Vanillin Content in Boiled Peanuts

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A high-performance liquid chromatographic (HPLC) method for determination of vanillin in boiled peanuts has been developed. Vanillin was extracted with acetonitrile by blending at high speed followed by purification of an aliquot of the extract on a minicolumn packed with Al_2O_3. Vanillin was quantitated by HPLC on silica gel with *n*-hexane/2-propanol/water/acetic acid (2100/540/37/2, v/v) as a mobile phase. The recovery of vanillin added to fresh peanut hulls at 0.50 and 2.50 μg/g was 78.7 ± 2.7 and 79.9 ± 3.1%, respectively. The detection limit of vanillin in boiled peanuts was estimated at 0.05 μg/g. UV-detector response to vanillin was linear to at least 2.5 μg/injection. Free vanillin has been found in two commercial brands of boiled peanuts at low ppm levels. Both the kernels and the hulls contained vanillin, which was formed during hydrolysis of lignin, one of the major constituents of the peanut hulls. Since vanillin has a low flavor threshold, it could be considered as one of the major ingredients that determines the flavor of boiled peanuts.

Keywords: *Vanillin; peanuts; groundnuts; Arachis hypogaea L.; flavor; HPLC analysis; lignin*

INTRODUCTION

Traditional consumption of peanuts as whole nuts and peanut butter is based on the use of roasted peanut seeds, which have a pleasant and unique flavor. Several hundred compounds that contribute to that flavor have been identified in roasted peanuts (*1*−*5*). Various aspects of peanut flavor have been discussed in a number of publications (5−*9*) that reflect our increased knowledge of the chemistry of roasted peanut flavor. In contrast, there is a lack of any data on the flavor quality of boiled peanuts, a highly popular product in the southeastern part of the U.S. Boiled peanuts are produced commercially, and they are sold frozen or in cans, nationwide.

The flavor of roasted and boiled peanuts can be fully judged only by sensory evaluation by human subjects. However, chemical analytical methods are extremely useful in quantifying compounds that contribute to, or detract from, flavor (*10*).

Our previous research on resveratrol content in peanuts (*11*) demonstrated that boiled peanuts contain some compounds that are not found in fresh peanuts or in any other kind of peanut products. Preliminary results based on sensory evaluation and HPLC analysis showed that the major volatile component of boiled peanut extract seemed to be vanillin.

Vanillin (3-methoxy-4-hydroxybenzaldehyde) occurs mainly as a glucoside in several plants such as *Vanilla planifolia*. Free vanillin develops as a result of fermentation during the curing process of vanilla beans (*12*). Vanillin is also obtained by oxidative hydrolysis of lignin, the chief noncarbohydrate polymer constituent of wood (*13*). Vanillin content in peanuts has not been reported.

Vanillin is widely used as a flavoring agent with the pleasant aromatic "vanilla" odor and taste in foods and beverages. Vanillin has a low flavor threshold value of 20 μg/L in water at 20 °C (*14*).

The purpose of this work was to conduct a chemical analysis of the vanillin content in boiled peanuts, and to determine its source.

