Changes in Free Amino Acid and Kjeldahl N Concentrations in Seeds from Vegetable-Type and Grain-Type Soybean Cultivars during the Cropping Season

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Immature soybean seeds of the vegetable-type (edamame) cultivars that are harvested 30−40 DAF and consumed as a vegetable are preferred by consumers over those of the grain-type cultivars. This study was carried out mainly to examine differences in the concentrations of free amino acids in seeds of the edamame and grain-type cultivars. Asparagine, alanine, and glutamic acid were the major components in immature seeds of the edamame and grain-type cultivars. The concentrations of these amino acids increased until 30−40 DAF and then decreased in seeds of the edamame cultivars, while in seeds of the grain-type cultivars, they decreased continuously starting 20−30 DAF toward mturity. The differences in free amino acid concentrations between immature seeds of the edamame and grain-type cultivars may indicate that these two types of soybean cultivars belong to genetically different ecotypes.

Keywords: *Glycine max; soybean; edamame; amino acid; water-soluble nitrogen*

INTRODUCTION

The quantitative improvement of the storage proteins and oils in soybean seeds has been the subject of several studies. One accomplishment has been the breeding of soybean lines that lacked or contained lower concentrations of lipoxygenases (Davis and Nielsen, 1986; Hajika et al., 1991; Kitamura, 1984; Wilson et al., 1981). These isozymes are related to flavor of foodstuffs such as synthetic milk. Another accomplishment has been an improvement in the storage protein composition, i.e., an increase in the 11S globulin subunit (glycinin) and an accompanying decrease in the 7S globulin subunit (β-conglycinin) (Harada et al., 1983; Ogawa et al., 1989; Takahashi et al., 1994). These biochemical changes resulted in increased concentrations of essential amino acids, such as methionine and threonine in the mature seeds.

Generally, many free cellular components such as sugars, polysaccharides, amino acids, carotinoids, various volatile substances, and so on may be related to the tastes of vegetables. However, in contrast to the above improvements, which have mainly been in grain-type cultivars, the quantitative improvement of the immature seeds of the edamame cultivars has received little attention, although recently some breeding trails were attempted by Takahashi (1991) and Shanmugasundaram et al. (1991). This may be because the edamame cultivars are a relatively new crop and have not yet become a major crop in the world (Lumpkin et al., 1993).

Amino acids are one of the important cellular components relating to the tastes (Ofuji et al., 1983; Masuda et al., 1988; Masuda, 1989). The objective of this study was to examine the changes in the concentrations of free amino acids of the seeds of the edamame and grain-type cultivars during the cropping season.

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MATERIALS AND METHODS

Experiment 1. In 1992, we selected 20 soybean [*Glycine max* (L.) Merr.] cultivars, 10 of which were of the edamame type and 10 of which were grain type (Table 1). The edamame and grain-type cultivars that are cultivated mainly in the Tohoku or Hokkaido (northern) area of Japan have a relatively similar range of flowering and maturity characteristics. The cultivation area of these cultivars ranges from 38° to 43° N lat. The maturity groups of cultivars (i.e., the 20 cultivars mentioned above) are shown in Table 1.

A chemical fertilizer containing N, P_2O_5, and K_2O in the ratio of 60, 180, and 240 g kg^{-1}, respectively, was applied at the rate of 15 kg ha^{-1} at plowing on 12 May. In order to ensure uniform growth, seedlings were grown in a greenhouse nursery bed for 2 weeks and then transplanted to an upland field (22 m above sea level) of Yamagata University on 25 May. Two seedlings were transplanted per hill. The hills were spaced 20−45 cm apart according to the plant height of cultivars, and all rows were 80 cm wide. One plot of a single cultivar consisted of 6 rows × 10 hills. All the cultivars were grown in 3 replicates. The 60 plots (20 cultivars × 3 replicates) were placed in a field adjacent to one another but in a completely randomized order.

