Case No.: IPR2023-00208
U.S. Patent No. 11,013,250

JOURNAL OF

AGRICULTURAL AND FOOD CHEMISTRY

Volume 51 No. 11 May 21, 2003

STE... MEMORIAL LIBRARY

Published by the American Chemical Society http://pubs.acs.org/JAFC

Motif Exhibit 1056, Page 1 of 12

Case No.: IPR2023-00208
U.S. Patent No. 11,013,250

JOURNAL OF
AGRICULTURAL AND FOOD CHEMISTRY

May 21, 2003

JAFCAU 51(11) 3197−3508 (2003) ISSN 0021-8561
Registered in U.S. Patent and Trademark Office
Copyright 2003 by the American Chemical Society

REVIEWS

3197 **Antimicrobial Properties of Basil and Its Possible Application in Food Packaging**
Panuwat Suppakul, Joseph Miltz, Kees Sonneveld, and Stephen W. Bigger*

ANALYTICAL METHODS

3208 **Preparation of a *t,t* Conjugated Linoleic Acid Methylester (CLA-Me) Isomers Mixture from Synthetic CLA by Methylation with BF_3/Methanol**
*Seck J. Kim, Sook J. Park, Jung K. Kim, Jeong H. Kim, Ki H. Shim, Chung G. Park, Jeong O. Kim, and Yeong L. Ha**

3215 **A Sensitive Sandwich ELISA for the Detection of Trace Amounts of Cashew (*Anacardium occidentale* L.) Nut in Foods**
*Yanhong Wei, Shridhar K. Sathe, Suzanne S. Teuber, and Kenneth H. Roux**

3222 **Determination of Thiamine and Its Esters in Beers and Raw Materials Used for Their Manufacture by Liquid Chromatography with Postcolumn Derivatization**
*Pilar Viñas, Carmen López-Erroz, Nuria Balsalobre, and Manuel Hernández-Córdoba**

3228 **Development of Enzyme-Linked Immunosorbent Assays for Isocupressic Acid and Serum Metabolites of Isocupressic Acid**
Stephen T. Lee, Dale R. Gardner, Massoud Garrosian, Kip E. Panter, Alessio N. Serrequi, Thomas K. Schoch, and Bryan L. Stegelmeier*

3234 **Honey Characterization and Adulteration Detection by Pattern Recognition Applied on HPAEC-PAD Profiles. 1. Honey Floral Species Characterization**
Christophe B. Y. Cordella, Julio S. L. T. Militão, Marie-Claude Clément, and Daniel Cabrol-Bass*

3243 **Quantitative Determination of Sulfur-Containing Wine Odorants at Sub Parts per Billion Levels. 2. Development and Application of a Stable Isotope Dilution Assay**
*Rémi Schneider, Yorgos Kotseridis, Jean-Loïc Ray, Christian Augier, and Raymond Baumes**

3249 **A Rapid Spectrofluorometric Screening Method for Enrofloxacin in Chicken Muscle**
Guoying Chen and Marilyn J. Schneider*

3254 **Determination of Albendazole and Its Major Metabolites in the Muscle Tissues of Atlantic Salmon, Tilapia, and Rainbow Trout by High Performance Liquid Chromatography with Fluorometric Detection**
Badar Shaikh, Nathan Rummel, and Renate Reimschuessel*

Case No.: IPR2023-00208
U.S. Patent No. 11,013,250

3260 **Characterization of Triterpene Alcohol and Sterol Ferulates in Rice Bran Using LC-MS/MS**
Nianbai Fang, Shanggong Yu, and Thomas M. Badger*

3268 **FTIR-ATR Analysis of Brewed Coffee: Effect of Roasting Conditions**
Donald J. Lyman, Robert Benck, Stacy Dell, Scott Merle, and Jacqueline Murray-Wijelath*

