

Articles

Analytical Methods

11895

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Analysis of Acid-Soluble Glycogen in Pork Extracts of Two *PRKAG3* Genotypes by ^1H Liquid-State NMR Spectroscopy and Biochemical Methods

Flemming H. Larsen,* Birgitta Essén-Gustavsson, Marianne Jensen-Waern, René Lametsch, Anders H. Karlsson, and Gunilla Lindahl

11903

dx.doi.org/10.1021/jf203201b

Fast Determination of Sudan Dyes in Chili Tomato Sauces Using Partial Filling Micellar Electrokinetic Chromatography

Tatiana S. Fukuji, Maria Castro-Puyana, Marina F. M. Tavares, and Alejandro Cifuentes*

11910



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Marzipan: Polymerase Chain Reaction-Driven Methods for Authenticity Control

Philipp Brüning, Ilka Haase,* Reinhard Matissek, and Markus Fischer

11918

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Evaluation of a Modified QuEChERS Extraction of Multiple Classes of Pesticides from a Rice Paddy Soil by LC-APCI-MS/MS

Sergiane S. Caldas, Cátia M. Bolzan, Maristela B. Cerqueira, Debora Tomasini, Eliana B. Furlong, Carlos Fagundes, and Ednei G. Primele*

11927

dx.doi.org/10.1021/jf2029364

Detection, Accumulation, Distribution, and Depletion of Furalfadone and Nifursol Residues in Poultry Muscle, Liver, and Gizzard

Jorge Barbosa, Andreia Freitas, Sara Moura, José Luis Mourão, Maria Irene Noronha da Silveira, and Fernando Ramos*

11935



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A Boron-dipyromethene-Based Fluorescent Probe for Colorimetric and Ratiometric Detection of Sulfite

Xianfeng Gu,* Chunhua Liu, Yi-Chun Zhu, and Yi-Zhun Zhu*

Bioactive Constituents

11940 [dx.doi.org/10.1021/jf202473e](https://doi.org/10.1021/jf202473e)

Effect of Commercial Lignosulfonate-Humate on *Zea mays* L. Metabolism
Andrea Ertani, Ornella Francioso, Vitalliano Tugnoli, Valeria Righi, and Serenella Nardi*

11949 [dx.doi.org/10.1021/jf203576g](https://doi.org/10.1021/jf203576g)

In Vitro Fermentation by Human Gut Bacteria of Proteolytically Digested Caseinomacropeptide Nonenzymatically Glycosylated with Prebiotic Carbohydrates
Oswaldo Hernandez-Hernandez, M. Luz Sanz, Sofia Kollida, Robert A. Rastall, and F. Javier Moreno*

11956 [dx.doi.org/10.1021/jf202890e](https://doi.org/10.1021/jf202890e)

Transport Across Caco-2 Cell Monolayer and Sensitivity to Hydrolysis of Two Anxiolytic Peptides from α_{s1} -Casein, α -Caseozepine, and α_{s1} -Casein-(f91–97): Effect of Bile Salts
Céline Cakir-Kiefer, Laurent Miclo, Frédérique Balandras, Annie Dary, Claire Soligot, and Yves Le Roux*

11966 [dx.doi.org/10.1021/jf202958r](https://doi.org/10.1021/jf202958r)

Echinacea Alkamides Prevent Lipopolysaccharide/β-Galactosamine-Induced Acute Hepatic Injury through JNK Pathway-Mediated HO-1 Expression
Chia-Chung Hou, Chi-Chang Huang, and Lie-Fen Shyur*

11975 [dx.doi.org/10.1021/jf2032439](https://doi.org/10.1021/jf2032439)

Dynamics of Nrf2 and Keap1 in ARE-Mediated NQO1 Expression by Wasabi 6-(Methylsulfinyl)hexyl Isothiocyanate
De-Xing Hou,[†] Yoshimi Korenori, Shunsuke Tanigawa, Tomoe Yamada-Kato, Masashi Nagai, Xi He, and Jianhua He

11983 [dx.doi.org/10.1021/jf2033329](https://doi.org/10.1021/jf2033329)

LC-MS/MS Quantification of Bioactive Angiotensin I-Converting Enzyme Inhibitory Peptides in Rye Malt Sourdoughs
Ying Hu, Achim Stromeck, Jussi Laponen, Daise Lopes-Lutz, Andreas Schieber, and Michael G. Glänze*

Biofuels and Bioproducts Chemistry

11990 [dx.doi.org/10.1021/jf2031633](https://doi.org/10.1021/jf2031633)

