Journal of Applied Horticulture, 11(1): 59-63, January-June, 2009

Extraction and determination of α-solanine in eggplant fruits

Zhiwen Li[1], Baoli Zhou[1]*, Yuwen Ding[1] and Xiang Liu[2]

*[1]College of Horticulture, Shenyang Agricultural University, Shenyang 110161, China; [2]College of Agriculture and Bioengineering, Tianjin University, Tianjin 300072, China. *E-mail: zblaaa@163.com*

Abstract

A simple and effective high-performance liquid chromatographic (HPLC) method for determination of α-solanine in eggplant fruits is described in our study. A new extraction method is established for extracting α-solanine in eggplant fruits. Single and orthogonal tests were designed to analyze the effect of different extraction methods and ultrasonic wave extraction condition on extraction of α-solanine in eggplant fruits. HPLC separation was achieved on a Waters Nova-pak C18 column with the mobile phase acetonitrile-0.05N potassium dihydrogen phosphate (55:45, V/V). The flow rate was 0.7mL min^{-1} and the UV absorbance was monitored at 202 nm. The optimal extraction method was ultrasonic wave extraction in 70% methanol for 60 minutes at 50°C, and with material to liquid ratio of 1:10. Under the optimal extraction conditions, the average content of α-solanine in skins and flesh of dried eggplant fruits was 0.107±0.006 and 0.626±0.004mg g^{-1}, respectively. The average recovery efficiency was 97.97%.

Key words: Eggplant, α-solanine, HPLC, extraction, ultrasonic wave

Introduction

Eggplants (*Solanum melomgena* L.) produce natural active substances called glycoalkaloids with biological activity. Glycoalkaloids, like many secondary metabolites, are thought to function in plant chemical defense, acting as nonspecific protectors or repellents against potential pest predators (Osman, 1980). Eggplants contain a type of glycoalkaloids like other nightshade species called α-solanine (Manuchair, 2006), which is an important active substance with many biological activities including anti-tumor (Tai, 2002, Ji *et al.*, 2005), lowering blood pressure and heart stimulation (Yang, 2004). In addition, α-solanine has been used in the treatment of asthma and epilepsy (Zhang *et al.*, 2002), and also has shown the effects of antifeedant (Beier, 1990), fungicide (Allen and Kúc, 1968), and pesticide (Birch *et al.*, 2002).

Consideration of the above demonstrates the importance of accurate and reliable analytical methods for α-solanine. Literatures suggest that α-solanine could be extracted with methanol (Kobayashi *et al.*, 1989; Saito *et al.*, 1990), methanol-chloroform (2:l v/v) (Bushway and Ponnampalam, 1981), heptanesulfonic and acetic acids (Carman *et al.*, 1986), 1.1% acetic acid (Filadelfi and Zitnak, 1982), and tetrahydrofuran (THF)-water-acetonitrile with 1% acetic acid (Bushway *et al.*, 1980a, 1983, 1986). However, the common soaking extraction procedure usually requires long time, high temperature and more energy. As a novel technique for sample pretreatment, ultrasound-assisted extraction has attracted more attention in recent years. Compared with other leaching techniques such as Soxhlet extraction, microwave-assisted extraction and supercritical fluid extraction, ultrasound-assisted extraction shows many potential advantages in various aspects, such as faster extracting rate, matter quality reservation and lower time and energy cost. It has been used in extracting anthraquinones from *Rheum palmatum* L, and cyanuric acid from pet food (Chen *et al.*, 2008; Wang *et al.*, 2008)

Many analytical methods for α-solanine are reported in literature, these methods include isotachyphoresis (Kvasnicka *et al.*, 1994), thin layer chromatographic scanning (Ferreira *et al.*, 1993), various colorimetric methods, gas chromatography (Lawson *et al.*, 1992), countercurrent chromatography (Fukuhara and Kubo, 1991), and high-pressure liquid chromatography (HPLC) (Everard *et al.*, 1996; Hitoshi *et al.*, 2005), and enzyme immunoassays (Plhak and Sporns, 1992). Each method has advantages and disadvantages. For example, the colorimetry and thin layer chromatographic scanning are not proper to quantitative analysis of glycoalkaloids because of low sensitivity and recovery; the gas chromatography and enzyme immunoassays treatment has complicated process, bad colour stability, low sensitivity. Compared to other methods, HPLC is a sensitive, simple, rapid, and relatively cheap detection method.

