DE GRUYTER
OPEN

Beiträge zur Tabakforschung International
Contributions to Tobacco Research
Volume 27 · No. 2 · April 2016
DOI: 10.1515/cttr-2016-0008

Nicotine Analysis in Several Non-Tobacco Plant Materials *

by

Serban C. Moldoveanu, Wayne A. Scott, and Darlene M. Lawson

R.J. Reynolds Tobacco Co., Winston-Salem NC, USA

SUMMARY

Present study describes the determination of nicotine in various plant samples with a low content of this compound. Nicotine is found naturally in plants from the *Solanaceae* family. The plants from *Nicotiana* genus contain large levels of nicotine. However, only low levels are present in plants from *Solanum* genus including potato, tomato, eggplant, and from *Capsicum* genus, which are used as food. Because the levels of nicotine in these materials are in the range of parts per billion, the measurements are difficult and the results are very different from study to study. The present study evaluated the level of nicotine in a number of plants (fruits, roots, leaves, tubers) from *Solanaceae* family (not including *Nicotiana* genus) and from several other vegetables commonly used as food. The analysis consisted of the treatment of plant material with an aqueous solution 5% NaOH at 70 °C for 30 min, followed by extraction with TBME containing d_3-nicotine as an internal standard. The TBME organic layer was analyzed on a 7890B/7000C GC-MS/MS system with a 30 m x 0.25 mm, 0.25 µm film CAM column. The MS/MS system worked in MRM positive ionization mode monitoring the transition 162 - 84 for nicotine and 165 - 87 for d_3-nicotine. Particular attention was given to the preservation of the intact levels of nicotine in the plant material. The plant material was analyzed as is, without drying and with minimal exposure to contaminations. Separately, the moisture of the plant material was measured in order to report the nicotine level on a dry-basis. Levels of nicotine around 180 ng/g dry material were obtained for tomatoes and eggplant (fruit) and lower levels were obtained for green pepper and potato. Similar levels to that in the tomato fruit were detected in tomato leaves. Materials from other plant families also showed traces of nicotine. [Beitr. Tabakforsch. Int. 27 (2016) 54–59]

KEYWORDS

Nicotine, *Solanaceae*, Tomato, Eggplant

ZUSAMMENFASSUNG

Die vorliegende Studie beschreibt die Analyse von Nikotin in verschiedenen Pflanzenproben mit einem geringen Gehalt dieser Substanz. Nikotin kommt von Natur aus in Pflanzen der Familie der Nachtschattengewächse vor. Pflanzen der Gattung *Nicotiana* enthalten große Mengen Nikotin. In Pflanzen der Gattung *Solanum*, wie Kartoffeln, Tomaten, Auberginen, sowie der Gattung *Capsicum*, die als Nahrungsmittel verwendet werden, sind jedoch nur geringe Mengen vorhanden. Da die Nikotinmengen in diesen Stoffen im Bereich von Milliardstel liegen, sind Messungen schwierig und die Ergebnisse von Studie zu Studie sehr verschieden. In der vorliegenden Untersuchung wurde der Nikotingehalt in einer Reihe von Pflanzen (Früchte, Wurzeln, Blätter, Knollen) der Familie der Nachtschattengewächse (*Solanaceae*, ohne die Gattung *Nicotiana*) sowie in diversen anderen Gemüsen, die allgemein als Nahrungsmittel verwendet werden, analysiert. Dazu wurde das Pflanzenmaterial mit einer wässrigen Lösung mit 5% NaOH bei 70 °C für 30 min behandelt, gefolgt von einer Extraktion mit TBME, welches d_3-Nikotin als internen Standard enthielt. Die organische TBME-Schicht wurde auf einem 7890B/7000C GC/MS/MS-System mit einer CAM-