Nonfeed Application of Rendered Animal Proteins for Microbial Production of Eicosapentaenoic Acid by the Fungus *Pythium irregulare*
Yi Liang, Rafael A. Garcia, George J. Piazza, and Zhiyou Wen*

Chemical Aspects of Biotechnology/Molecular Biology

11997 [dx.doi.org/10.1021/jf202457r](https://doi.org/10.1021/jf202457r)

Branched α -(1,4) Glucans from *Lentinula edodes* (L10) in Combination with Radiation Enhance Cytotoxic Effect on Human Lung Adenocarcinoma through the Toll-like Receptor 4 Mediated Induction of THP-1 Differentiation/Activation
Tiffany Chien-Ting Lo, Feng-Ming Hsu, C. Allen Chang, and Jason Chia-Hsein Cheng*

Chemical Aspects of Food Safety

12006 [dx.doi.org/10.1021/jf202919a](https://doi.org/10.1021/jf202919a)

Development of Gold Nanoparticle-Based Rapid Detection Kit for Melamine in Milk Products
Qingqing Zhou, Nan Liu, Zhiwei Qie, Ying Wang, Baoan Ning, and Zhixian Gao*

12012 [dx.doi.org/10.1021/jf202975x](https://doi.org/10.1021/jf202975x)

Residue Analysis and Degradation Studies of Fenbuconazole and Myclobutanil in Strawberry by Chiral High-Performance Liquid Chromatography–Tandem Mass Spectrometry
Hu Zhang, Xinquan Wang, Mingrong Qian, Xiangyun Wang, Hao Xu, Mingfei Xu, and Qiang Wang*

Chemical Changes Induced by Processing/Storage

12018 [dx.doi.org/10.1021/jf203582k](https://doi.org/10.1021/jf203582k)

Identification and Thermal Degradation Kinetics of Chlorophyll Pigments and Ascorbic Acid from Dita Nectar (*Detarium senegalense* J.F. Gmel)
Nafissatou Diop Ndiaye, Claudie Dhuique-Mayer, Mady Cissé, and Manuel Dornier*

12028 [dx.doi.org/10.1021/jf203390e](https://doi.org/10.1021/jf203390e)

Effects of High Hydrostatic Pressure on Some Functional and Nutritional Properties of Soy Protein Isolate for Infant Formula
Huijing Li, Kexue Zhu, Hulming Zhou,* and Wei Peng

12037 [dx.doi.org/10.1021/jf202515k](https://doi.org/10.1021/jf202515k)

Determination of Advanced Glycation Endproducts by LC-MS/MS in Raw and Roasted Almonds (*Prunus dulcis*)
Gong Zhang, Guangwei Huang, Lu Xiao, and Alyson E. Mitchell*

12047 [dx.doi.org/10.1021/jf202582t](https://doi.org/10.1021/jf202582t)

Lycopene Bioaccessibility and Starch Digestibility for Extruded Snacks Enriched with Tomato Derivatives
Zeinab Dehghan-Shoar,* Tafadzwa Mandimika, Allan K. Hardacre, Gordon W. Reynolds, and Charles S. Brennan

Chemical Composition of Foods/Feeds

12054 [dx.doi.org/10.1021/jf2037104](https://doi.org/10.1021/jf2037104)

Broth from Canned Clams Is Suitable for Use as an Excellent Source of Free Vitamin B₁₂
Kazumi Ueta, Shigeo Takenaka, Yukinori Yabuta, and Fumio Watanabe*

12059 [dx.doi.org/10.1021/jf202556p](https://doi.org/10.1021/jf202556p)

UHPLC-PDA-ESI/HRMS/MSⁿ Analysis of Anthocyanins, Flavonol Glycosides, and Hydroxycinnamic Acid Derivatives in Red Mustard Greens (*Brassica juncea* Cass Variety)
Long-Ze Lin,* Jianghao Sun, Pei Chen, and James Harnly

12073 [dx.doi.org/10.1021/jf202969e](https://doi.org/10.1021/jf202969e)

Variation of Polyphenols and Betaines in Aerial Parts of Young, Field-Grown *Amaranthus* Genotypes

Stine Krogh Steffensen, Hans Albert Pedersen, Rodrigo Labouriau, Arne G. Mortensen, Bente Laursen, Rosa M. de Troiani, Elke J. Noellemeier, Dagmar Janovska, Helena Stavelikova, Andreu Taberner, Carsten Christophersen, and Inge S. Fomsgaard*

12083 [dx.doi.org/10.1021/jf203187v](https://doi.org/10.1021/jf203187v)

Update on the Healthful Lipid Constituents of Commercially Important Tree Nuts

Katherine S. Robbins, Eui-Cheol Shin, Robert L. Shewfelt, Ronald R. Eitenmiller, and Ronald B. Pegg*