The objective of this study was to establish a concise, reproducible method for the routine extraction and quantification of α-solanine in eggplants.

Materials and methods

Materials: The seeds of eggplant 'Liaoqie-7' (purple eggplant) were bought from Fuyou Seed Company in Liaoning Province, China. The seeds were sown in soil and transplanted on 60th days in the greenhouse in Shenyang Agricultural University. Eggplant fruit samples were harvested at mature stage. In order to separate skin from flesh, a knife was used to peel fruits. Then scrape the inside of skin to remove the left flesh. So the content of α-solanine in skin didn't contain the flesh. And the content of α-solanine in flesh didn't contain the skin. The skin and flesh were porphyrized to powder after drying in the air. The rate of dried weight to fresh weight in samples was 1:10. The powder was then passed through a 40-mesh screen, and stored in the extractor at 4°C.

The α-solanine stock solution (3 mg mL^{-1}) was prepared through dissolving α-solanine (Sigma-Aldrich chemical Co., USA) in methanol. All solutions were prepared with ultrapure water (Barnstead Diamind, USA). All chemicals were AnalaR grade except for special notifications.

Extraction procedure: The extraction technique was adopted from Bushway *et al.* (1980b) with some modifications. 25g of dried powder was stirred with 250 mL of 70% methanol and 1mg mL^{-1} sodium bisulfitein in a mixer (Corning PC-420D, USA) for 15 min. The mixture was treated by different treatments which were ultrasonic wave extraction, microwave extraction, soxhlet extraction, oscillation extraction and soaking extraction at 50° for 60 min, and then vacuum filtrated. The filter was collected and concentrated to approximately 10-15 mL by rotary evaporator (Eyela N-1000, Japan). The concentrate was then added with 10-15 mL of 0.2 NHCl while being stirred. The extraction was centrifuged at 8000 rpm at 20° for 15 min (HERMLE Z 36 HK, German), and the pH of the suspension was adjusted to 10-11 (Horiba F-53, Japan) with concentrated ammonium hydroxide before the sample was placed into a water bath at 70° for 30min. The solution was then cooled in an ice-water bath for 30 min and centrifuged at 10000 rpm at 20° for 30 min. The precipitate was washed with 5 mL of 1% ammonium hydroxide and centrifuged at 10000 rpm at 20° for 15 min, and the pellet was collected. The air-dried pellet was then dissolved in 10 mL of methanol (HPLC grade, Merck, German) and treated in the ultrasonic treatment unit at 50° for 30min. The suspension was filtered through a 0.45 μm filter membrane. The filtrate was used for HPLC. In this experiment we adopted different factors which were three solvent (70% Methanol, 5% Acetic acid and Methanol: chloroform = 2:1), three extraction time (40, 50 and 60 min), three temperature (30, 40 and 50°C) and three material to liquid ratio (1:8, 1:9 and 1:10) to find the best condition of extracting α-solanine from eggplant fruit with ultrasonic wave by orthogonal testa.

HPLC Procedure. The HPLC procedure was adopted from Friedman and Dao (1992) with some modifications**.** Spectra of α-solanine in the adopted mobile phase showed maxima absorbance at 202 nm. Therefore, 202 nm was used for quantification of α-solanine. A Waters 600 controller HPLC system with a 600 pump and degasser, an automatic injector with a final volume loop of 10 μL, a Nova-pak Cl8 coupled cartridges column (250×4.6mm, 5μm) and a Waters 2487 UV-vis variable-wavelength detector was used. System management and hardware interface for data acquisition were performed by the Millennium32 computer software package from Waters. The mobile phase was acetonitrile (HPLC grade, Merck, German)-0.05 N potassium dihydrogen phosphates (55:45 v/v) adjusted to pH 4.5 with 1% phosphoric acid. This was passed through a 0.45 μm filter and degassed for 20 min under reduced pressure. The HPLC flow rate was 0.7 mL min^{-1}, and the UV absorbance was measured at 202 nm.