MATERIALS AND METHODS

Apparatus. A high-performance liquid chromatograph equipped with a pump (model LC-10AT; Shimadzu), a diode array detector in the 220−450-nm range (model SPD-M10A with EZChrom software, version 3.2; Shimadzu), and an autosampler (model 717 plus; Waters) was used. The separation was performed on a Zorbax Rx-SIL analytical column (250 × 4.6 mm i.d., packed with 5-μm silica gel; MAC-MOD Analytical, Inc.) using *n*-hexane/2-propanol/water/acetic acid (2100/540/37/2, v/v) as the mobile phase at a flow rate of 1.5 mL/min at room temperature. The column was equilibrated with the mobile phase within 2 h at 1.5 mL/min. Cleanup procedure was performed using a rotary evaporator (Rotavapor-R; Brinkmann; bath temperature, 40 °C), a cleanup column composed of a glass Pasteur pipet (borosilicate, length 145 mm with a small cotton plug placed at its bottom) packed with 1 g of Al_2O_3 (neutral; Brockman activity 1, 80−200 mesh; Fisher), an evaporating unit (Reacti-Vap Evaporating Unit, model 18780 with Reacti-Therm Heating Module; Pierce), an ultrasonic bath (Ultrasonic Cleaner, model T-9; L & R), a high-speed blender (13000 rpm, with a 1-L glass jar; General Electric), a vial (1 mL, 40 × 8 mm dia., borosilicate glass clear Autosampler Vial with cap; Waters), and a glass pipet (1 mL capacity; Fisher). Thin-layer chromatography (TLC) was performed on TLC plates (Kieselgel 60, 10 × 10 cm; E. Merck) with two mobile phases: hexane−acetone (4 + 1, v/v) and chlorofom−methanol (99 + 1, v/v). A Fisher Acumet Basic pH meter was used to measure the acidity of boiled peanut broth.

Reagents and Products. Solvents for HPLC and extraction (*n*-hexane, 2-propanol, benzene, water, and acetic acid) were HPLC grade (Fisher). An acid-washed diatomaceous earth (Sigma) was used. Compressed nitrogen was used as a gas for extract evaporation (UN 1066; Air Products and Chemicals). Standard of vanillin (3-methoxy-4-hydroxybenzaldehyde) was purchased from Sigma. A stock solution was prepared by dissolving 5.00 mg of the standard in 100 mL of

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the HPLC mobile phase. Working solutions were prepared daily by mixing 100 μL of the stock solutions with 2.9 mL of the HPLC mobile phase; 5 to 50 μL were injected into HPLC system. To visualize vanillin spots on TLC plates, 2,4-dinitrophenylhydrazine (0.4% solution in 2 N hydrochloric acid) and *o*-dianisidine in glacial acetic acid (all from Sigma) were used. Canned boiled peanuts and roasted peanuts were purchased locally from grocery stores.

Sampling Procedure. Boiled peanuts were separated into kernels and hulls by hand, and were blotted with a paper towel before weighing.

Hydrolysis and Extraction Procedure. The following materials were used for acidic and basic hydrolysis: peanut hulls, hardwood (oak) sawdust, softwood (pine) sawdust, fresh (green) peanut kernels without skins, roasted peanut kernels without skins, and filter paper as a control. Peanut hulls, fresh peanut kernels, and roasted peanut kernels were ground in a high-speed mill before hydrolysis.

For basic hydrolysis, one gram of each product was placed into a separate 50-mL polypropylene vial followed by addition of 20 mL of 1 M KOH and sealed with a cap. The vials were incubated in a water bath at 90 °C for 3 h and then cooled to room temperature. The content of each vial was neutralized with 15% HCl to pH 1−2 and then extracted with benzene (2 × 20 mL). Combined benzene layers were dried over anhydrous Na_2SO_4, filtered through filter paper, and evaporated to dryness with a rotary evaporator at 40 °C. The residue was dissolved in the HPLC mobile phase and injected into the HPLC system.

Acidic hydrolysis was performed in a manner similar to the above procedure, except that 20 mL of 2 M HCl for 1 g of each product was used and the neutralization step was omitted. The strong emulsion that was formed during extraction of the fresh peanut kernels hydrolysate was broken by addition of anhydrous Na_2SO_4.

Cleanup Procedure. Boiled peanuts (50 g) or hulls (25 g) and 150 mL of CH_3CN were placed in a blender jar, blended for 3 min, and filtered through a filter paper. Blending of roasted peanut samples was performed in the presence of 10 g of diatomaceous earth. The filtrate (2 mL) was transferred to the cleanup column and allowed to drain by gravity into a vial. The column then was eluted with 1 mL of CH_3CN. Combined eluates were evaporated to dryness under a nitrogen steam at 35 °C in an evaporating unit. The residue was dissolved in 500 μL of HPLC mobile phase, sonicated in an ultrasonic bath for 10 s, and transferred into a glass vial.