12093 [dx.doi.org/10.1021/jf203363q](https://doi.org/10.1021/jf203363q)

Identification of Olive (*Olea europaea*) Pulp Proteins by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry and Nano-Liquid Chromatography Tandem Mass Spectrometry

Clara Esteve, Benito Cañas, Estefanía Moreno-Gordaliza, Carmen Del Río, María Concepción García, and María Luisa Marina*

12102 [dx.doi.org/10.1021/jf203428t](https://doi.org/10.1021/jf203428t)

Determination of Coenzyme Q₁₀, Coenzyme Q₉, and Melatonin Contents in Virgin Argan Oils: Comparison with Other Edible Vegetable Oils

Carmen Venegas, Carmen Cabrera-Vique, Laura García-Corzo, Germaine Escames, Darío Acuña-Castroviejo, and Luis Carlos López*

Environmental Chemistry

12109 [dx.doi.org/10.1021/jf2026555](https://doi.org/10.1021/jf2026555)

A Comparative Study on the Interference of Two Herbicides in Wheat and Italian Ryegrass and on Their Antioxidant Activities and Detoxification Rates

Daniele Del Buono,* Gerardina Ioli, Luigi Nasini, and Primo Proietti

12116 [dx.doi.org/10.1021/jf202924a](https://doi.org/10.1021/jf202924a)

Cosorption of Phenanthrene and Mercury(II) from Aqueous Solution by Soybean Stalk-Based Biochar

Huoliang Kong, Jiao He, Yanzheng Gao,* Hui Fang Wu, and Xuezhu Zhu

Flavors and Aromas/Chemosensory Perception

12124 [dx.doi.org/10.1021/jf202813r](https://doi.org/10.1021/jf202813r)

Quality of Sour Cherry Juice of Different Clones and Cultivars (*Prunus cerasus* L.) Determined by a Combined Sensory and NMR Spectroscopic Approach

Morten R. Clausen, Bjarne H. Pedersen, Hanne C. Bertram, and Ulla Kidmose*

12131 [dx.doi.org/10.1021/jf2029835](https://doi.org/10.1021/jf2029835)

Establishment of a New Cell-Based Assay To Measure the Activity of Sweeteners in Fluorescent Food Extracts

Yasuka Toda, Shinji Okada, and Takumi Misaka*

Food Chemistry/Biochemistry

12139 [dx.doi.org/10.1021/jf2019825](https://doi.org/10.1021/jf2019825)

Postprandial Glycemia, Insulinemia, and Satiety Responses in Healthy Subjects after Whole Grain Rye Bread Made from Different Rye Varieties. 1

Liza A. H. Rosén, Elin M. Östman,* Peter R. Shewry, Jane L. Ward, Annika A. M. Andersson, Vleno Piironen, Anna-Majla Lampi, Marianne Rakszegi, Zoltan Bedő, and Inger M. E. Björck

12149 [dx.doi.org/10.1021/jf2019837](https://doi.org/10.1021/jf2019837)

Postprandial Glycemia, Insulinemia, and Satiety Responses in Healthy Subjects after Whole Grain Rye Bread Made from Different Rye Varieties. 2

Liza A. H. Rosén, Elin M. Östman,* and Inger M. E. Björck

12155 [dx.doi.org/10.1021/jf203950d](https://doi.org/10.1021/jf203950d)

Compositional Changes in 'Bartlett' Pear (*Pyrus communis* L.) Cell Wall Polysaccharides As Affected by Sunlight Conditions

María D. Raffo, Nora M. A. Ponce, Gabriel O. Sozzi, Ariel R. Vicente, and Carlos A. Stortz*

12163 [dx.doi.org/10.1021/jf2024769](https://doi.org/10.1021/jf2024769)

Antioxidant Activity of Betanidin: Electrochemical Study in Aqueous Media

Slawomir Wybraniec,* Pawel Stalica, Aneta Sporna, Boris Nemzer, Zbigniew Pietrzkowski, and Tadeusz Michalowski

12171 [dx.doi.org/10.1021/jf202679y](https://doi.org/10.1021/jf202679y)

Combined Effects of Prefermentative Skin Maceration and Oxygen Addition of Must on Color-Related Phenolics, Volatile Composition, and Sensory Characteristics of Airén White Wine

María Jesús Cejudo-Bastante,* Lucía Castro-Vázquez, Isidro Hermosín-Gutiérrez, and María Soledad Pérez-Coello

12183 [dx.doi.org/10.1021/jf202808r](https://doi.org/10.1021/jf202808r)