Spiking Experiments: A series of spiking experiments were carried out to establish the extent of recovery of added α-solanine from eggplants. Specifically, 20 g dried eggplant fruit powder was added in different experiments with 1, 2, 3, 4 and 5 mg α-solanine, respectively. The samples were thoroughly mixed, extracted, and analyzed by HPLC for recovery of the added alkaloids.

TLC Procedure: Preparative thin-layer chromatography (TLC) was performed on silica gel precoated plates, 0.25 mm×20cm×20 cm (Merck, Darmstadt, Germany). A 25-μL methanol concentrated extract was spotted on the plate along with α-solanine standards. Then the plate was developed with a bottom layer of benzene-methanol-1 % ammonium hydroxide (10:2 v/v). After that, the dried plate was sprayed with 1% bismuth potassium iodide with visible violet red spot.

Results

The methanol concentrated extracts were spotted on a TLC plate along with α-solanine standards. And the skin and flesh extracts were analyzed. After development, all plates showed only one spot corresponding to α-solanine (R = 0.35).

Fig. 1 shows linear relationships in the range 0.15-3 mg mL^{-1} between concentrations of α-solanine (r = 0.995) and the peak height on HPLC chromatograms. Fig. 2 illustrates the separation of α-solanine on the HPLC columns in standard solution, extract and extract added with standard of eggplant fruit, with the retention time of 2.8 min. These data suggested that minimal error was related to HPLC.

The highest extracting rates of α-solanine in both skin and flesh were obtained using ultrasonic wave (Table 1), with virtually 0.107 and 0.626 mg g^{-1}, whereas common extraction gave only 0.0114 and 0.192 mg g^{-1}, respectively. None of the other extraction means used gave extracting rates as high as those obtained with the ultrasonic wave extraction.

The effect of extracting α-solanine from eggplant fruit with ultrasonic wave was dependent on solvent, extraction time, extraction temperature and material to liquid ratio. For both skin and flesh, the effect of these four factors on extracting rate was solvent (A) > material to liquid ratio (D) > time (B) > temperature (C) (Table 2). The best treatment was A1B3C3D3, with extraction

Table 1**.** Extracting rates of different extraction means on skin and flesh of eggplant[a]

Extraction method	α-solanine extracting rate (mg g^{-1})	
	Skin	Flesh
Ultrasonic wave extraction	0.107±0.006 a A b	0.626±0.004 a A
Microwave extraction	0.0764±0.002 b B	0.432±0.003 b B
Soxhlet extraction	0.0422±0.0004 c C	0.358±0.006 c C
Oscillation extraction	0.0405±0.001 c C	0.343±0.007 d D
Common Extraction	0.0114±0.001 d D	0.192±0.003 e E

[a] Results values are means ± standard errors (n=3)

Different lowercase letters in the same column indicate significant difference at *P*=0.05 and different capital letters indicate significant difference at level of *P*=0.01