HPLC Quantitation. Peanut extract (50−100 μL) was injected into the HPLC system and vanillin was quantitated at 308 nm by reference to the peak area of an external authentic standard of vanillin.

Data Analyses. Statistical analyses were conducted using the SigmaStat software program, version 1.00 (Jandel Corp., San Rafael, CA). An unpaired *t* test and one way ANOVA were used to compare two and three groups of data.

RESULTS AND DISCUSSION

An HPLC method for analysis of vanillin in peanuts was developed. Extraction of vanillin from boiled peanuts and hulls with acetonitrile followed by a one-step purification procedure on a minicolumn resulted in an eluate with sufficient purity for HPLC quantitation of vanillin. The recovery of the vanillin standard added to fresh peanut hulls at 0.50 and 2.50 μg/g was 78.7 ± 2.7 and 79.9 ± 3.1% (mean ± SD; $n = 3$), respectively. The detection limit of vanillin in boiled peanuts was estimated at 0.05 μg/g (signal-to-noise ratio 5:1). A typical chromatogram of purified acetonitrile extract of boiled peanuts (Figure 1) shows sufficient separation of vanillin from impurities. Use of the combination of a photodiode array detector and a UV transparent mobile phase (from 215 nm) allowed for reliable quantitation of vanillin. The UV-detector response to vanillin was linear to at least 2.5 μg/injection. Vanillin showed maximum adsorption at 232, 279, and 308 nm in the proposed HPLC mobile phase. Quantitation of vanillin was performed at 308 nm to increase selectivity.

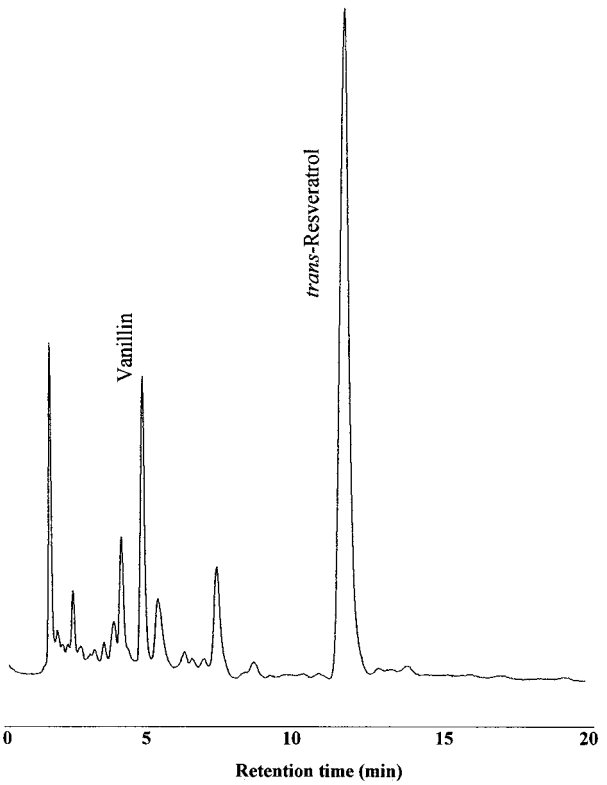

Figure 1. HPLC of purified acetonitrile extract of whole boiled peanuts at 308 nm. Vanillin concentration is 1.28 μg/g.