Effect of the Simultaneous Interaction among Ascorbic Acid, Iron and pH on the Oxidative Stability of Oil-in-Water Emulsions

Gabriel F. Branco, Maria I. Rodrigues, Luiz A. Gioielli, and Inar A. Castro*

12193 [dx.doi.org/10.1021/jf202837g](https://doi.org/10.1021/jf202837g)

An Antihypertensive Peptide from Tilapia Gelatin Diminishes Free Radical Formation in Murine Microglial Cells

Thanh-Sang Vo, Dai-Hung Ngo, Jung-Ae Kim, BoMi Ryu, and Se-Kwon Kim*

12198 [dx.doi.org/10.1021/jf202844t](https://doi.org/10.1021/jf202844t)

Species-Specific Myoglobin Oxidation

Shuang Yin, Cameron Faustman,* Nantawat Tattijaborworntham, Ranjith Ramanathan, Naveena B. Maheswarappa, Richard A. Mancini, Poulson Joseph, Surendranath P. Suman, and Qun Sun

12204 [dx.doi.org/10.1021/jf202942h](https://doi.org/10.1021/jf202942h)

Studies on the Key Aroma Compounds in Soy Milk Made from Three Different Soybean Cultivars

Shu Kaneko,* Kenji Kumazawa, and Osamu Nishimura

12210 [dx.doi.org/10.1021/jf2039185](https://doi.org/10.1021/jf2039185)
Potent in Vivo Antifungal Activity against Powdery Mildews of Pregnane Glycosides from the Roots of *Cynanchum wilfordii*
 Mi-Young Yoon, Nam Hee Choi, Byung Sun Min, Gyung Ja Choi, Yong Ho Choi, Kyoung Soo Jang, Seong-Sook Han, Byeongjin Cha, and Jin-Cheol Kim*

12217 [dx.doi.org/10.1021/jf203022f](https://doi.org/10.1021/jf203022f)
Flavonoids in Tropical Citrus Species
 Surl Roowl and Alan Crozier*

12226 [dx.doi.org/10.1021/jf203139s](https://doi.org/10.1021/jf203139s)
High Molecular Weight Glutenin Subunits in Some Durum Wheat Cultivars Investigated by Means of Mass Spectrometric Techniques
 Vera Muccilli,* Marisol Lo Bianco, Vincenzo Cunsolo, Rosaria Saletti, Giulia Gallo, and Salvatore Foti

12238  [dx.doi.org/10.1021/jf203145p](https://doi.org/10.1021/jf203145p)
Ferrochelatase Catalyzes the Formation of Zn-protoporphyrin of Dry-Cured Ham via the Conversion Reaction from Heme in Meat
 Tuan Thanh Chau, Mutsumi Ishigaki, Takao Kataoka, and Shigeru Taketani*

12246 [dx.doi.org/10.1021/jf203556s](https://doi.org/10.1021/jf203556s)
Analysis and Comparison of Glucocerebroside Species from Three Edible Sea Cucumbers Using Liquid Chromatography–Ion Trap–Time-of-Flight Mass Spectrometry
 Jie Xu, Jingjing Duan, Changhu Xue,* Tingyu Feng, Ping Dong, Tatsuya Sugawara, and Takashi Hirata

Molecular Nutrition

12254 [dx.doi.org/10.1021/jf203136j](https://doi.org/10.1021/jf203136j)
Inhibitory Effect of Blueberry Polyphenolic Compounds on Oleic Acid-Induced Hepatic Steatosis in Vitro
 Yixiang Liu, Dan Wang, Di Zhang, Yechun Lv, Ying Wei, Wei Wu, Feng Zhou, Miaomiao Tang, Ting Mao, Mengmeng Li, and Baoping Ji*

Toxicology in Agriculture and Food

12264 [dx.doi.org/10.1021/jf204035y](https://doi.org/10.1021/jf204035y)
Occurrence of *Fusarium* spp. and Fumonisin in Durum Wheat Grains
 Sofia A. Palacios, Maria L. Ramirez,* Mariel Cabrera Zalazar, Maria C. Farnochi, Diego Zappacosta, Stella M. Chiacchiera, Maria M. Reynoso, Sofia N. Chulze, and Adriana M. Torres

Analysis of Acid-Soluble Glycogen in Pork Extracts of Two *PRKAG3* Genotypes by ¹H Liquid-State NMR Spectroscopy and Biochemical Methods

Flemming H. Larsen,^{1,†} Birgitta Essén-Gustavsson,⁵ Marianne Jensen-Waern,⁵ René Lametsch,¹ Anders H. Karlsson,¹ and Gunilla Lindahl^{1,7,*}