We sampled 100 pods at the middle positions of the culm from the central 6 hills of the middle 2 rows excluding the border of 2 rows and 2 hills in each plot. Sampling occurred 30−40 DAF depending on when a suitable softness was attained for consumption as a vegetable (edamame) for both the vegetable-type and grain-type cultivars. Suitable softness for eating as an edamame (which is normally boiled for 2 min) was determined by chewing the raw immature seeds and comparing them to commercially harvested seeds. After removal of the hulls, seeds that were pooled for each plot were freeze-dried using an EYELA FD-80 freezer (Hitachi-koki Co., Ltd., Ibaraki, Japan) and then pulverized to 150 μm flour using a Cyclone Sample Mill (U.D. Co., Boulder, CO).

1 g of seed flour was incubated and shaken with 25 mL of 700 mL of ethanol/L at 20 °C for 1 h. The suspension was precipitated by the addition of 10 mL of 5 g of $MgSO_4$/L into the ethanol−flour mixture to obtain the milky component and filtered using Toyo-Roshi No. 2 filter paper (Toyo-Roshi, Co., Ltd., Tokyo, Japan). The filtrate was vacuum-dried and then dissolved in 2 mL of 1:1 (v/v) solution of TFA (13.094 mM/L) and acetonitrile. A Sep-Pak18 plus C18 cartridge (Millipore Co., MA, U.S.A.) was used to fractionate free amino acids from the extract according to the manufacturer's instructions. 1

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Table 1. Growth Characteristics of Edamame and Grain-Type Soybean Cultivars Grown in 1992[a]

cultivar	flowering onset	flowering end	plant height (cm)	number of pods (plant^{-1})	weight of pods (g plant^{-1})
edamame					
Shirayama-dadacha	16 July	24 July	59.9 ± 2.2	32.0 ± 5.1	58.9 ± 5.2
Sapporo-midori	18 June	26 June	19.5 ± 0.7	20.3 ± 0.9	38.5 ± 2.1
Wase-midori	24 June	2 July	30.3 ± 2.0	29.0 ± 1.0	63.7 ± 3.5
Wase-Shirayama-dadacha	4 July	14 July	53.3 ± 1.2	30.0 ± 3.0	58.8 ± 0.1
Shonai 5	16 July	24 July	59.7 ± 2.7	48.7 ± 3.5	106.6 ± 10.3
Shiroge-green	18 June	30 July	22.2 ± 1.1	18.0 ± 1.0	39.3 ± 1.8
Shinsetsu-midori	20 June	28 June	27.7 ± 0.4	16.0 ± 0.6	39.1 ± 2.4
Ezo-midori	20 June	30 June	30.0 ± 1.0	23.0 ± 2.1	39.6 ± 4.8
Hakucho	24 June	4 July	32.4 ± 1.6	29.0 ± 3.0	68.6 ± 7.3
Hokko-midori 2	24 June	4 July	30.7 ± 0.9	22.3 ± 3.7	49.7 ± 3.8
grain-type					
Tohoku 70	24 June	10 July	37.3 ± 1.6	22.3 ± 6.8	53.2 ± 2.8
Akita-ani	16 July	13 Aug.	111.7 ± 4.1	90.0 ± 17.4	88.1 ± 20.1
Fuji-musume	10 July	1 Aug.	57.1 ± 1.4	107.0 ± 15.5	111.9 ± 8.6
Shin 3	10 July	28 July	59.4 ± 2.8	105.0 ± 4.9	105.6 ± 7.2
Wase-shiroge*	10 July	28 July	67.7 ± 2.2	80.7 ± 12.2	89.1 ± 10.3
Kokeshijiro*	16 July	5 Aug.	53.4 ± 1.7	134.3 ± 34.8	143.7 ± 24.0
Raiko*	14 July	9 Aug.	75.6 ± 2.6	98.0 ± 13.6	137.1 ± 23.0
Miyagi-shirome**	28 July	21 Aug.	94.2 ± 3.0	190.0 ± 14.2	355.2 ± 16.4
Kurakake**	28 July	26 Aug.	61.7 ± 2.1	97.0 ± 11.5	184.8 ± 13.5
Nakasen-nari**	28 July	26 Aug.	91.9 ± 3.3	137.7 ± 7.2	219.1 ± 8.2