*Received: 29th May 2015 – accepted: 1st April 2016

Säule von 30 m x 0,25 mm und 0,25 µm Filmdicke analysiert. Das MS/MS-System lief im MRM-Modus mit positiver Ionisierung zur Messung des Übergangs 162 - 84 für Nikotin und 165 - 87 für d_3-Nikotin. Ein besonderes Augenmerk wurde auf die Erhaltung der intakten Nikotinkonzentrationen im Pflanzenmaterial gelegt. Letzteres wurde im Ist-Zustand, ohne Trocknung und unter minimaler Exposition gegenüber Kontaminationsquellen analysiert. Die Feuchtigkeit des Pflanzenmaterials wurde separat gemessen, um die Nikotinkonzentration auf Trockenbasis anzugeben. Für Tomaten und Auberginen (in der Frucht) wurden Nikotinkonzentrationen um 180 ng/g Trockenmaterial ermittelt, niedrigere Werte wurden für grüne Paprika und Kartoffeln gemessen. Ähnliche Mengen an Nikotin wie in der Tomatenfrucht wurden in den Blättern der Tomatenpflanze festgestellt. In Material von weiteren Pflanzenfamilien fanden sich Spuren von Nikotin, die wahrscheinlich von Kontaminationen herrührten. [Beitr. Tabakforsch. Int. 27 (2016) 54–59]

RESUME

La présente étude décrit une analyse de la nicotine dans divers échantillons de plantes présentant une faible teneur de ce composé. La nicotine est naturellement présente dans les plantes de la famille des solanacées. Les plantes du genre *Nicotiana* contiennent de grandes concentrations de nicotine. Cependant, seules de faibles concentrations sont observées dans les plantes du genre *Solanum* telles que la pomme de terre, la tomate, l'aubergine et dans les plantes du genre *Caspicum*, qui sont utilisées à des fins alimentaires. Sachant que les concentrations en nicotine dans ces matières sont de l'ordre de parties par milliards, les mesures s'avèrent difficiles et les résultats varient grandement d'une étude à l'autre. La présente étude évalua la concentration de nicotine dans un certain nombre de plantes (fruits, racines, feuilles, tubercules) de la famille des solanacées (à l'exception des plantes du genre *Nicotania*) ainsi que dans plusieurs autres légumes communément consommés à des fins alimentaires. L'analyse consista en un traitement de la matière végétale à l'aide d'une solution aqueuse 5% NaOH à 70 °C durant 30 minutes, suivi d'une extraction avec de l'éther *tert*-butylique méthylique (ETBM) contenant de la nicotine d_3 en guise d'étalon interne. La couche organique d'ETBM fut analysée sur un système 7890B/7000C GC/MS/MS (Agilent) avec une colonne CAM (dimensions du film: 30 m x 0,25 mm, 0,25 µm). Le système de spectrométrie de masse en tandem fonctionna en mode d'ionisation positive MRM (suivi des réactions multiples), surveillant la transition 162-84 de la nicotine et 165-87 de la nicotine d_3. Une attention particulière fut portée à la préservation de concentrations intactes de nicotine dans la matière végétale. La matière végétale fut analysée en l'état, sans séchage et avec une exposition minimale aux contaminations. De façon séparée, l'humidité de la matière végétale fut mesurée afin de recenser la concentration de nicotine sur une base sèche. Des concentrations de nicotine avoisinant 180 ng/g par matière sèche furent obtenues pour les tomates et les aubergines (fruits) et des concentrations plus faibles furent mesurées pour le poivron vert et la pomme de terre. Des concentrations similaires à celles observées dans le fruit de la tomate furent détectées dans les feuilles de tomate. Les matières provenant d'autres familles végétales présentèrent des traces de nicotine probablement imputables à des contaminations. [Beitr. Tabakforsch. Int. 27 (2016) 54–59]

INTRODUCTION

Nicotine is known to be present naturally in plants from the *Solanaceae* family, with large levels in plants from *Nicotiana* genus (*N. tabacum* 2–4% or higher nicotine, *N. rustica* up to 9% nicotine, *N. alata*, *N. sylvestris*, etc., and other 63 species). Only low levels are present in plants from other *Solanaceae* genera which are used as food such as *Solanum* genus including potato, tomato, eggplant, or *Capsicum* genus that includes peppers. A number of studies are dedicated to the significance of nicotine intake from non-tobacco plants used as food (1–3), and therefore the determination of nicotine in non-tobacco plant materials is of interest.