¹Department of Food Science, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

²Department of Food Science and ³Department of Clinical Sciences, Swedish University of Agricultural Sciences, P.O. Box 7051, SE-750 07 Uppsala, Sweden

ABSTRACT: Meat extracts with acid-soluble glycogen (macroglycogen) from *M. longissimus dorsi* of carriers and noncarriers of the *PRKAG3* mutation (*RN*⁻ and *rn*⁺ genotype) were analyzed by both ¹H liquid-state NMR spectroscopy and a biochemical method. The ¹H NMR analysis revealed that shorter polymers (dimers, trimers, etc.) of α -1,4-linked glucose were generated 24–48 h post-mortem. This is not possible to elucidate with the biochemical method, by which only the total amount of hydrolyzed glucose residues is determined. The shorter polymers were primarily formed in carriers of the *PRKAG3* mutation, suggesting different post-mortem glycogen degradation mechanisms in the two genotypes.

KEYWORDS: glycogen; ¹H nuclear magnetic resonance (¹H NMR); enzymatic analysis; pork; *RN*-gene; *PRKAG3* mutation

INTRODUCTION

Glycogen is the cellular energy storage molecule in animals and serves the same purpose as starch does in plants. Both starch and glycogen are α -linked polymers of glucose, but whereas starch is composed of linear amylose and more branched amylopectin, glycogen is a large branched polymer of glucose units, similar to amylopectin, built up on a core protein, glycogenin.¹

Glycogen has been divided into two molecular subgroups according to its solubility in trichloroacetic acid or perchloric acid: acid-insoluble glycogen, called proglycogen, with a protein content of about 10% (molecular weight 400 kDa) and acid-soluble glycogen, called macroglycogen (molecular weight 10⁷ Da) with only 0.35% protein content.² Methods for the analysis of glycogen in biological tissues are traditionally based on the hydrolysis of glycogen to glucose units either by acid or enzymatic hydrolysis. Total glycogen as well as pro- and macroglycogen have reliably been measured by both of these methods.² However, these biochemical methods do not reveal the actual size and size distribution as well as the configuration or structure of the glycogen molecules. By ¹H liquid-state nuclear magnetic resonance (NMR) spectroscopy hydrogens in various chemical environments in solution can be identified and quantified, for example, the anomeric proton involved in the α -1,4 glycosidic bond in glycogen. Previously, this approach has been used for structural analysis of rabbit liver glycogen.³

Recent microscopy studies^{4,5} demonstrated that two kind of particles can be present in glycogen: β -particles having diameters in the range of 10–30 nm and supramolecular complexes of β -particles, the so-called α -particles, having diameters up to 300 nm. The α -particles are so far only observed in liver glycogen, whereas glycogen from skeletal muscles contain only β -particles.⁵ Similar to amylopectin, the glucose units in glycogen are connected by linear chain α -1,4 and branched α -1,6 glycosidic bonds. The glycogen molecule is formed by two different

kinds of chains, A- and B-chains.⁴ The A-chains are not branched, having only α -1,4 bonds, whereas the B-chains are branched, each of them with two branching points with α -1,6 bonds, which create new either A- or B-chains. There are four glucose residues between the branches and a tail of approximately four residues after the second branch in the B-chains. The glycogen molecule is spherical and organized into concentric tiers. Every B-chain is in the inner tiers, and every A-chain is within the outer tier. Each A- or B-chain has 12–14 glucose residues, and there are 12 tiers in a molecule. The number of chains in any tier is twice that of the previous one, as a consequence of two branching points at each chain.⁶

The dominant *PRKAG3* mutation (previously known as the *RN*⁻ mutation) in pigs of the Hampshire breed results in very high glycogen content, especially in glycolytic muscles, compared with the wild type Hampshire pigs.⁷ Furthermore, the post-mortem pH decline has been shown to be slightly faster and the ultimate pH lower in *M. longissimus dorsi* (LD) of carriers of the *PRKAG3* mutation compared with noncarriers.^{8–11} Essén-Gustavsson et al.¹² studied post-mortem degradation of total glycogen in LD of carriers and noncarriers of the *PRKAG3* mutation using a biochemical method. As expected, they found a higher glycogen content in carriers compared with noncarriers of the *PRKAG3* mutation, but the amount of glycogen degraded from 10 min to 24 h post-mortem did not differ between the genotypes. Samples of LD from the same pigs as in the study of Essén-Gustavsson et al.¹² were in the present study homogenized with perchloric acid and centrifuged, and the soluble glycogen in the supernatant (macroglycogen) was then analyzed

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