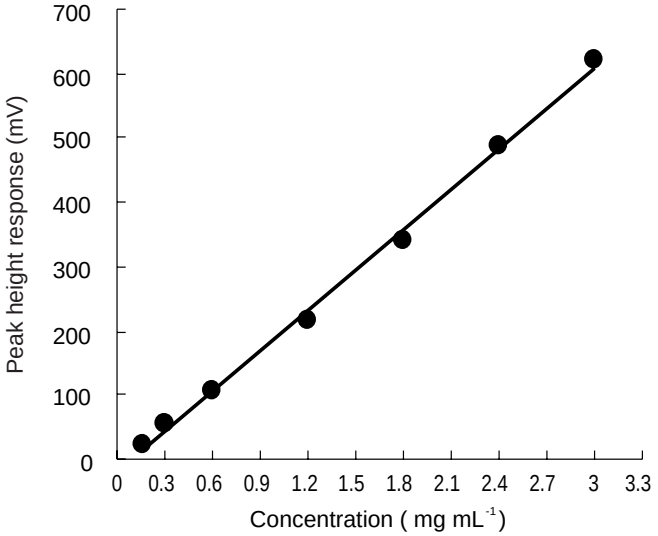

Fig. 1. Standard α-solanine peak height response with HPLC

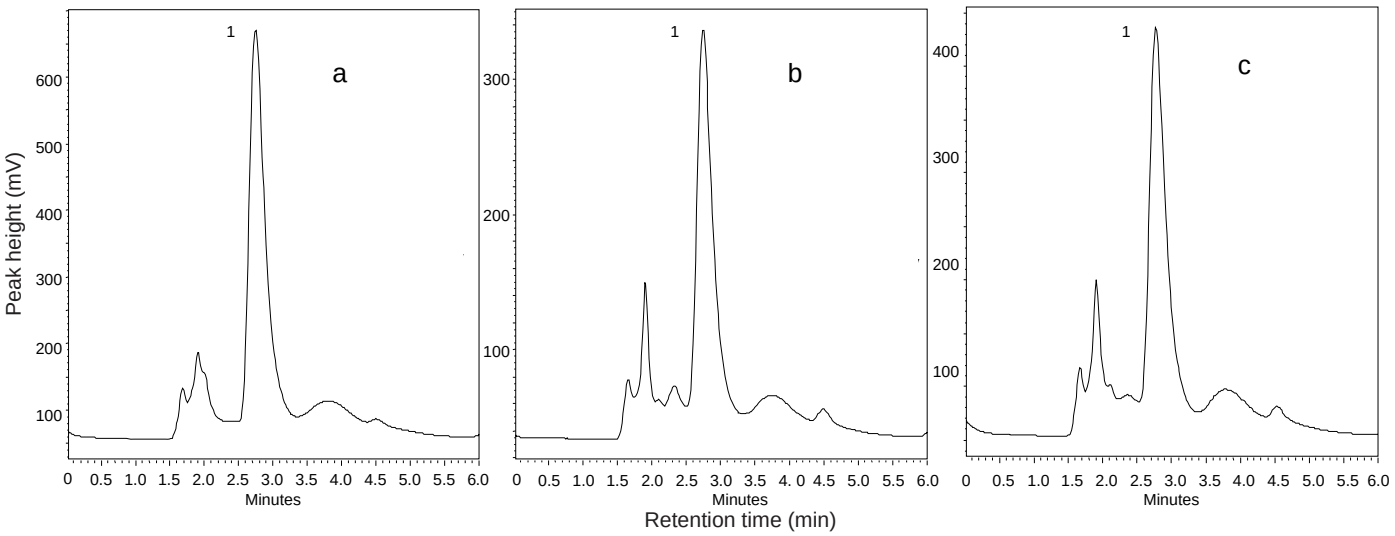

Fig. 2. Chromatograms of α-solanine standard (a), α-solanine in eggplant fruit (b) and α-solanine in mixture of eggplant fruit extracting solution added with α-solanine standard (c). Peak: 1, α-solanine

rates 0.107 and 0.626 mg g^{-1}, extracted at 50° for 60 minutes in 70% methanol with the material to liquid 1:10.

Spiking experiments (Table 3) revealed that the modified method could recover a 96-99% of added α-solanine. In the range of 1- 5 mg of α- solanine / 20 g dried eggplant fruit powder (flesh and skin), the extent of recovery increased with the amount added before extraction. The average recovery was 97.97%.

Discussion

Since eggplant fruits contain approximately 93-94% water, the air-dried samples provide more than 10 times concentration of α-solanine. Air-dried samples can be easily grinded and stored for longer periods of time prior to measurements. Considering these advantages, air-dried fruits were used in this study.

The recovery of added and native α-solanine in skin and flesh of eggplant fruts increased by approximately 2% in the condition of without washing the precipitate with 1% ammonium hydroxide. Washing aimed to remove most brown pigments in the final extraction product when eggplants flesh was used as starting materials. The brown pigment does not seem to interfere in the analysis except for turning into dark. To minimize possible interference of pigment in skins, the purple precipitate formed during evaporation of the extracts was first removed by centrifugation and filtration. The partially evaporated clear filtrate was then analyzed for alkaloid content.

