

## Articles

### Analytical Methods

8047 [dx.doi.org/10.1021/jf201501x](http://dx.doi.org/10.1021/jf201501x)

**LC-MS/MS Quantification of Sulforaphane and Indole-3-carbinol Metabolites in Human Plasma and Urine after Dietary Intake of Selenium-Fortified Broccoli**

Johanna Hauder, Stefanie Winkler, Achim Bub, Corinna E. Rüfer, Marc Pignitter, and Veronika Somoza\*

8058 [dx.doi.org/10.1021/jf2022397](http://dx.doi.org/10.1021/jf2022397)

**Qualitative and Quantitative Analysis of Steroidal Saponins in Crude Extract and Bark Powder of *Yucca schidigera* Roez.**

Mariusz Kowalczyk,\* Lukasz Pecio, Anna Stochmal, and Wiesław Oleszek

8065 [dx.doi.org/10.1021/jf200754f](http://dx.doi.org/10.1021/jf200754f)

**A Simple Analytical Method for Dhurrin Content Evaluation in Cyanogenic Plants for Their Utilization in Fodder and Biofumigation**

Gina Rosalinda De Nicola, Onofrio Leoni, Lorena Malaguti, Roberta Bernardi, and Luca Lazzeri\*

8070 [dx.doi.org/10.1021/jf2023947](http://dx.doi.org/10.1021/jf2023947)

**Analytical Study of Azadirachtin and 3-Tigloylazadirachtol Residues in Foliage and Phloem of Hardwood Tree Species by Liquid Chromatography–Electrospray Mass Spectrometry**

Susana Grimalt, Dean G. Thompson,\* Melanie Coppens, Derek T. Chartrand, Thomas Shorney, Joe Meating, and Taylor Scarr

8078 [dx.doi.org/10.1021/jf201129j](http://dx.doi.org/10.1021/jf201129j)

**Determination of Alachlor and Its Metabolite 2,6-Diethylaniline in Microbial Culture Medium Using Online Microdialysis Enriched-Sampling Coupled to High-Performance Liquid Chromatography**

Chi-Zen Chen, Cheing-Tong Yan, Ponnusamy Vinoth Kumar, Jenn-Wen Huang, and Jen-Fon Jen\*

8086 [dx.doi.org/10.1021/jf201158k](http://dx.doi.org/10.1021/jf201158k)

**Analysis of Alkylamides in *Echinacea* Plant Materials and Dietary Supplements by Ultrafast Liquid Chromatography with Diode Array and Mass Spectrometric Detection**

Elizabeth Mudge, Daise Lopes-Lutz, Paula Brown, and Andreas Schlieber\*

8095 [dx.doi.org/10.1021/jf2011936](http://dx.doi.org/10.1021/jf2011936)

**Analysis of Isomeric Forms of Oxidized Triacylglycerols Using Ultra-High-Performance Liquid Chromatography and Tandem Mass Spectrometry**

Jukka-Pekka Suomela,\* Heidi Leskinen, and Heikki Kallio

8101  dx.doi.org/10.1021/jf201410p  
Podophyllotoxin and Deoxydopodophyllotoxin in *Juniperus bermudiana* and 12 Other *Juniperus* Species: Optimization of Extraction, Method Validation, and Quantification  
Sullivan Renouard,\* Tatiana Lopez, Oktavia Hendrawati, Patricia Dupre, Joëi Doussot, Annie Falguieres, Clotilde Ferroud, Daniel Hagege, Frédéric Lamblin, Eric Laine, and Christophe Hano

8108 dx.doi.org/10.1021/jf201745a  
Determination of Parent and Substituted Polycyclic Aromatic Hydrocarbons in High-Fat Salmon Using a Modified QuEChERS Extraction, Dispersive SPE and GC-MS  
Norman D. Forsberg, Glenn R. Wilson, and Kim A. Anderson\*