[a] Each value in the three columns is mean ± SE. "*" or "**" after the cultivar name indicates the estimated maturity group (I or II, respectively). Other cultivars were Group 00.

mL of extract was passed through the cartridge. 1 mL of TFA (13.094 mM/L) was used to elute the hydrophilic free amino acids, and then 1 mL of TFA (13.094 mM/L):acetonitrile [1:1 (v/v)] solution was used to elute the hydrophobic free amino acids. The total amino acids were dissolved in 20 μL of an ethanol:H_2O:triethylamine solution [2:1:1 (v/v)] and freeze-dried. Amino acids were labeled with PITC by the addition of 20 μL of ethanol:H_2O:triethylamine:PITC [7:1:1:1 (v/v)] to the dried sample for 20 min at room temperature. The PITC-labeled amino acids, called "PTC-amino acids", were dissolved in 100 μL of a solution of 0.05 mol of Na_2HPO_4 and 953.7 mM/L acetonitrile at pH 5.8 and passed through an ultrafilter unit with a pore size of 0.2 or 0.5 μm (Nihon Millipore Co., Ltd., Tokyo, Japan) for purification. The PTC-amino acids were then analyzed using a Protein-Pak 300 column (7.8 mm in diameter × 15 cm in length, Millipore Co. Ltd., MA) and an ultraviolet detector at 254 nm in a Pico-Tag HPLC system (Waters Nihon Millipore Co., Ltd., Tokyo, Japan) in 3 replicates from 3 plots of each cultivar. The elution and recovery times of this system were 16 and 13 min, respectively. A modified amino acid mixture standard solution (H-type, Wako Chemical Ind. Ltd., Doshu-machi, Osaka), into which asparagine and glutamine (2.50 mol/mL) were added, was used for identification of amino acids in the samples.

Experiment 2. In 1993, we selected four cultivars, Sapporo-midori and Shirayama-dadacha from the edamame cultivars and Tohoku 70 and Akita-ani from the grain-type cultivars examined in 1992. Shirayama-dadacha and Akita-ani are native cultivars in Japan, while Sapporo-midori and Tohoku 70 represent cultivars that have been moderately improved and intensively improved, respectively, through crossbreeding. These four cultivars are the major ones in the Tohoku area of Japan. Management of seedlings, transplanting procedures, and the level of fertilizer application were similar to those used in 1992. One plot of a single cultivar consisted of 6 rows × 25 hills. All the cultivars were grown in 3 replicates. Twenty adjacent plots that consisted of 3 replicates × 2 cultivars for the edamame plus 3 × 2 for the grain-type cultivars were arranged in a completely randomized design in a field. Transplanting was conducted on 26 May 1993, and fertilizer was applied on 13 May 1993.

We sampled 100 pods at the middle positions of the culm from the 2 rows and 21 hills excluding the border of 2 rows and 2 hills, 20, 30, 40, and 50 DAF. Flour preparation, PITC-labeling, and extraction and analysis of free amino acids were done as in the 1992 experiment. Concentrations of the three major amino acids observed in 1992 (asparagine, alanine, and glutamic acid) were analyzed at the different grain filling stages.

