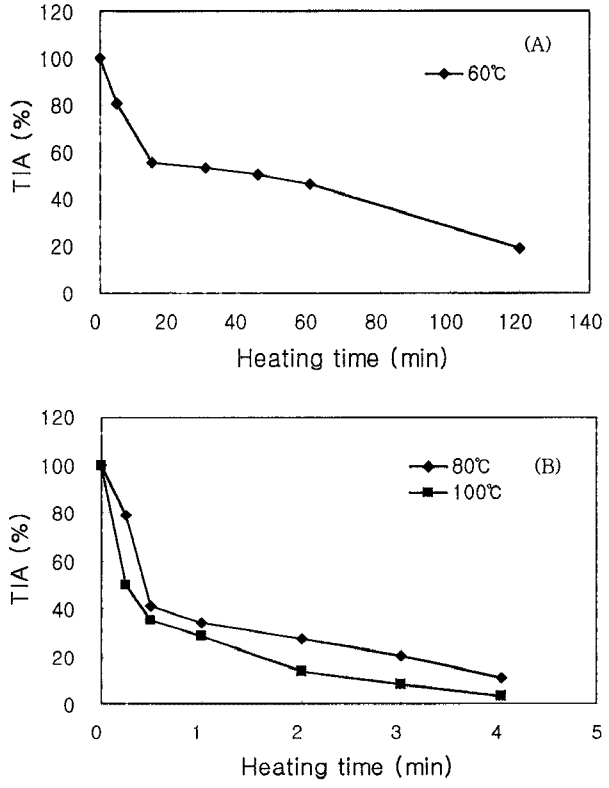

Fig. 1. Trypsin inhibitor activities (TIA, %) of potatoes (1×1×1 cm) after moist cooking at different temperatures (A, 60°C; B, 80 and 100°C).

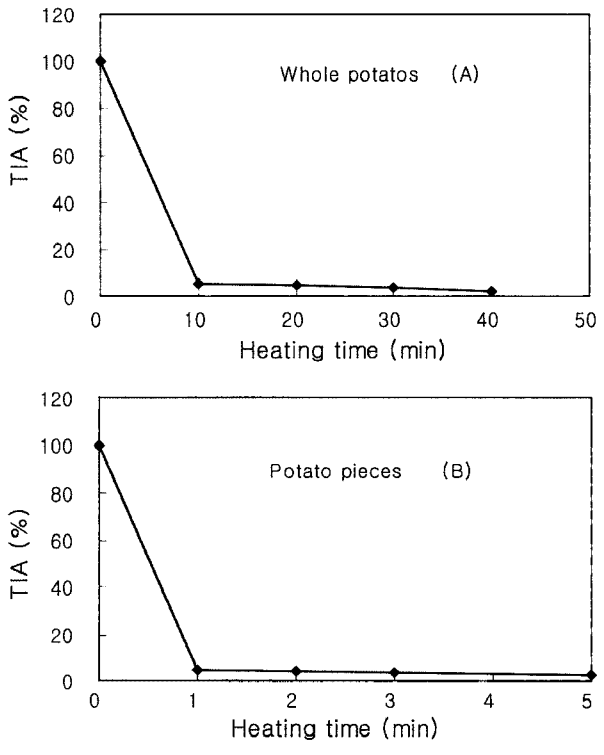

Fig. 2. Trypsin inhibitor activity (TIA, %) of whole potatoes (A) and potato pieces (B) after oven baking at 200°C.

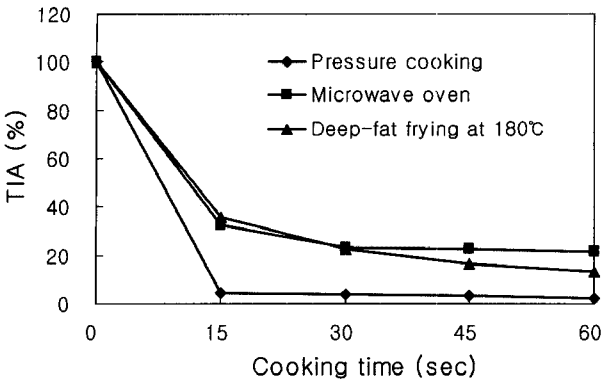

Fig. 3. Trypsin inhibitor activities (TIA, %) of potatoes (1×1×1 cm) after deep-fat frying at 180°C, pressure cooking at 120°C, and microwave cooking at medium heat.

Table 3 Time required to reach 50% inhibition (IT_{50}) of TIA according to cooking methods

Cooking methods	IT_{50} (min)
Moist heating, 60°C	45.415
80°C	0.449
100°C	0.340
Pressure heating, 120°C (with higher 1 kg_f/m^2)	0.194
Microwave cooking	0.185
Deep-fat frying, 160°C	0.263
170°C	0.262
180°C	0.131
190°C	0.131
200°C	0.130
Baking (1×1×1 cm), 200°C	0.526
Baking (whole), 200°C	5.280

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 (23). Figure 3 shows the decrease in the TIA of potatoes at medium heat in a 2,450 MHz microwave oven. TIA was decreased to 32% of control after 15 sec. These results were remarkably different from the reports that the TIA in soybean grain at 49.7% moisture was not completely inactivated even after 8 min by microwave treatment. However, the effects of microwave cooking are notably dependent on the techniques and conditions, including time, temperature, moisture content, and pH (24). In addition, The TIA of potato was completely inactivated by moist heating at 100°C within 5 min, whereas the pressure cooking at 120°C and deep-fat frying at 180°C within 60 and 30 sec, respectively. Based on our results, deep-fat frying is the most effective cooking method to reduce TIA in potatoes.

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