### Bioactive Constituents

8117 dx.doi.org/10.1021/jf2018935  
Bioactive Phytochemicals of Leaf Essential Oils of *Cinnamomum osmophloeum* Prevent Lipopolysaccharide/*D*-Galactosamine (LPS/*D*-GalN)-Induced Acute Hepatitis in Mice  
Yu-Tang Tung, Chi-Chang Huang, Shang-Tse Ho, Yueh-Hsiung Kuo, Chi-Chen Lin, Chien-Tsong Lin, and Jyh-Hong Wu\*

8124 dx.doi.org/10.1021/jf2006358  
Effect of  $\beta$ -Phenylethyl Isothiocyanate from Cruciferous Vegetables on Growth Inhibition and Apoptosis of Cervical Cancer Cells through the Induction of Death Receptors 4 and 5  
Le Diem Huong, Jung-Hyung Shim, Kyeong-Hee Choi, Ji-Ae Shin, Eun-Sun Choi, Hyung-Seop Kim, Sook-Jeong Lee, Sun-Ju Kim, Nam-Pyo Cho, and Sung-Dae Cho\*

8132 dx.doi.org/10.1021/jf200177q  
Seasonal Variations in the Deoxydopodophyllotoxin Content and Yield of *Anthriscus sylvestris* L. (Hoffm.) Grown in the Field and under Controlled Conditions  
Oktavia Hendrawati, Herman J. Woerdenbag, Jacques Hille, Wim J. Quax, and Oliver Kayser\*


8140 dx.doi.org/10.1021/jf2009342  
Agrowaste-Based Nanofibers as a Probiotic Encapsulant: Fabrication and Characterization  
Wai-Yee Fung, Kay-Hay Yuen, and Min-Tze Liang\*

8148 dx.doi.org/10.1021/jf2009815  
Potential Fungal Inhibition by Immobilized Hydrolytic Enzymes from *Trichoderma asperellum*  
Bárbara Dumas S. Silva, Cirano J. Ulhoa, Karla A. Batista, Fábio Yamashita, and Kátia F. Fernandes\*

8155 dx.doi.org/10.1021/jf201073g  
Storage Elevates Phenolic Content and Antioxidant Activity but Suppresses Antiproliferative and Pro-apoptotic Properties of Colored-Flesh Potatoes against Human Colon Cancer Cell Lines  
Gaurav P. Madiwale, Lavanya Reddivari, David G. Holm, and Jairam Vanamala\*

8167 dx.doi.org/10.1021/jf201459u  
Influence of Cultivar and Concentration of Selected Phenolic Constituents on the in Vitro Chemopreventive Potential of Olive Oil Extracts  
Roberto Fabiani,\* Maria V. Sepporta, Teresa Mazza, Patrizia Rosignoli, Raffaella Fucelli, Angelo De Bartolomeo, Mariëna Crescimanno, Agnese Taticchi, Sonia Esposito, Maurizio Servili, and Guido Morozzi

8175 dx.doi.org/10.1021/jf2015683  
One-Step Production of a Biologically Active Novel Furan Fatty Acid from 7,10-Dihydroxy-8(E)-octadecenoic Acid  
Joel B. Ellamar, Kyung-Sik Song, and Hak-Ryul Kim\*

8180  dx.doi.org/10.1021/jf202561p  
Structure and Function of the Oxidation Products of Polyphenols and Identification of Potent Lipoxigenase Inhibitors from Fe-Catalyzed Oxidation of Resveratrol  
Yoshimi Shingai, Aya Fujimoto, Mitsuhiro Nakamura, and Toshiya Masuda\*

8187 dx.doi.org/10.1021/jf2018929  
Subamolide E from *Cinnamomum subavenium* Induces Sub-G1 Cell-Cycle Arrest and Caspase-Dependent Apoptosis and Reduces the Migration Ability of Human Melanoma Cells  
Hui-Min Wang, Chien-Chih Chiu, Pei-Fang Wu, and Chung-Yi Chen\*

8193 dx.doi.org/10.1021/jf201927t  
Adulticidal Activity of Phthalides Identified in *Cnidium officinale* Rhizome to B- and Q-Biotypes of *Bemisia tabaci*  
Song-Hwa Chae, Soon-Il Kim, Seong-Hum Yeon, Si-Woo Lee, and Young-Joon Ahn\*