Separation and retention of the α-solanine increased by raising the buffer pH. However, this resulted in reduced sensitivity of the system due to low solubility of α-solanine. No alkaloid was detected above pH 7 because of precipitation upon injection onto the column. The optimum pH was found to be 4-5. Reducing the amount of acetonitrile in the mobile phase also improved separation. Using 55% acetonitrile, 45% buffer gave maximum separation. α-solanine was insoluble at low acetonitrile ratios.

Table 2. Results of orthogonal test[a] of α-solanine extracted from eggplant skin and flesh with ultrasonic wave[b]

Treatment	A Solvent	B Time (min)	C Temperature (°C)	D Material to liquid (g:mL)	Extracting rate in skins (mg g^{-1})	Extracting rate in flesh (mg g^{-1})
1	A1 70% Methanol	B1 (40)	C1 (30)	D1 (1:8)	0.0867±0.004	0.524±0.002
2	A1	B2 (50)	C2 (40)	D2 (1:9)	0.0958±0.003	0.554±0.003
3	A1	B3 (60)	C3 (50)	D3 (1:10)	0.1070±0.006	0.626±0.004
4	A2 5% Acetic acid	B1	C2	D3	0.0776±0.003	0.386±0.006
5	A2	B2	C3	D1	0.0535±0.0003	0.147±0.004
6	A2	B3	C1	D2	0.0547±0.0003	0.158±0.005
7	A3 Methanol:chloroform 2:1	B1	C3	D2	0.0395±0.001	0.139±0.007
8	A3	B2	C1	D3	0.0458±0.001	0.151±0.006
9	A3	B3	C2	D1	0.0220±0.001	0.0635±0.004
R_α	0.4542	0.0708	0.0528	0.1390	—	—
R_α	0.0608	0.0067	0.0044	0.0228	—	—

[a]$L_9(3)4$ design was adopted in this orthogonal test

[b]Results are the mean extracting rate of standard ± standard errors, n=3

Table 3. Results of recoveries of α-solanine added to dried sample[a]

Sample (mg)	Added (mg)	Detected (mg)	Recovery[b] (%)
15.09±0.05	1	15.90±0.05	98.82±1.1
14.27±0.05	2	16.18±0.05	99.45±0.9
14.25±0.05	3	16.70±0.05	96.81±1.3
14.65±0.05	4	18.24±0.05	97.80±0.8
14.89±0.05	5	19.29±0.05	96.98±1.2
Mean±SD			97.97±1.1

[a]20 g powder was added the known amount of α-solanine. The samples were mixed, extracted, and analyzed with HPLC.

[b]Results are the mean recoveries of standard ± standard errors, n=3

The composition of the mobile phase used for HPLC was important in ensuring full separation of α-solanine. A number of published methods were conducted with Tris-HCl at near-neutral pH (Jonker *et al.*, 1992). However, under such conditions the HPLC stability was not good and the linear relationships did not perform well.

Many of extraction solvents were utilized in the published methods and most were based on some solvents or salts in a weak solution of acetic acid. Heptanesulfonic acid and aqueous acetic acid (Everard *et al.*, 1996) or 5% acetic acid (Hitoshi *et al.*, 2005) were commonly added. However, as α-solanine is probably stored within the aqueous phase of the eggplant cell and is readily soluble in solvents like methanol with the addition of water through some special extraction means like ultrasonic wave extraction, the use of acid is unnecessary. In addition, the solution of acetic acid under ultrasonic wave condition may lead to hydrolyzation of α-solanine resulting in a reduced recovery. In this experiment, the extracting rate of 70% methanol as solvent was higher than that of 5% acetic acid. Sodium bisulfite was used to reduce oxidation of the extract (Hellenas, 1986), and also used in these investigations.

Ultrasound is an efficient non-thermal alternative (Kim und Zayas, 1989; Yan *et al.*, 2002; Knorr *et al.*, 2002). Ultrasonic cavitation creates shear forces that break cell walls mechanically and increase material transfer. In this experiment, ultrasonic wave extraction with methanol as extraction solvent gave the highest extracting rate and optimal recovery.