The procedures for the determination of traces of nicotine included radioimmunoassay (RIA) (4), gas chromatography (GC) with nitrogen-phosphorus detector (NPD) (5–9), GC with mass spectrometric (MS) detection (10, 11), GC-MS/MS (12), and liquid chromatography with mass spectrometric detection (LC-MS/MS) with solid phase extraction (SPE) concentration (13). Most procedures involved extraction of the homogenized plant material with 0.2 M HCl, treatment of the mixture with a base, and extraction with an organic solvent such as toluene or dichlorometane. Some procedures also involved solid phase extraction cleanup. Because the levels of nicotine in various plant materials (not including *Nicotiana*) are in the range of parts per billion, the measurements are difficult and the reported results differ from study to study. Besides the fact that the nicotine level in a plant material may show some natural variability, another reason for the differences in the results is that the analysis of nicotine at very low levels, in the range of parts per billion, presents several challenges. The first such challenge is related to the potential contamination with nicotine from the surroundings and during sample preparation, such as during drying and grinding. The problem of contamination of sample with exogenous nicotine was also addressed in the literature (12, 14). Special care must be given to the cleanliness of glassware and any equipment getting in contact with the samples. The second challenge is related to the need for a complete extraction of nicotine from the plant material. For complete extraction the plant material must be made available to an extracting solvent without having nicotine encapsulated in the plant cell or as a result of the formation of clathrates with proteins. Finally, the low levels of nicotine in some samples required a very sensitive method of analysis that does not require the use of large amounts of sample and elaborate sample preparation procedures for nicotine concentration. The goal of present study was to establish a reliable method for the determination of nicotine at ng/g levels in various plant materials, and compare the results with previously reported ones that differ from author to author.

EXPERIMENTAL

Materials and instrumentation

The extracting solvent used for this analysis was *tert*-butyl methyl ether (TBME) obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, USA). Pure water (18.2 MΩ·cm) was obtained from a Barnsted water purification unit (Thermo Fisher Scientific, Waltham, MA, USA). A solution of 5% NaOH in water was prepared by diluting a 50% solution of NaOH also from Aldrich. Nicotine and d_3-nicotine were obtained from Toronto Research Chemicals (TRC, Toronto, ON, Canada). The moisture of the sample was removed using a Lindberg/Blue convection oven from Fisher Scientific. A heating block from Thermoline Scientific (Thermoline, Wetherill Park NSW, Australia) was used to heat the samples. The samples were centrifuged using a Sorval Legend XTR centrifuge (Thermo Scientific). The analysis was performed using a 7890B/7000C GC-MS/MS system from Agilent (Agilent Technologies Inc. Wilmington, DE, USA) equipped with a 30 m x 0.25 mm, 0.25 µm film CAM column (polyethylene glycol type phase specifically designed for amines analysis) from J&W (Agilent). The inlet GC was a multimode type, and the liner utilized was ultra-inert splitless, single taper with wool. Screw-cap vials of 50 mL and of 2 mL (GC vials), glass vial inserts of 250 µL, as well as 50 mL polypropylene conical tubes were also used during the analysis.

Sample preparation

The first step before performing the nicotine level determination was the measurement of sample moisture. The plant materials were placed in aluminum pans (14.5 cm × 8.4 cm × 4.7 cm). For fruits, roots, and tubers, about 50 g of plant material were precisely weighed. For leaves, between 10 g and 20 g were precisely weighed. The plant materials were placed for 24 hours in a convection oven at 102 °C. Longer drying times showed no variation in the final sample weight. The moisture (water content) was calculated from the weight difference (moist *vs.* dry).