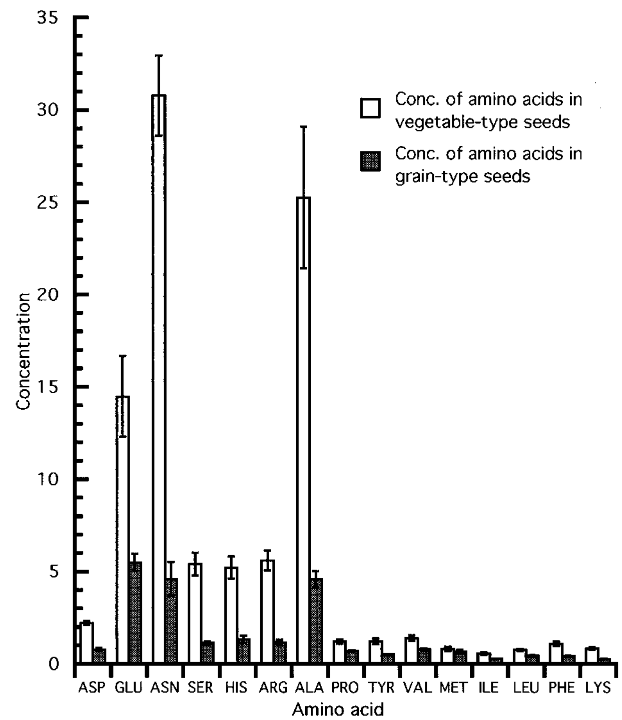

Figure 1. Mean concentrations (mM/kg dry weight) of free amino acids in the immature seeds of vegetable-type and grain-type soybean cultivars in 1992. Concentrations are the mean of 10 cultivars of each type. Vertical bars indicate standard error.

1 g of seed flour was incubated and shaken with 25 mL of distilled water at 75 °C for 1 h. The milky components were precipitated as they were in 1992. 10 mL of the filtrate and 0.2 g of 150 μm flour were used to determine the Kjeldahl water-soluble and total N concentrations, respectively, using a Kjeltec System 1026 (Nippon General Co. Ltd., Tokyo, Japan) in 3 replicates from 3 plots of each cultivar.