Some researches showed that during ultrasonic extraction, the relationships between extraction time and extracting rate are as follows (Guo, 1995; Guo, 1997): (1) The extracting rate increased as time prolongs; (2) The extracting rate increasment slowed as time prolonging to certain extent; (3) The extracting rate decreased as time prolonging to critical stage. The reason for extracting rate decrease is probably due to the fact that prolonged time may decompose the active components and increase their impurity, leading to low extracting rate (Duan and Feng, 1992). In a preparatory experiment, the extracting rate increased rapidly within 60 minutes extraction, slowly in 60-90 minutes extraction, and decreased after 90 minutes.

The above method aim at a number of problems with HPLC analysis of α-solanine, and utilizes a new extracting way to improve sample recoveries and got good results. It can be used in eggplant breeding, production and medical care research. Laboratory also can use this method to investigate the response of eggplant alkaloid concentrations to environmental conditions during cultivation and some biological activity of eggplant.

References

Allen, E.H. and J. Kúc, 1968. α-solanine and α-chaconine as fungitoxic compounds in extracts of Irish potato tubers. *J. Phytopathol.*, 58: 776-781.

Beier, R.C. 1990. Natural pesticides and bioactive components in foods. *Rev. Environ. Contam. Toxicol.*, 113: 47-137.

Birch, N.E., I.E. Geoghegan, D.W. Griffiths and J.W. Mcnicol, 2002. The effect of genetic transformations for pest resistance on foliar solanidine-based glycoalkaloids of potato (*Solatium tuberosuni*). *J. Annals Appl. Biol.*, 14: 143-149.

Bushway, R.J. and R. Ponnampalam, 1981. α-chaconine and α-solanine content of potato products and their stability during several modes of cooking. *J. Agr. Food Chem.*, 29: 814-817.

Bushway, R.J., E.S. Barden, A.W. Bushway and A.A. Bushway, 1980a. The mass extraction of potato glycoalkaloids from blossoms. *Am. Potato J.*, 157: 175-180.

Bushway, R.J., E.S. Barden, A.M. Wilson and A.A. Bushway, 1980b. Analysis of potato glycoalkaloids by high-performance liquid chromatography. *J. Food Sci.*, 45: 1088-1089.

Bushway, R.J., J.L. Bureau and J. King, 1986. Modification of the rapid high-performance liquid chromatographic method for the determination of potato glycoalkaloids. *J. Agr. Food Chem.*, 34: 277-279.

Bushway, R.J., J.L. Bureau and D.F. McGann, 1983. α-Chaconine and α-solanine content of potato peels and potato peel products. *J. Food Sci.*, 48: 84-86.

Carman, A.S., S.S. Kuan, G.M. Ware, O.J.J. Francis and G.P. Kirschenheuter, 1986. Rapid HPLC determination of the potato glycoalkaloids α-solanine and α-chaconine. *J. Agr. Food Chem.*, 34: 279-282.

Chen Y., L. Zhua, J. Xiaoa, H. Tanga, G. Guob, Q. Zengb and X.Wang, 2009. Ultrasonic extraction and determination of cyanuric acid in pet food. *Fd. Control*, 20(3): 205-208.

Duan, G.M. and C.Q. Feng, 1992. Glycoalkaloids in potatoes. *J. Plant Physiol. Commun.*, 28: 457-461.

Everard, J., Edwards and H.Cobb. Andrew, 1996. Improved high-performance liquid chromatographic method for the analysis of potato (*Solanum tuberosum*) glycoalkaloids. *J. Agr. Food Chem.*, 44: 2705-2709.

Ferreira, F., P. Moyna, S. Soule and A. Vazquez, 1993. Rapid determination of solanum glycoalkaloids by thin-layer chromatographic scanning. *J. Chromatogr.*, 653(2): 380-384.

Filadelfi M.A. and A. Zitnak, 1982. Preparation of chaconines by enzymic hydrolysis of potato berry alkaloids. *J. Phytochemistry*, 21: 250-251.

Friedman, M. and L. Dao, 1992. Distribution of glycoalkaloids in potato plants and commercial potato products. *J. Agric. Food Chem.*, 40: 419-423.