3273 **Assays for Hydrophilic and Lipophilic Antioxidant Capacity (oxygen radical absorbance capacity ($ORAC_{FL}$)) of Plasma and Other Biological and Food Samples**
Ronald L. Prior, Ha Hoang, Liwei Gu, Xianli Wu, Mara Bacchiocca, Luke Howard, Maureen Hampsch-Woodill, Dejian Huang, Boxin Ou, and Robert Jacob*

3280 **Capillary Electrophoresis of α-Lactalbumin in Milk Powders**
Jose E. N. Gutierrez and Lisa Jakobovits*

BIOACTIVE CONSTITUENTS

3287 **Purification and Structural Characterization of 3-Hydroxypropionaldehyde and Its Derivatives**
Sabine Vollenweider, Guido Grassi, Iwo König, and Zdenko Puhan*

3294 **Volatile Metabolites from *Salvia fruticosa* as Antifungal Agents in Soilborne Pathogens**
Danae Pitarokili, Olga Tzakou, Anargyros Loukis, and Catherine Harvala*

3302 **Antioxidative and Hepatoprotective Effects of *Antrodia camphorata* Extract**
*George Hsiao, Ming-Yi Shen, Kuan-Hung Lin, Ming-Huang Lan, Li-Yu Wu, Duen-Suey Chou, Chien-Huang Lin, Ching-Hua Su, and Joen-Rong Sheu**

3309 **Dihydrochalcones: Evaluation as Novel Radical Scavenging Antioxidants**
Yoshimasa Nakamura, Shigeo Watanabe, Nobuyuki Miyake, Hiroyuki Kohno, and Toshihiko Osawa*

3313 **Stoichiometric and Kinetic Studies of Phenolic Antioxidants from Andean Purple Corn and Red-Fleshed Sweetpotato**
*Bolívar A. Cevallos-Casals and Luis Cisneros-Zevallos**

3320 **Food Matrix Effects on Bioactivity of Broccoli-Derived Sulforaphane in Liver and Colon of F344 Rats**
*Anna-Sigrid Keck, Qingyan Qiao, and Elizabeth H. Jeffery**

BIOTECHNOLOGY

3328 **Endophytic Fungi Associated with Mediterranean Plants as a Source of Mycelium-Bound Lipases**
*Mercè Torres, Marta M. Dolcet, Nuria Sala, and Ramon Canela**

3334 **Protection against Trp-P-2 DNA Adduct Formation in C57bl6 Mice by Purpurin Is Accompanied by Induction of Cytochrome P450**
Tim Marczylo, Chitose Sugiyama, and Hikoya Hayatsu*

CHEMICAL AND PHYSICAL CHANGES INDUCED BY PROCESSING/STORAGE

3338 **Commercial Scale Pulsed Electric Field Processing of Tomato Juice**
*Seacheol Min, Z. Tony Jin, and Q. Howard Zhang**

Case No.: IPR2023-00208
U.S. Patent No. 11,013,250

3345 **Chemometric Studies of Vinegars from Different Raw Materials and Processes of Production**
Ramón Natera, Remedios Castro, María de Valme García-Moreno, María Jesús Hernández, and Carmelo García-Barroso*

3352 **Model Studies on the Stability of Folic Acid and 5-Methyltetrahydrofolic Acid Degradation during Thermal Treatment in Combination with High Hydrostatic Pressure**
*Minh Thuy Nguyen, Indrawati, and M. Hendrickx**

3358 **Thermal Decomposition of Specifically Phosphorylated D-Glucoses and Their Role in the Control of the Maillard Reaction**
Varoujan A. Yaylayan, David Machiels, and Louis Istasse*

3367 **Volatile Emissions of Navel Oranges as Predictors of Freeze Damage**
David M. Obenland, Louis H. Aung, David L. Bridges, and Bruce E. Mackey*

COMPOSITION OF FOODS/FEEDS

3372 **Anthocyanin Composition of Cabernet Sauvignon and Tempranillo Grapes at Different Stages of Ripening**
*José-María Ryan and Eugenio Revilla**