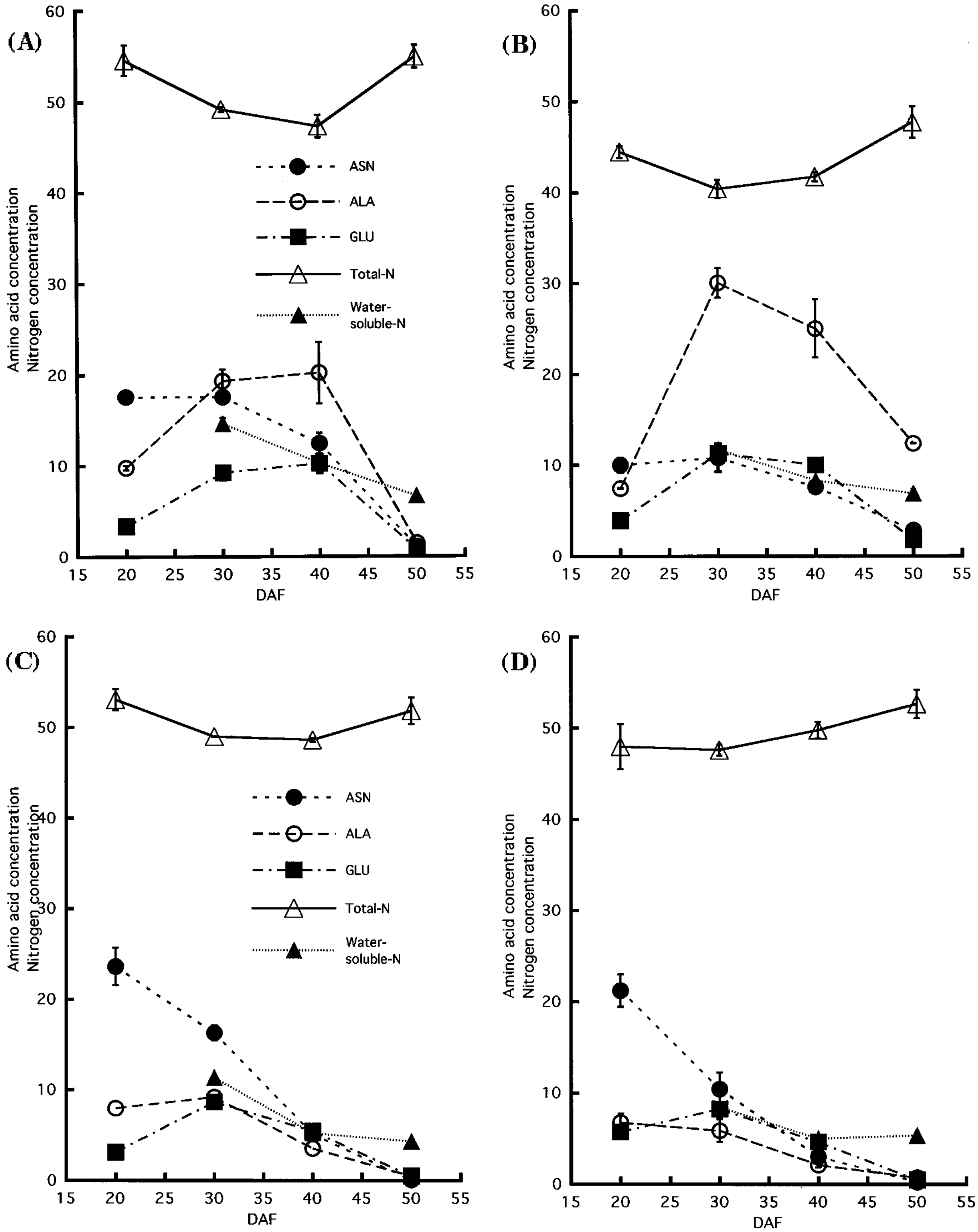

Figure 2. (A) Concentrations (mM/kg dry weight) of the three major free amino acids and concentrations (g/kg dry weight) of the total and water-soluble N in seeds of an edamame cultivar (Sapporo-midori) at the ripening stage in 1993. Vertical bars indicate standard error calculated on the basis of three replications. (B) Concentration (mM/kg dry weight) of the three major free amino acids and concentrations (g/kg dry weight) of the total and water-soluble N in seeds of an edamame cultivar (Shirayama-dadacha) at the ripening stage in 1993. Vertical bars indicate standard error calculated on the basis of three replications. (C) Concentrations (mM/kg dry weight) of the three major free amino acids and concentrations (g/kg dry weight) of the total and water-soluble N in seeds of a grain-type cultivar (Tohoku 70) at the ripening stage in 1993. Vertical bars indicate standard error calculated on the basis of three replications. (D) Concentrations (mM/kg dry weight) of the three major free amino acids and concentrations (g/kg dry weight) of the total and water-soluble N in seeds of a grain-type cultivar (Akita-ani) at the ripening stage in 1993. Vertical bars indicate standard error calculated on the basis of three replications.

RESULTS

Experiment 1. Variance analyses showed that cultivar variations in free amino acid concentrations in the immature soybean seeds were highly significant in both the edamame and grain-type cultivars, except for tyrosine in the grain-type cultivars in 1992 (data not shown). The major free amino acids in the immature seeds of the edamame cultivars were asparagine, alanine, and glutamic acid, each with concentrations over 15 mM/kg dry weight, followed by serine, histidine, and arginine, each with concentrations of about 5 mM/kg dry weight or less (Figure 1). Other amino acids except for those shown in Figure 1 were not detected. In the immature seeds of the grain-type cultivars, the major amino acids were also asparagine, alanine, and glutamic acid, but the concentrations (about 5 mM/kg dry weight) were lower, while serine, histidine, and arginine were present at concentrations below 3 mM/kg dry weight (Figure 1).

Concentrations of other amino acids (aspartic acid, proline, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine) were about 2 mM/kg dry weight or less in both the edamame and grain-type cultivars. However, the concentrations of these amino acids tended to be almost twice as high in the former than in the latter (Figure 1).

Experiment 2. Changes in the concentrations of asparagine, alanine, and glutamic acid, which were the three major amino acids in the immature seeds of both the edamame and grain-type cultivars in 1992, were examined in two edamame-type cultivars and two grain-type cultivars in 1993 during the cropping season (Figure 2A–D). The concentrations of these amino acids in seeds of the two edamame cultivars, Shirayama-dadacha and Sapporo-midori, increased until 30 or 40 DAF and then decreased linearly with maturity. However, their concentrations of amino acids in seeds of the two grain-type cultivars, Tohoku 70 and Akita-ani, tended to gradually decrease with the ripening stage.