For nicotine level determination, the plant material obtained from the market was carefully washed in order to eliminate potential external contaminations. The washing was performed first using water with 1–2 drops/L of Fisher Versa-Clean multi-purpose laboratory cleaner, followed by thorough rinsing with tap water, rinsing with distilled water, and then rapidly rinsing with about 50 mL methanol (for about 50 g plant material). After rinsing, the plant material was allowed to dry in a clean environment. Special precautions were taken not to contaminate the samples with external sources of nicotine, assuring the cleanliness of the utensils used to cut or handle the samples (knives, tweezers).

Based on the water content, samples equivalent to 300 mg dry material were placed in 50 mL screw-cap vials. The procedure for extracting nicotine from plant material samples has been previously reported and validated (14, 15). To each vial containing the sample were added 10 mL aqueous solution 5% NaOH. The samples were placed in a heating block for 30 min at 70 °C with occasional agitation. After the samples were cooled, 2 mL *tert*-butyl methyl ether (TBME) and 20 µL of a TBME solution of d_3-nicotine (as internal standard, I.S.) were added to the sample solutions, and the mixtures were agitated for 30 min on a wrist-action shaker. The d_3-nicotine solution contained 16 µg/mL d_3-nicotine. This solution was prepared by dissolving 1.6 mg compound in 100 mL TBME. The sample solutions plus extractant were transferred to 50 mL polypropylene conical tubes and centrifuged for 5 min at 3000 rpm. This sample preparation procedure is similar to that for a typical nicotine determination. The separation of the organic layer was good, and allowed the transfer of about 200 µL from each sample into vial inserts placed in 2 mL GC vials. These extracts were submitted for GC-MS/MS analysis.

For the preparation of nicotine standards, a stock solution of nicotine containing 531 µg/mL in TBME was initially prepared. From this solution, samples of different dilutions in TBME were obtained.

Determination of nicotine content

The determination of nicotine level in the sample extracts or in standard solutions was performed using a GC-MS/MS working in multiple reaction monitoring (MRM) positive mode. The conditions for the GC used for nicotine analysis are described in Table 1. The conditions for the tandem mass spectrometer detection are given in Table 2.

Using the conditions from Tables 1 and 2, the extracted ion chromatogram for ion 84 for a standard containing 10.4 ng/mL nicotine is shown in Figure 1. The S/N ratio (signal to noise) for the peak at 15.45 min is 1379. This ratio indicates the excellent sensitivity of the analytical measurement.

The quantitation for nicotine levels was performed using calibrations of amount of nicotine *vs.* (Peak area nicotine/Peak area I.S.). For this purpose, a set of 12 standards starting with the concentration of 2655.0 ng/mL to 2.6 ng/mL nicotine was used. The standard solutions contained

Table 1. Conditions for the GC analysis.

Parameter	Description
Initial oven temperature	50 °C
Initial time	1.0 min
Oven ramp rate	10 °C/min
Final oven temperature	240 °C
Final time	5.0 min
Total run time	25.0 min
Inlet temperature	250 °C
Inlet mode	Pulsed splitless
Pulse pressure	20 psi
Pulse time	0.75 min
Purge flow to split vent	15.0 mL/min
Carrier gas	Helium
Injection volume	1.0 µL
Flow mode	Constant flow
Flow rate	1.0 mL/min
Nominal initial pressure	12.05 psi
Average velocity	25.485 cm/sec
GC outlet	MS/MS
Auxiliary temperature	240 °C
Solvent delay	4.0 min