Table 1. Vanillin Content in Commercial Boiled Peanuts

brand/material analyzed	vanillin content, μg/g (mean ± SD; $n = 3$)
Roddenbery's peanut patch green	
whole	1.35 ± 0.04
hulls	2.11 ± 0.05
kernels	1.67 ± 0.06
Roddenbery's peanut patch Cajun style	
whole	1.54 ± 0.02
hulls	2.46 ± 0.06
kernels	1.71 ± 0.05

The method was applied to boiled peanuts as well as hydrolyzed peanut kernels, hulls, and wood pulp. The data obtained showed that all analyzed boiled peanut samples of two major brands contained vanillin. The concentration of vanillin in whole pods varied from 1.28 to 1.56 μg/g (Table 1). Hulls showed significantly higher ($P < 0.001$) vanillin content when compared to that of whole pods and kernels, varying from 2.09 to 2.50 μg/g (Table 1). Vanillin content in kernels was also higher ($P < 0.005$) than that in whole pods. A lower concentration of vanillin in whole peanut pods compared to that in kernels and hulls can be explained by higher water content in the former. This extra amount of water significantly reduced the amount of analyzed matter, and thus, the apparent concentration of vanillin. It should be noted that no significant amount of vanillin was found in the broth. This can be explained by the lower solubility of vanillin in water than in oils. Boiled peanut kernels contain about 40% oil.

The identity of vanillin was confirmed by its unique UV spectrum that perfectly matched that of an authen-

tic standard of vanillin. TLC with two mobile phases composed of solvents of different nature followed by spraying with specific dyeing reagents confirmed the presence of vanillin as well. In addition to the above, the vanillin fraction isolated from boiled peanut extract by means of HPLC possessed a unique, aromatic "vanilla" odor.

In contrast with boiled peanuts, fresh (green) peanuts, as well as roasted peanuts, did not contain any trace of vanillin. The apparent explanation of this fact is that boiled peanut kernels are processed in hot water in the presence of hulls. The hulls apparently were the source of free vanillin because they contained about 23% lignin (http://www.cfe.cornell.edu/compost/calc/lignin.noframes.html) that is susceptible to hydrolysis. Upon hydrolysis, lignins from different plant sources may produce vanillin as one of the major products (*13*). To confirm that hull lignin was the source of free vanillin, a set of hydrolysis experiments was performed. Fresh (green) peanut kernels and hulls, as well as roasted peanut kernels, were examined. Softwood and hardwood pulps known to produce vanillin upon hydrolysis, and filter paper that was not supposed to contain any lignin were examined as well. These experiments demonstrated that upon both basic and acidic hydrolysis, only peanut hulls, softwood, and hardwood produced free vanillin. These substrates are known to contain high concentrations of lignin that is susceptible to hydrolysis. The amount of free vanillin was not determined quantitatively; however, the softwood showed higher lignin content than the hardwood on the basis of the concentration of vanillin after the hydrolysis under identical conditions. In contrast, fresh peanut kernels, roasted peanut kernels, and filter paper did not produce any detectable vanillin. This could be indicative of a lack in fresh kernels of lignin or any other potential source of vanillin, such as vanillin-D-glucoside. Thus, lignin of peanut hulls is the most likely source of vanillin in boiled peanuts.

Lignin carbohydrate linkages are hydrolytically unstable even under mildly acidic conditions (*15*). The broth in the cans of both brands was slightly acidic, with the pH varying from 5.68 to 5.76. The hydrolysis of peanut-hull lignin and formation of free vanillin could take place at high temperatures (close or above 100 °C) and increased acidity. Significantly higher solubility of vanillin in oils and in hot water (6.3% at 80 °C) compared to that in cold water (1.0% at 25 °C) suggests that vanillin migrates to peanut kernels from the hulls during and/or after hydrolysis. Slowly forming vanillin may be readily released from the peanut hulls (that are almost oil-free at the beginning of the process) into hot water during hydrolysis. At the same time, exchange of oil from the kernels to peanut hulls, with which they are in close contact, may take place. During cooling to ambient temperature the solubility of vanillin in water decreases, and at this point it may be dissolved in the oil of the peanut kernels.

Vanillin possesses a desirable flavor character, and at the concentrations that significantly exceed its flavor threshold, it could be considered a major flavor ingredient of boiled peanuts.