8199 dx.doi.org/10.1021/jf201948v  
Enhanced Hypolipidemic Effect and Safety of Red Mold *Dioscorea* Cultured in Deep Ocean Water  
Chun-Lin Lee, Yi-Hsin Kung, Jyh-Jye Wang, Tzu-Ying Lung, and Tzu-Ming Pan\*

### Biofuels and Bioproducts Chemistry

8208 dx.doi.org/10.1021/jf201589y  
Xylan-rich Hemicelluloses-graft-Acrylic Acid Ionic Hydrogels with Rapid Responses to pH, Salt, and Organic Solvents  
Xin-Wen Peng, Jun-Li Ren,\* Lin-Xin Zhong, Feng Peng, and Run-Cang Sun\*

### Chemical Aspects of Biotechnology/Molecular Biology

8216 dx.doi.org/10.1021/jf2012584  
Growth Suppression of HER2-Overexpressing Breast Cancer Cells by Berberine via Modulation of the HER2/PI3K/Akt Signaling Pathway  
Han-Peng Kuo, Tzu-Chao Chuang, Ming-Han Yeh, Shih-Chung Hsu, Tzong-Der Way, Po-Yuan Chen, Shan-Shue Wang, Yung-Hsien Chang, Ming-Ching Kao,\* and Jah-Yao Liu\*

### Chemical Aspects of Food Safety

8225 dx.doi.org/10.1021/jf201595q  
Acrylamide Formation in Almonds (*Prunus dulcis*): Influences of Roasting Time and Temperature, Precursors, Varietal Selection, and Storage  
Gong Zhang, Guangwei Huang, Lu Xiao, James Seiber, and Alyson E. Mitchell\*

## Chemical Changes Induced by Processing/Storage

8233 [dx.doi.org/10.1021/jf200732p](https://doi.org/10.1021/jf200732p)

**Bioactivity of Antioxidants in Extruded Products Prepared from Purple Potato and Dry Pea Flours**  
Balunkeswar Nayak, Rui Hai Liu, Jose De J Berrios, Juming Tang,\* and Christopher Derito

8244 [dx.doi.org/10.1021/jf201000x](https://doi.org/10.1021/jf201000x)

**Ultraviolet-Induced Oxidation of Ascorbic Acid in a Model Juice System: Identification of Degradation Products**  
Rohan V. Tikekar, Ramaswamy C. Anantheswaran, Ryan J. Elias, and Luke F. LaBorde\*

8249 [dx.doi.org/10.1021/jf201041z](https://doi.org/10.1021/jf201041z)

**Formation of Dextran Deposits in Brazilian Sugar Cane Spirits**  
Francisco Wendel Batista de Aquino and Douglas Wagner Franco\*

8256 [dx.doi.org/10.1021/jf2011134](https://doi.org/10.1021/jf2011134)

**Impact of Low Hydration of Barley Grain on  $\beta$ -Glucan Degradation and Lipid Transfer Protein (LTP1) Modifications During the Malting Process**  
Jean-Luc Runavot, Benedicte Bakan,\* Nathalie Geneix, Luc Sauvier, Keltouma Moco, Fabienne Guillon, Françoise Corbineau, Patrick Boivin, and Didier Marion

8265 [dx.doi.org/10.1021/jf201333a](https://doi.org/10.1021/jf201333a)

**Role of Water State and Mobility on the Antiplasticization of Green and Roasted Coffee Beans**  
Pietro Rocculi, Giampiero Sacchetti, Luca Venturi, Mauro Cremonini, Marco Dalla Rosa, and Paola Pittla\*

8272 [dx.doi.org/10.1021/jf201845b](https://doi.org/10.1021/jf201845b)

**Extrusion-Induced Changes to the Chemical Profile and Viscosity Generating Properties of Citrus Fiber**  
R. J. Redgwell, D. Curtl, F. Robin,\* L. Donato, and N. Pineau