3379 **Effect of Selenium on the Yield and Quality of Green Tea Leaves Harvested in Early Spring**
*Qiuhui Hu, Juan Xu, and Genxin Pang**

3382 **Variation in Isoflavone of Soybean Cultivars with Location and Storage Duration**
*Sun Joo Lee, Joung Kuk Ahn, Seung Hyun Kim, Jung Tae Kim, Sang Jun Han, Mun Yhung Jung, and Ill Min Chung**

ENVIRONMENTAL CHEMISTRY

3390 **Kinetics for Substrate Utilization and Methane Production during the Mesophilic Anaerobic Digestion of Two Phases Olive Pomace (TPOP)**
Rafael Borja, Antonio Martín, Bárbara Rincón, and Francisco Raposo*

3396 **Dissipation of Sulfosulfuron in Soil and Wheat Plant under Predominant Cropping Conditions and in a Simulated Model Ecosystem**
Atmakuru Ramesh and Sinthalapadi Thulasiramaraja Maheswari*

3401 **Predicting Regional Emissions and Near-Field Air Concentrations of Soil Fumigants Using Modest Numerical Algorithms: A Case Study Using 1,3-Dichloropropene**
S. A. Cryer, I. J. van Wesenbeeck, and J. A. Knuteson*

3410 **Sulfentrazone Adsorbed on Micelle−Montmorillonite Complexes for Slow Release in Soil**
Tamara Polubesova, Shlomo Nir, Onn Rabinovitz, Mikhail Borisover, and Baruch Rubin*

FLAVORS AND AROMAS

3415 **Evaluation of Aroma Compounds Contributing to Muskmelon Flavor in Porapak Q Extracts by Aroma Extract Dilution Analysis**
Yasuyoshi Hayata, Takayuki Sakamoto, Chamorn Maneerat, Xinxian Li, Hiroshi Kozuka, and Koji Sakamoto*

3419 **Impact Odorants of Different Young White Wines from the Canary Islands**
*Ricardo López, Natalia Ortín, Juan Pedro Pérez-Trujillo, Juan Cacho, and Vicente Ferreira**

Case No.: IPR2023-00208
U.S. Patent No. 11,013,250

3426 **Aroma Compounds Formed from 3-Methyl-2,4-nonanedione under Photooxidative Conditions**
*Isabelle A. Sigrist, Giuseppe G. G. Manzardo, and Renato Amadò**

3429 **Analysis of Volatiles in Meat from Iberian Pigs and Lean Pigs after Refrigeration and Cooking by Using SPME-GC-MS**
*Mario Estévez, David Morcuende, Sonia Ventanas, and Ramón Cava**

3436 **Aroma Extract Dilution Analysis of Cv. Marion (*Rubus* spp. *hyb*) and Cv. Evergreen (*R. laciniatus* L.) Blackberries**
*Keith Klesk and Michael Qian**

3442 **Distribution of Volatile Compounds in the Pulp, Cloud, and Serum of Freshly Squeezed Orange Juice**
*Pierre Brat, Barbara Rega, Pascaline Alter, Max Reynes, and Jean-Marc Brillouet**

FOOD CHEMISTRY/BIOCHEMISTRY

3448 **Distribution of Hydroxycinnamic Acid Conjugates in Fruit of Commercial Eggplant (*Solanum melongena* L.) Cultivars**
Bruce D. Whitaker and John R. Stommel*

3455 **The Effect of SO_2 on the Production of Ethanol, Acetaldehyde, Organic Acids, and Flavor Volatiles during Industrial Cider Fermentation**
*Mónica Herrero, Luis A. García, and Mario Díaz**

3460 **Antiphoto-oxidative Activity of Sesamol in Methylene Blue- and Chlorophyll-Sensitized Photo-oxidation of Oil**
*Joo Yeon Kim, Dong Seong Choi, and Mun Yhung Jung**