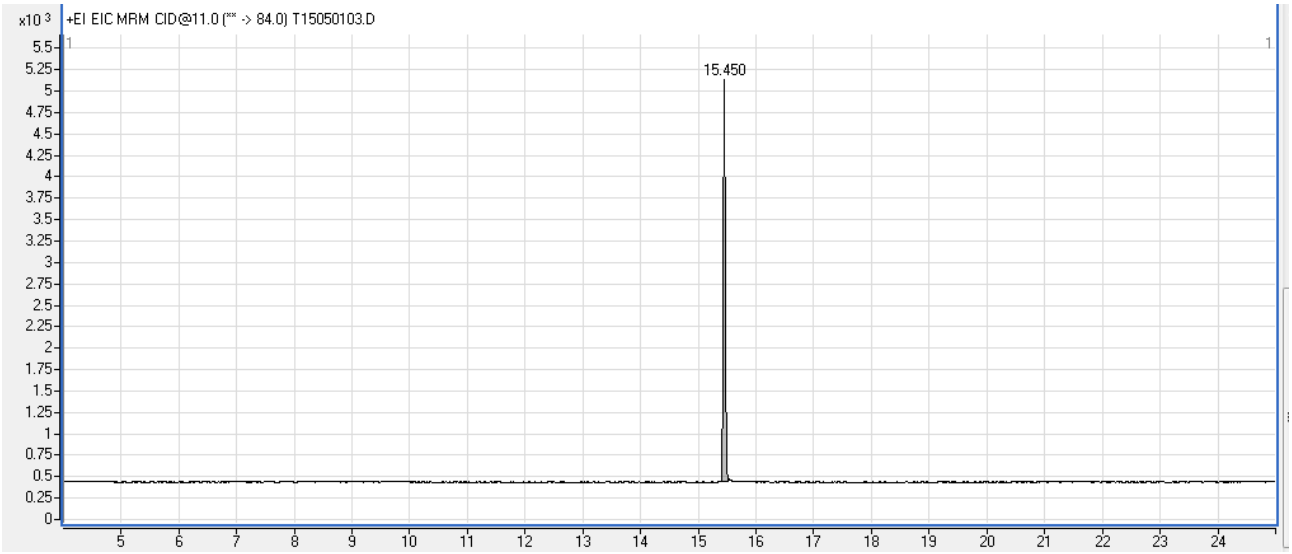

Figure 1. Extracted ion m/z = 84 from a nicotine standard containing 10.4 ng/mL.

Table 2. Conditions for the MS/MS detection.

Parameter	Description
Ion source	EI
Acquisition mode	MRM (positive)
Source temperature	230 °C
Collision cell helium flow	2.25 mL/min
N_2 collision gas flow	1.5 mL/min
Gain factor	10
d_3-Nicotine precursor ion	165.1
d_3 Nicotine product ion	87.1
MS1 resolution	Unit
MS2 resolution	Unit
Dwell time	120 ms
CE	11 V
Nicotine precursor ion	162.1
Nicotine product ion	84.1

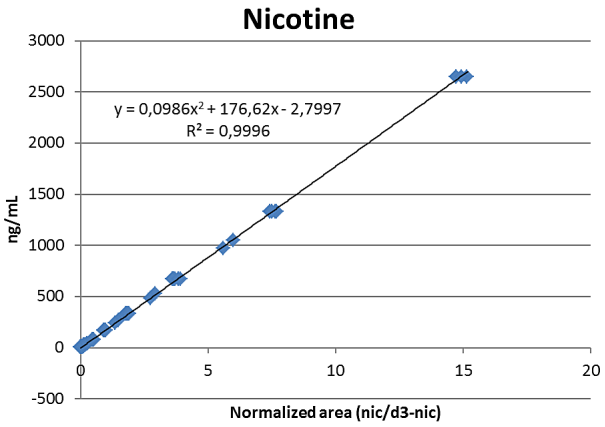

Figure 2. Calibration line for the quantitation of nicotine in the range 2.6 ng/mL to 2655.0 ng/mL.

160 ng/mL d_3-nicotine. The calibration curve obtained from several runs of the standards is shown in Figure 2.