## Chemical Composition of Foods/Feeds

8280 [dx.doi.org/10.1021/jf201520t](https://doi.org/10.1021/jf201520t)

**Chemometric Classification of the Fat Residues from the Conditioning Operations of Table Olive Processing, Based on their Minor Components**  
Amparo Cortés-Delgado, Antonio Garrido-Fernández, and Antonio López-López\*

8289 [dx.doi.org/10.1021/jf201052u](https://doi.org/10.1021/jf201052u)

**Allium Species from Central and Southwest Asia Are Rich Sources of Marasmin**  
Jan Kusterer, Reinhard M. Fritsch, and Michael Keusgen\*

8298 [dx.doi.org/10.1021/jf2012634](https://doi.org/10.1021/jf2012634)

**Effect of Different Vitamin D Supplementations in Poultry Feed on Vitamin D Content of Eggs and Chicken Meat**  
Pirjo H. Mattila,\* Eija Valkonen, and Jarmo Valaja

8304 [dx.doi.org/10.1021/jf201278r](https://doi.org/10.1021/jf201278r)

**Soil Water Availability in Rainfed Cultivation Affects More than Cultivar Some Nutraceutical Components and the Sensory Profile of Virgin Olive Oil**  
Pierluigi Bucelli,\* Edoardo A. C. Costantini, Roberto Barbetti, and Elena Franchini

8314 [dx.doi.org/10.1021/jf201753k](https://doi.org/10.1021/jf201753k)

**Phenolic Composition of the Brazilian Seedless Table Grape Varieties BRS Clara and BRS Morena**  
Ellen Silva Lago-Vanzela, Roberto Da-Silva, Eleni Gomes, Esteban García-Romero, and Isidro Hermosin-Gutiérrez\*

## Environmental Chemistry

8324 [dx.doi.org/10.1021/jf201454w](https://doi.org/10.1021/jf201454w)

**Evaluation of Cadmium Phytoremediation Potential in Chinese Cabbage Cultivars**  
Weitao Liu, Qixing Zhou,\* Zhineng Zhang, Tao Hua, and Zhang Cai

## Flavors and Aromas/Chemosensory Perception

8331 [dx.doi.org/10.1021/jf201171b](https://doi.org/10.1021/jf201171b)

**Characterization of Geosmin as Source of Earthy Odor in Different Aroma Type Chinese Liquors**  
Hai Du, Wenlai Fan, and Yan Xu\*

8338 [dx.doi.org/10.1021/jf200762h](https://doi.org/10.1021/jf200762h)

**The Compositions of Volatiles and Aroma-Active Compounds in Dried *Omiya* Fruits (*Schisandra chinensis* Baillon) According to the Cultivation Areas**  
Hyun Jeong Lee, In Hee Cho, Kyung Eun Lee, and Young-Suk Kim\*

8347 [dx.doi.org/10.1021/jf201184m](https://doi.org/10.1021/jf201184m)

**Volatile Composition of Four Southern Highbush Blueberry Cultivars and Effect of Growing Location and Harvest Date**  
Xiaofen Du, Anne Plotto,\* Mei Song, James Olmstead, and Russell Rouseff

8358 [dx.doi.org/10.1021/jf201469c](https://doi.org/10.1021/jf201469c)

**Characterization of the Bound Volatile Extract from Baby Kiwi (*Actinidia arguta*)**  
Coralia V. Garcia, Siew-Young Quek,\* Ralph J. Stevenson, and Robert A. Wenz

8366 [dx.doi.org/10.1021/jf201685m](https://doi.org/10.1021/jf201685m)

**Taste Modulating *N*-(1-Methyl-4-oxoimidazolidin-2-ylidene)  $\alpha$ -Amino Acids Formed from Creatinine and Reducing Carbohydrates**  
Christof Kunert, Alesia Walker, and Thomas Hofmann\*

8375  dx.doi.org/10.1021/jf2024038  
**Topography of the Casein Micelle Surface by Surface Plasmon Resonance (SPR) Using a Selection of Specific Monoclonal Antibodies**