3466 **Phaseolin In Vitro Pepsin Digestibility: Role of Acids and Phenolic Compounds**
*Mahesh Venkatachalam and Shridhar K. Sathe**

NUTRITIONAL CHEMISTRY

3473 **Assessment of Egg Nutrient Compositional Changes and Residue in Eggs, Tissues, and Excreta Following Oral Administration of Atorvastatin to Laying Hens**
Robert G. Elkin, Emily J. Furumoto, and Charles R. Thomas*

SAFETY/TOXICOLOGY

3482 **Substrate Suitability of Different Genotypes of Sorghum in Relation to *Aspergillus* Infection and Aflatoxin Production**
C. V. Ratnavathi and R. B. Sashidhar*

3493 **Repeated Oral Administration of High Doses of the Pomegranate Ellagitannin Punicalagin to Rats for 37 Days Is Not Toxic**
*Begoña Cerdá, José J. Cerón, Francisco A. Tomás-Barberán, and Juan Carlos Espín**

3502 **Fluorescence of Dietary Porphyrins as a Basis for Real-Time Detection of Fecal Contamination on Meat**
Kyle D. Ashby, Jin Wen, Pramit Chowdhury, Thomas A. Casey, Mark A. Rasmussen, and Jacob W. Petrich**

Case No.: IPR2023-00208
U.S. Patent No. 11,013,250

CORRECTION

3508 **Sulfite Analysis of Fruits and Vegetables by High-Performance Liquid Chromatography (HPLC) with Ultraviolet Spectrophotometric Detection**

R. F. McFeeters and A. O. Barish*

■ Supporting Information is available free of charge via the Internet at http://pubs.acs.org.

* In papers with more than one author, the asterisk indicates the name of the author to whom inquiries about the paper should be addressed.

Motif Exhibit 1056, Page 6 of 12

Case No.: IPR2023-00208
U.S. Patent No. 11,013,250

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

This material may be protected by Copyright law (Title 17 U.S. Code)

Aroma Extract Dilution Analysis of Cv. Marion (*Rubus* spp. *hyb*) and Cv. Evergreen (*R. laciniatus* L.) Blackberries

KEITH KLESK AND MICHAEL QIAN*

Department of Food Science & Technology, Oregon State University, 100 Wiegand Hall, Corvallis, Oregon 97331-6602

Cultivar Marion and Evergreen blackberry aromas were analyzed by aroma extract dilution analysis. Sixty-three aromas were identified (some tentatively) by mass spectrometry and gas chromatography–retention time; 48 were common to both cultivars, and 27 have not been previously reported in blackberry fruit. A comparison of cultivars shows that both have comparable compound types and numbers but with widely differing aroma impacts, as measured by flavor dilution (FD) factors. Ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, hexanal, furanones (2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone, 2-ethyl-4-hydroxy-5-methyl-3-(2*H*)-furanone, 4-hydroxy-5-methyl-3-(2*H*)-furanone, 4,5-dimethyl-3-hydroxy-2-(5*H*)-furanone, and 5-ethyl-3-hydroxy-4-methyl-2-(5*H*)-furanone), and sulfur compounds (thiophene, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, 2-methylthiophene, and methional) were prominent in Evergreen (FD 512–2048). Except for ethyl 2-methylpropanoate, these same compounds were also prominent in Marion, but the FD factors varied significantly (FD 8–256) from Evergreen. The aroma profile of blackberry is complex, as no single volatile was unanimously described as characteristically blackberry.