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LITERATURE CITED

(1) Walradt, J. P.; Pittet, A. O.; Kinlin, T. E.; Muralidhara, R.; Sanderson, R. Volatile Components of Roasted Peanuts. *J. Agric. Food Chem*. **1971**, *19*, 972−979.

(2) Buckholz, L. L. Jr.; Daun, H.; Stier, E.; Trout, R. Influence of Roasting Time on Sensory Attributes of Fresh Roasted Peanuts. *J. Food Sci.* **1980**, *45*, 547−554.

(3) Buckholz, L. L. Jr.; Daun, H. Instrumental and Sensory Characteristics of Roasted Peanut Flavor Volatiles. In *Quality of Selected Fruits and Vegetables of North America*; Teranishi, R., Barrera-Benitez, H., Eds; ACS Symp. Series 170; American Chemical Society: Washington, DC, 1981; pp 163−181.

(4) Ho, C.-T.; Lee, M.-H.; Chang, S. S. Isolation and Identification of Volatile Compounds from Roasted Peanuts. *J. Food Sci*. **1982**, *47*, 127−133.

(5) Vercellotti, J. R.; Crippen, K. L.; Lovegren, N. V.; Sanders, T. H. Defining Roasted Peanut Flavor Quality. Part 1. Correlation of GC Volatiles with Roast Color as an Estimate of Quality. In *Food Science and Human Nutrition*; Charalambous, G., Ed.; Elsevier: Amsterdam, The Netherlands, 1992; pp 183−209.

(6) Pattee, H. E.; Singleton, A. Peanut Quality: Its Relationship to Volatile Compounds. In *Quality of Selected Fruits and Vegetables of North America*; Teranishi, R., Barrera-Benitez, H., Eds; ACS Symp. Series 170. American Chemical Society: Washington, DC, 1981; pp 147−161.

(7) Ahmed, E. M.; Young, C. T. Composition, Quality, and Flavor of Peanuts. In *Peanut Science and Technology*; Pattee, H. E., Young, C. T., Eds.; APRES: Yoakum, TX, 1982; pp 655−688.

(8) Sanders, T. H.; Vercellotti, J. R.; Blankenship, P. D.; Crippen, K. L.; Civille, G, V. Interaction of Maturity and Curing Temperature on Descriptive Flavor of Peanuts. *J. Food Sci*. **1989**, *54*, 1066−1069.

(9) Ory, R. L.; Crippen, K. L.; Lovegren, N. V. Off-Flavors in Peanuts and Peanut Products. In *Off-Flavors in Foods and Beverages*; Charalambous, G., Ed.; Elsevier: Amsterdam, The Netherlands, 1992; pp 57−75.

(10) Sanders, T. H.; Pattee, H. E.; Vercellotti, J. R.; Bett, K. L. Advances in Peanut Flavor Quality. In *Advances in Peanut Science*; Pattee, H. E., Stalker, H. T., Eds.; APRES: Stillwater, OK, 1995; pp 529−533.

(11) Sobolev, V. S.; Cole, R. J. *trans*-Resveratrol Content in Commercial Peanuts and Peanut Products. *J. Agric. Food Chem*. **1999**, *47*, 1435−1439.

(12) Brady, G. S. Vanilla Beans. In *Materials Handbook*; Brady, G. S., Ed.; McGraw-Hill Book Co.: New York, 1963; p 798.

(13) Fargues, C.; Mathias, A.; Rodrigues, A. E. Kinetics of Vanillin Production from Kraft Lignin Oxidation. *Ind. Eng. Chem. Res*. **1996**, *35*, 28−36.

(14) Belitz, H.-D., Grosch, W., Eds. *Food Chemistry*; Springer Verlag : Berlin, Germany, 1987; pp 257−304.

(15) Peng, P. P.; Argyropoulos, D. S. On the Interaction of UV Screens with the Lignocellulosic Matrix. *Photochem. Photobiol*. **2000**, *71*, 149−156.

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