Didier Dupont,\* Annette Johansson, Stephane Marchin, Odile Rolet-Repecaud, Sylvie Marchesseau, and Joelle Leonil

8385 dx.doi.org/10.1021/jf200872r  
**Effects of Tyrosinase and Laccase on Oat Proteins and Quality Parameters of Gluten-free Oat Breads**

Laura Flander,\* Ulla Holopainen, Kristlina Kruus, and Johanna Buchert

8391 dx.doi.org/10.1021/jf200931t  
**Chrysin Protects Epidermal Keratinocytes from UVA- and UVB-Induced Damage**


Nan-Lin Wu, Jia-You Fang, Marcelo Chen, Chia-Jung Wu, Chieh-Chen Huang, and Chi-Feng Hung\*

8401 dx.doi.org/10.1021/jf201953v  
**Use of *Tetragenococcus halophilus* as a Starter Culture for Flavor Improvement in Fish Sauce Fermentation**

Natteewan Udomsil, Sureelak Rodtong, Yeung Joon Choi, Yanglin Hua, and Jirawat Yongsawatdigul\*

8409 dx.doi.org/10.1021/jf201054p  
**Relationship between Red Wine Grade and Phenolics. 2. Tannin Composition and Size**

Stella Kassara and James A. Kennedy\*

8413  dx.doi.org/10.1021/jf201198s  
**Rennet-Induced Aggregation of Heated pH-Adjusted Skim Milk**

Skelte G. Anema,\* Siew Kim Lee, and Henning Klostermeyer

8423 dx.doi.org/10.1021/jf201871w  
**Up-regulated Desaturase and Elongase Gene Expression Promoted Accumulation of Polyunsaturated Fatty Acid (PUFA) but Not Long-Chain PUFA in *Lates calcarifer*, a Tropical Euryhaline Fish, Fed a Stearidonic Acid- and  $\gamma$ -Linoleic Acid-Enriched Diet**


Ramez Alhazzaa,\* Andrew R. Bridle, Peter D. Nichols, and Chris G. Carter

8435 dx.doi.org/10.1021/jf201398t  
**Flavanols and Methylxanthines in Commercially Available Dark Chocolate: A Study of the Correlation with Nonfat Cocoa Solids**

Swen Langer, Lisa J. Marshall,\* Andrea J. Day, and Michael R. A. Morgan

8442 dx.doi.org/10.1021/jf201494b  
**Food Matrix Effects on in Vitro Digestion of Microencapsulated Tuna Oil Powder**

Zhiping Shen,\* Christina Apriani, Rangika Weerakkody, Luz Sanguanri, and Mary Ann Augustin

8450  dx.doi.org/10.1021/jf201556e


**Multielement Fingerprinting as a Tool in Origin Authentication of PGI Food Products: Tropea Red Onion**

Emilia Furla, Attilio Naccarato, Giovanni Sindona, Gaetano Stabile, and Antonio Tagarelli\*

8458 dx.doi.org/10.1021/jf201637u

**Luteolin Inhibits the Release of Glutamate in Rat Cerebrocortical Nerve Terminals**

Tzu Yu Lin, Cheng Wei Lu, Chia Chien Chang, Shu Kuei Huang, and Su Jane Wang\*

8467  dx.doi.org/10.1021/jf201870z

**Effect of pH, NaCl, CaCl<sub>2</sub> and Temperature on Self-Assembly of  $\beta$ -Lactoglobulin into Nanofibrils: A Central Composite Design Study**

S. M. Loveday,\* X. L. Wang, M. A. Rao, S. G. Anema, and H. Singh

8475 dx.doi.org/10.1021/jf202006a

**Physicochemical and Flavor Characteristics of Flavoring Agent from Mungbean Protein Hydrolyzed by Bromelain**

Chanikan Sonklin, Natta Laohakunjit,\* and Orapin Kerdchoechuen

### Molecular Nutrition

8484 dx.doi.org/10.1021/jf201536v

**Noncovalent Interaction of Dietary Polyphenols with Bovine Hemoglobin in Vitro: Molecular Structure/Property–Affinity Relationship Aspects**