Fukuhara, K. and I. Kubo, 1991. Isolation of steroidal glycoalkaloids from *Solanum incanum* by two countercurrent chromatographic methods. *J. Phytochemistry*, 30: 685-687.

Guo, X.W. 1995. Effect on extraction yield of berberine by ultrasound-assisted extraction. *J. Chin. Materia*, 20: 673-675.

Guo, X.W., 1997. Comparison of effect on rutin component by ultrasound-assisted and hot alkali extraction. *J. Chin. Traditional Herbal Drugs*, 28: 88-89.

Hellenas, K. 1986. A simplified procedure for the quantification of potato glycoalkaloids in tuber extracts by HPLC, comparison with ELISA and a colorimetric method. *J. Sci. Food Agr*., 37: 776-782.

Hitoshi, K., S. Keiitsu, N. Nobumitsu, Y. Shigeo and T. Youichi, 2005. Simple and sensitive method for determination of glycoalkaloids in potato tubers by high-performance liquid chromatography with chemiluminescence detection. *J. Chromatogr. A*, 1100(1): 26-31.

Ji, Y.B., H.L. Wang and S.Y. Gao, 2005. Effect of solanine on DNA and RNA in tumor cell of tumor-bearing mice. *J. Chin. Traditional Herbal Drugs*, 36: 1200.

Jonker, H.H., A.J. Koops and J.C. Hoogendoorn, 1992. A rapid method for the quantification of steroidal glycoalkaloids by reversed phase HPLC. *J. Potato Res.*, 35: 451-455.

Kim, S.M. and J.F. Zayas, 1989. Processing parameter of chymosin extraction by ultrasound. *J. Food Sci.*, 54: 700.

Knorr, D., B.I.O. Ade-Omowaye and V. Heinz, 2002. Nutritional improvement of plant foods by non-thermal processing. *J. Proceedings Nutr. Soc.*, 61: 311-318.

Kobayashi, K., A.D. Powell, M. Toyoda and Y. Saito, 1989. HPLC method for the simultaneous analysis of α-solanine and α-chaconine in potato plants cultured *in vitro*. *J. Chromatogr.*, 462: 357-364.

Kvasnicka, F., K.R. Price, K. Ng and G.R. Fenwick, 1994. Determination of potato glycoalkaloids using isotachophoresis and comparison with a HPLC method. *J. Liquid Chromatogr. Related Technol.*, 17: 1941-1951.

Lawson, D.R., W.A. Erb and A.R. Millar, 1992. Analysis of *Solanum* alkaloids using internal standardization and capillary gas chromatography. *J. Agric. Food Chem.*, 40: 2186-2191.

Manuchair, S.E., 2006. *Pharmacodynamic basis of herbal medicine*, US: CRC Press, 309.

Osman, S.F., 1980. Glycoalkaloids of the Solanaceae. *Recent Adv. Phytochem*., 14: 75-96.

Plhak, L.C. and P. Sporns, 1992. Enzyme immunoassay for potato glycoalkaloids. *J. Agric. Food Chem.*, 40: 2533-2540.

Saito, K., M. Horie, Y. Hoshino, N. Nose and H. Nakazawa, 1990. HPLC determination of glycoalkaloids in potato products. *J. Chromatogr.*, 508: 141-147.

Tai, J.R. 2002. The effect of cancer prevention and resistance of main vegetables. *J. Southwest Hort.*, 30: 28-29.

Wang, L., D. Li, C. Bao, Z. Wang, Y. Shi and H. Zhang, 2008. Ultrasonic extraction and separation of anthraquinones from *Rheum palmatum* L. *Ultrason. Sonochem*., 15: 738-746.

Yan, W., S.F. Li and S.J. Tian, 2002. Ultrasound-assisted extraction technology. *J. Chem. Ind. Eng. Progress*, 21: 649-651.

Yang, X.W. 2004. *Alkaloids.* Beijing, Chemical Industry Press, 393.

Zhang, C.Y., S. Li, X. Chen and C.M. Wang, 2002. Studies on the anti-asthma action mechanism of nightshade alkaloids. *J. Theory and Practice of Chinese Medicine*, 2002: 488-489.