The concentrations of the three major amino acids in seeds increased and/or remained at higher levels in the edamame cultivars during the period 30–40 DAF. Seeds of the edamame cultivars were especially characterized by a higher concentration of alanine during this period. In contrast, seeds of the grain-type cultivars exhibited a linear decrease in asparagine, and a low level of alanine. The three major amino acids in seeds of Sapporo-midori and in the two grain-type cultivars became negligible, but alanine in Shirayama-dadacha remained at a fairly high level 50 DAF (Figure 2A–D).

The concentrations of water-soluble Kjeldahl N in seeds of the four cultivars decreased linearly toward maturity. In contrast, the total Kjeldahl N concentration in the four cultivars decreased 30–40 DAF, except for Akita-ani in which the total Kjeldahl N tended to decrease only 30 DAF (Figure 2A–D).

DISCUSSION

Previously, studies on the eating quality of edamame-type soybean seeds focused on the effects of different storage procedures (Abe and Okuda, 1986; Akazawa and Fukushima, 1991; Iwata and Shirahata, 1979; Masuda et al., 1988; Masuda, 1989). Thus, no consideration has been given to the cultivar variations in the accumulation of free amino acids among different cultivars. However, the free amino acids and other N-containing cellular components, such as peptides of various lengths, are involved in complicated processes of turnover, processing, synthesis, and incorporation into high molecular weight substances, such as proteins, at the early stages of soybean seed development. Concentrations of three amino acids (asparagine, alanine, and glutamic acid) were higher in the vegetable-type (edamame) cultivars than in the grain-type cultivars in the immature stages when the edamame is harvested and consumed. These findings appear to indicate that differences between the vegetable-type and grain-type cultivars in the accumulation of free amino acids in seeds are cultivar-specific. Thus, the edamame cultivars may be useful for examining gene regulation processes of protein synthesis in the different stages of seed development.

The edamame is one of the most common vegetables in Asian countries, although it is still a new vegetable in many of these countries (Lumpkin et al., 1993). Compared with the mature seeds of the grain-type cultivars, the edamame is simple to prepare as it can be eaten after boiling for 2 min. The three major free amino acids in the edamame cultivars may be associated with their palatability. It has been reported that alanine is highly correlated with sweetness, and asparagine and glutamic acid are highly correlated with taste (Ofuji et al., 1983; Masuda et al., 1988; Masuda, 1989). These characteristics may be one of the reasons that the edamame cultivars are preferred by consumers, although the threshold values of the concentrations of these amino acids with respect to sweetness and taste have not been determined.

Isoleucine, leucine, threonine, methionine, lysine, phenylalanine, tryptophane, histidine, arginine, and valine, all of which were found in the present experiments, are essential for the maintenance and growth of animals including humans. However, another essential amino acid (cysteine) was not detected in the present experiments. Although these amino acids were less abundant than those of the three major free amino acids in the immature seeds, their concentrations in the edamame were almost double, and in some cases more than double, those in the immature seeds of the grain-type cultivars. Thus, considering only free amino acids, the immature seeds of edamame cultivars may supply more essential amino acids than do those of the grain-type cultivars. This benefit of the edamame cultivars appears to be similar to the improvement of other cultivars through increases in globulin subunits (11S), which contain more essential amino acids, such as methionine and threonine (Harada et al., 1983; Ogawa et al., 1989), and decreases in β-conglycinin (7S), which is poor in essential amino acids. However, total amino acid compositions are needed to compare the nutritive values of the vegetable-type and grain-type cultivars. These problems remain for further experiments.

Why the total Kjeldahl N slightly decreased during the period 30–40 DAF remains to be explained. The decrease may be associated with the rapid growth of seeds during this period, as suggested by the accumulation of several amino acids.

ABBREVIATIONS USED

DAF, days after flowering; HPLC, high-performance liquid chromatography; PITC, phenylisothiocyanate; TFA, trifluoroacetic acid.

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