Jian Bo Xiao,\* Jiang Lei Huo, Fan Yang, and Xiao Qing Chen\*

8491 dx.doi.org/10.1021/jf201775v

**Improvement of Mitochondrial Function in Muscle of Genetically Obese Rats after Chronic Supplementation with Proanthocyanidins**

David Pajuelo, Anabel Fernández-Iglesias, Sabina Díaz, Helena Quesada, Anna Arola-Arnal, Cinta Bladé, Josepa Salvadó, and Lluís Arola\*

### Toxicology in Agriculture and Food

8499 dx.doi.org/10.1021/jf201796x

**Investigation on the Interaction between Ilaprazole and Bovine Serum Albumin without or with Different C-Ring Flavonoids from the Viewpoint of Food–Drug Interference**

Yuping Zhang, Shuyun Shi,\* Xiaoqin Chen, Wei Zhang, Kelong Huang, and Mijun Peng

8507 dx.doi.org/10.1021/jf200970s

**Study on the Binding of Propiconazole to Protein by Molecular Modeling and a Multispectroscopic Method**

Chao Wang and Ying Li\*

8513 dx.doi.org/10.1021/jf2010673

**Polychlorodibenzodioxin and -furan (PCDD and PCDF) and Dioxin-like Polychlorobiphenyl (DL-PCB) Congener Levels in Milk of Grazing Sheep as Indicators of the Environmental Quality of Rural Areas**

Gianfranco Brambilla,\* Vittorio Abate, Stefania Paola De Filippis, Anna Rita Fulgenzi, Anna Laura Iamiciell, Alessandro Mazzette, Roberto Miniero, and Giuseppe Pulina

8518 dx.doi.org/10.1021/jf201342t

**Residue Potential of Norsesquiterpene Glycosides in Tissues of Cattle Fed Austral Bracken (*Pteridium esculentum*)**

Mary T. Fletcher,\* Keith G. Reichmann, Ian J. Brock, Ross A. McKenzie, and Barry J. Blaney

8524 dx.doi.org/10.1021/jf201938d

**Phlorhizin Protects against Erythrocyte Cell Membrane Scrambling**

Sergios Gatidis, Anja Meier, Kashif Jilani, Elisabeth Lang, Christine Zelenak, Syed M. Qadri, and Florian Lang\*

**LC-MS/MS Quantification of Sulforaphane and Indole-3-carbinol Metabolites in Human Plasma and Urine after Dietary Intake of Selenium-Fortified Broccoli**Johanna Hauder,<sup>†</sup> Stefanie Winkler,<sup>§</sup> Achim Bub,<sup>§</sup> Corinna E. Rüfer,<sup>§</sup> Marc Pignitter,<sup>‡</sup> and Veronika Somoza<sup>\*†</sup><sup>†</sup>German Research Center for Food Chemistry, Lise-Meitner-Strasse 34, D-84354 Freising, Germany<sup>§</sup>Institute of Nutritional Physiology, Federal Research Centre for Nutrition and Food, Haad-und-Neu-Strasse 9, D-76131 Karlsruhe, Germany<sup>‡</sup>Department of Nutritional and Physiological Chemistry, University of Vienna, Althanstrasse 14 (UZA II), A-1090 Vienna, Austria

**ABSTRACT:** This study aimed at developing a sensitive LC-MS/MS method for the quantification of sulforaphane (SFN) and indole-3-carbinol metabolites in plasma and urine after dietary intake of regular and selenium-fertilized broccoli using stable isotope dilution analysis. In a three-armed, placebo-controlled, randomized human intervention study with 76 healthy volunteers, 200 g of regular (485  $\mu\text{g}$  of total glucosinolates and  $<0.01 \mu\text{g}$  of selenium per gram fresh weight) or selenium-fertilized broccoli (589  $\mu\text{g}$  of total glucosinolates and 0.25  $\mu\text{g}$  of selenium per gram fresh weight) was administered daily for 4 weeks. Glucoraphanin and glucobrassicin metabolites quantified in plasma and urine were SFN-glutathione, SFN-cysteine, SFN-cysteinylglycine, SFN-acetylcysteine, and indole-3-carboxaldehyde, indole-3-carboxylic acid, and ascorbigen, respectively. Dietary intake of selenium-fertilized broccoli increased serum selenium concentration analyzed by means of atomic absorption spectroscopy by up to 25% ( $p < 0.001$ ), but affected neither glucosinolate concentrations in broccoli nor their metabolite concentrations in plasma and urine compared to regular broccoli.