Validation of the analytical procedure

For the validation of the analytical procedure, typical steps for this process were followed (see e.g. (16)). The selectivity of the nicotine analysis is assured by the use of MS/MS detection with specific selection of the precursor and product ion in MRM operation mode. The precision of the method gave excellent results for pure standards. For example, ten repeated analyses of a standard containing 10.4 ng/mL nicotine generated a relative standard deviation, RSD% = 3.9%. Also, for standards, the lowest limit of detection (LOD) as well as that of lowest limit of quantitation (LOQ) are very low (see S/N for the 10.4 ng/mL standard). For real samples, the precision was also very good for samples at levels above 20–30 ng/g nicotine, with RSD% lower than 6%. However, at lower levels, around 10 ng/g nicotine in the sample, random problems appear with the contamination from reagents, sample handling, or other sources, and RSD% for samples with this level of nicotine ranges around 20–25%. Repeatability of the technique follows the same trend as precision. Linearity of the calibration is also very good. As seen in Figure 2, the R^2 for the trendline of the calibration is $R^2 = 0.9996$ with a range of concentration covering three orders of magnitude. The recovery from the plant material using treatment with a NaOH solution followed by extraction with TBME was previously evaluated and reported (14, 15). However, the recovery was once more evaluated for *Magnolia grandiflora* leaf samples. Leaves from *Magnolia grandiflora* (from private garden) were selected as a control material that was not exposed to nicotine contamination. The choice was made since this plant does not contain nicotine and the handling by an unknown person was avoided. For three replicates, a recovery of 98.6% was obtained when the samples were spiked with 435 ng nicotine. The procedure has been applied for the analysis of trace nicotine for a period of about 6 month, and generated the same results,

Table 3. Levels of nicotine in ng/g reported in the literature for several *Solanaceae* plants used as food, and the calculated levels of dry weight basis assuming 90% moisture.

Food source	Ref. (4)	Ref. (9)	Ref. (6)	Ref. (7)	Ref. (11)	Ref. (13)
Weight basis	*wet*	*dry*	*dry*	*wet*	*dry*	*wet*
Potato (whole)	not reported	not detected	76	7.1 ± 5.9	not detected–38.9	4.5–12.3
Potato skin	not reported	9500–16060	24	not reported	not reported	not reported
Tomato (ripe)	7.9–9.8	1450–3210	78–148	4.1 ± 1.8	not detected–48.4	2.8–11.5
Eggplant	100	1880–3000	not detected	not reported	not detected–44.7	5.2–15.0
Green pepper	5.7	1310–3940	not detected	not detected	48.4–149.1	not reported
Weight basis	*dry*	*dry*	*dry*	*dry*	*dry*	*dry*
Potato (whole)	not reported	not detected	76	71 ± 59	not detected–38.9	45–123
Potato skin	not reported	9500–16060	24	not reported	not reported	not reported
Tomato (ripe)	79–98	1450–3210	78–148	41 ± 18	not detected–48.4	28–115
Eggplant	1000	1880–3000	not detected	not reported	not detected–44.7	52–150
Green pepper	57	1310–3940	not detected	not detected	48.4–149.1	not reported

demonstrating the stability of this method. No other problems such as carry-over, sample decomposition, signal decrease in time, etc. were noticed during the application of this analytical procedure.

RESULTS AND DISCUSSION

Levels of nicotine on both wet basis and on dry basis are reported in the literature for several plant materials used as food. For tomatoes the results at different degrees of ripening are also reported (11). The results from six different studies are shown in Table 3. Some studies do not show the level of nicotine on dry weight basis, and for comparison, Table 3 also gives all the results reported on dry weight bases. The calculation for levels reported only for the plant as is (wet basis) was done assuming 90% moisture. As indicated in Table 3, some reported levels differ significantly, and plant variability cannot explain all the differences. Besides plant variability, various explanations may be given to such discrepancies such as: incomplete extraction of nicotine from plant material, contamination with exogenous nicotine (for the high levels), variability in the method for analysis of traces. The potential for possible contamination has been previously reported with nicotine detected in dry mushrooms (12), or in green tea and instant tea (9, 11).

Leaves from *Magnolia grandiflora* (five replicates) were processed identical to other plant materials, without grinding or freeze drying. The average result for nicotine in these samples was 6.1 ng/g with RSD% = 11.6%. This level of nicotine above zero can be caused by random contamination or background of nicotine in reagents. The same type of samples subject to grinding (with a new, clean coffee grinder) showed a level of nicotine around 6.4 ng/g (RSD% = 19.9%). The same type of samples subject to freeze drying were analyzed for nicotine and showed a significant level of contamination. For this reason, the method of sample preparation was selected such that the plant material was analyzed as is, to avoid any sample processing before treatment with NaOH, and with independent moisture measurement.