**KEYWORDS:** broccoli, glucosinolates, LC-MS/MS, glucobrassicin metabolites, glucoraphanin metabolites, sulforaphane, indole-3-carbinol, selenium

**INTRODUCTION**

Numerous human studies have shown an association between a diet rich in cruciferous vegetables (e.g., broccoli, cabbage, and Brussels sprouts) and a decreased risk for prostate cancer.<sup>1,2</sup> This chemopreventive activity of cruciferous vegetables is attributed to their contents in glucosinolates<sup>3</sup> and probably selenium (Se).<sup>4</sup>

Among the glucosinolates, glucoraphanin and glucobrassicin represent the quantitatively dominating groups in broccoli. After dietary intake, glucoraphanin and glucobrassicin undergo a myrosinase-catalyzed hydrolysis, yielding sulforaphane (SFN) and indole-3-carbinol (I3-C), respectively. Once absorbed in the gastrointestinal tract, SFN and I3-C can be further metabolized into a broad spectrum of metabolites.<sup>5–7</sup> SFN is primarily metabolized through the mercapturic acid pathway, whereby its glutathione conjugate (SFN-GSH) is formed intracellularly and is sequentially degraded to form cysteinylglycine (SFN-Cys-Gly), cysteine (SFN-Cys), and *N*-acetylcysteine (SFN-NAC) conjugates in the upper intestinal lumen.<sup>5–7</sup>

Glucobrassicin metabolites are also generated through the enzymatic activity of the myrosinase that releases indole-3-acetonitrile (I3-ACN) under acidic conditions.<sup>8</sup> At neutral pH, the myrosinase catalyzes the formation of I3-C via the unstable intermediate indole-3-methyl isothiocyanate.<sup>9</sup> I3-C might undergo oxidative metabolism, yielding the corresponding aldehyde and/or carboxylic acid (I3-CAL, I3-CA).<sup>10</sup> Numerous *in vitro* studies using the free compound I3-C also showed its oligomerization into dimers, linear and cyclic trimers, and higher oligomers

at acidic pH values as they occur in the stomach.<sup>9,11</sup> Furthermore, I3-C has also been demonstrated to react with 1-ascorbic acid to form ascorbigen (ASG) *in vitro*.<sup>12</sup>

Glucoraphanin metabolites in biological samples are commonly quantified by analytical methods based on HPLC coupled to UV or mass detection.<sup>13–15</sup> Recently, Egner et al. reported a very sensitive and well-validated method for quantification of SFN and its mercapturic acid pathway conjugates in urine samples using a stable isotope dilution assay (SIDA) after bolus administration of broccoli infusions containing 175 mg of glucoraphanin to healthy volunteers.<sup>16</sup>

Glucobrassicin metabolites after dietary intake have been quantified by means of less sensitive analytical methods that require higher doses for reliable quantification. In one of the previously reported studies, ASG was quantified in rat plasma after dietary administration of approximately 117 mg of ASG/kg of body weight for 7 days using HPLC-MS.<sup>17</sup> Reed et al.<sup>18</sup> administered amounts of 400–1200 mg of I3-C to human subjects and were able to quantify only one of the glucobrassicin metabolites, 3,3'-diindolylmethane (DIM), by means of HPLC-MS.<sup>18</sup> We aimed at elucidating whether other metabolites are formed *in vivo* after dietary intake of glucobrassicin-containing

Received: December 23, 2010

Revised: June 18, 2011

Accepted: June 19, 2011

Published: July 06, 2011