The plant materials analyzed were commercially available on the market (The Fresh Market, Greensboro, NC, USA). The results for nicotine in ng/g expressed on a dry weight basis are given in Table 4. Table 4 also lists results for the level of nicotine in blank samples. This level was subtracted from the reported levels for each plant. The results were calculated on dry weight basis using the sample moisture % also given in Table 4.

For tomatoes, potatoes and green pepper, some of the results previously reported in literature (11, 13) indicate the same range as the values shown in Table 4. Several studies (4, 11) show that green tomatoes are higher in nicotine than ripe tomatoes. No green tomatoes were analyzed in present study. For eggplant the results from present study are in the same range as those reported in one literature reference (13). One of the studies reported in the literature (9) shows very high levels of nicotine in all analyzed samples, which was very likely caused by contamination that can be easily

Table 4. Levels of water content % and of nicotine in ng/g (on a dry weight basis) in several plant materials.

Sample	No. of samples	Average water (%)	Average nicotine (ng/g)	RSD%
Blank	5	—	6.1	11.6
Tomato	4	95.88	181.9	3.4
Tomato organic	2	95.44	82.3	2.8
Tomato leaf (young plant)	4	88.45	184.4	4.3
Eggplant	2	93.76	174.3	1.6
Baby eggplant	2	95.27	94.6	5.9
Potato (whole)	2	82.80	42.6	1.0
Green pepper	2	95.22	74.1	1.0
Carrot (root)	2	88.55	18.2	3.1
Carrot leaf	2	86.64	44.6	2.6
Banana	2	79.95	8.9	24.9
Pear	2	86.76	10.5	3.9
Green apple	2	86.78	1.9	10.8
Blueberry	2	88.36	8.9	2.7
Strawberry	2	94.20	23.5	8.6

produced for samples with low nicotine level. Similarly high levels were seen in this present study when the plant material was subject to freeze drying in an instrument used alternatively for freeze drying tobacco products.

For some of the plant materials reported in this present study there are no available data in the literature for comparison. One of these materials is tomato plant leaf, which shows a similar level of nicotine as in the tomato fruit. Other plant materials such as carrot (root), banana, pear, and blueberries showed a level around 10 ng/g of nicotine. This low level is possibly caused by some kind of contamination during the analysis. Even some pure solvents (e.g., methanol, ethyl acetate, etc.) appear to contain traces of nicotine. Although the blank level of nicotine was subtracted from all the analyzed samples, this level may have some fluctuation from sample to sample, leading to an apparent level of detectable trace nicotine. For practical purposes, all levels of nicotine that are below 10 ng/g should be considered "nicotine absent". However, the carrot leaf and strawberries showed levels that cannot be assumed as caused only by contamination during the analysis. It cannot be determined the source of nicotine in these plant material, which can be inherent to the plant or caused by some contamination due to insecticide use, or from other sources. Also, in spite of the fact that the surface of each plant material was carefully washed, a complete elimination of contamination during handling toward the market cannot be assured.

CONCLUSIONS

A sensitive method for nicotine level determination has been developed with the goal of analyzing this compound in various plant materials. Sample preparation for the analysis is similar to that for a typical nicotine determination. Special precautions were taken to avoid any potential contamination of samples with nicotine. In spite of this effort, levels of nicotine in the range of 2 ng/g to 10 ng/g were still detected in some plant material not expected to contain nicotine. For practical purposes, all the levels that are below 10 ng/g should be considered "nicotine absent". Tomato, tomato leaf, and eggplant have nicotine at levels around 180 ng/g (on a dry weight basis). Other plant materials (also from *Solanaceae* family) such as green pepper and potato show levels around 50–60 ng/g in plant material. The results obtained for nicotine levels in tomatoes, potatoes, and green pepper are in the same range with some of the results previously reported in literature.

REFERENCES

1. Maffei, M. (ed.): Dietary Supplements of Plant Origin. A Nutrition and Health Approach; Taylor & Francis, London, UK, 2003, ISBN 9780415308359
2. Henningfield, J.E.: More on the Nicotine Content of Vegetables; N. Engl. J. Med. 329 (1993) 1581–1582.
3. Searles Nielsen, S., G.M. Franklin, W.T. Longstreth, P.D. Swanson, and H. Checkoway: Nicotine from Edible *Solanaceae* and Risk of Parkinson Disease; Ann. Neurol. 74 (2013) 472–477. doi: 10.1002/ana.23884
4. Castro, A. and N. Monji: Dietary Nicotine and its Significance in Studies on Tobacco Smoking; Biomed. Arch. 2 (1986) 91–97.
5. Davis, R.A.: The Determination of Nicotine and Cotinine in Plasma; J. Chromatogr. Sci. 24 (1986) 134–141.
6. Davis, R.A., M.F. Stiles, J.D. deBethizy, and J.H. Reynolds: Dietary Nicotine: A Source of Urinary Cotinine; Food Chem. Toxicol. 29 (1991) 821–827. DOI: 10.1016/0278-6915(91)90109-K
7. Domino, E.F., E. Hornbach, and T. Demena: Relevance of Nicotine Content of Common Vegetables to the Identification of Passive Tobacco Smokers; Med. Sci. Res. 21 (1993) 571–572.
8. Domino, E.F., E. Hornbach, and T. Demena: The Nicotine Content of Common Vegetables; N. Engl. J. Med. 329 (1993) 437. DOI: 10.1056/NEJM199308053290619
9. Sheen, S.J.: Detection of Nicotine in Foods and Plant Materials; J. Food Sci. 53 (1988) 1572–1573. DOI: 10.1111/j.1365-2621.1988.tb09328.x
10. Siegmund, B., E. Leitner, and W. Pfannhauser: Development of a Simple Sample Preparation Technique for Gas Chromatographic - Mass Spectrometric Determination of Nicotine in Edible Nightshades (*Solanaceae*); J. Chromatogr. A 840 (1999) 249–260. DOI: 10.1016/S0021-9673(99)00213-7
11. Siegmund, B., E. Leitner, and W. Pfannhauser: Determination of the Nicotine Content of Various Edible Nightshades (*Solanaceae*) and Their Products and Estimation of the Associated Dietary Nicotine Intake; J. Agric. Food Chem. 47 (1999) 3113–3120. DOI: 10.1021/jf990089w
12. Müller, C., F. Bracher, and F. Plössl: Determination of Nicotine in Dried Mushrooms by Using a Modified QuEChERS Approach and GC-MS-MS; Chromatographia 73 (2011) 807–811. DOI: 10.1007/s10337-011-1948-z
13. Liu, W., R. Zhao, B. Li, G. Wu, and Y. Xue: Determination of the Nicotine Content in *Solanaceae* Vegetables by Solid-Phase Extraction Coupled with Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry; Food Anal. Meth. 6 (2013) 643–647. DOI: 10.1007/s12161-012-9457-8
14. Jones, N.M., M.G. Bernardo-Gil, and M.G. Lourenco: Comparison of Methods for Extraction of Tobacco Alkaloids; J. AOAC Int. 84 (2001) 309–316.
15. Chen, P.X., N. Qian, H.R. Burton, and S.C. Moldoveanu: Analysis of Minor Alkaloids in Tobacco: A Collaborative Study; Beitr. Tabakforsch. Int. 21 (2005) 369–379. DOI: 10.2478/cttr-2013-0803
16. Moldoveanu, S. and V. David: Modern Sample Preparation for Chromatography; Elsevier, Amsterdam, The Netherlands, 2015. DOI: 10.1016/B978-0-444-54319-6.12001-8

Corresponding author:

Serban C. Moldoveanu
R.J. Reynolds Tobacco Co.
Winston-Salem NC, USA
E-mail: moldovs@rjrt.com