KEYWORDS: Marion; Evergreen; blackberry aroma; AEDA; GC/O; GC-MS

INTRODUCTION

Blackberries are popular fruits because of their flavor and nutritional content; they have been used as food and medicine for hundreds of years (*1*). Used fresh and processed into a variety of food products, blackberries are extensively cultivated; yet, their aroma compositions have not been detailed as compared to the aromas of other small fruits such as raspberry and strawberry (*2–6*). Independent analyses of blackberries examined anthocyanins (*1*), sugars and acids (*7*, *8*), and volatile and bound volatile compounds in fresh or processed blackberries (*9–17*). Most of the studies investigated volatile compounds (*9–14*) in the Evergreen cultivar. A total of 147 volatiles have been reported in fresh blackberries (*18*), but very few studies were about aroma active compounds, and few compounds were specifically described as "blackberry-like".

The Marion cultivar blackberry (*Rubus* spp. hyb) has a flavor greatly preferred by consumers; consequently, it has replaced cv. Thornless Evergreen (*Rubus laciniatus* Willd.) as the predominant cultivar planted in the Pacific Northwest (*19*, *20*). Consumer preference for the Marion has stimulated research to correlate quantifiable blackberry flavor characteristics to berry genetic makeup, to breed new thornless blackberry cultivars with Marion flavor. Because the aroma differences between Marion and Evergreen have been only subjectively described (*20*), the purpose of this investigation was to identify, rank, and compare the odor active compounds in the two cultivars using aroma extract dilution analysis (AEDA) and gas chromatography–mass spectrometry (GC-MS).

MATERIALS AND METHODS

Chemicals. Authentic aroma standards were obtained as follows: butyl acetate, limonene, octyl acetate, octyl formate, 2-heptanone, and 2-undecanone were from K&K Laboratories (Jamaica, NY). Methyl hexanoate and octanol were from Eastman (Rochester, NY). Acetaldehyde, acetic acid, β-ionone, butanoic acid, *l*-carvone, 2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone, dimethyl disulfide, dimethyltrisulfide, ethyl acetate, ethyl butanonate, ethyl hexanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl 2-methylpropanonate, eugenol, 2-heptanol, hexanal, hexanoic acid, *t*-2-hexenal, linalool, *p*-methylacetophenone, 3-methylbutanal, methyl butanoate, 2-methylbutanoic acid, nonanal, *t*-2-nonenal, octanol, 1-octen-3-ol, 1-octen-3-one, and phenethyl alcohol were from Aldrich Chemical Co. Inc. (Milwaukee, WI). Diacetyl and methional were from Sigma Chemical Co. (St. Louis, MO).

Blackberry Samples. Marion and Evergreen blackberries were grown in Woodburn, OR, from 5 to 10 year old plants. The fruits (both machine and hand-harvested) were washed, graded, individually quick frozen (IQF), and stored at −18 °C. One box of each cultivar (13.6 kg, frozen for 5 months) was transported on ice to the laboratory, where they were stored at −23 °C. Samples had been frozen for 9 months when analyzed.

Extraction of Volatile Compounds. For each cultivar, 1 kg of IQF blackberries was thawed at room temperature in a single layer for 3 h. The berries were combined with 100 g of NaCl and 10 g of $CaCl_2$ in a commercial blender and blended by pulsing for a total of 3 min at

* To whom correspondence should be addressed.Tel: 541 737 9114. Fax: 541 737 1877. E-mail: michael.qian@orst.edu.

10.1021/jf0262209 CCC: $25.00 © 2003 American Chemical Society
Published on Web 04/25/2003

Case No.: IPR2023-00208
U.S. Patent No. 11,013,250

Table 1. AEDA of Cv. Marion and Evergreen Blackberries (Stabilwax Column)

RI	compd[a]	aroma descriptors this study	basis of identification[b]	FD factors	
				Marion	Evergreen
811	ethyl acetate[r]	floral, fruity	MS, RI	16	2
910	3-methylbutanal	fresh grass, fruity, leaf	MS, RI		4
935	dimethyl sulfide[*,T]	garlic bologna, cabbage	RIL	16	128
965	ethyl 2-methylpropanoate[*]	sweet, fruity, berry, floral	RI		256
973	methyl butanoate	fruity, sweet	RI	4	
998	2,3-butanedione (diacetyl)[*,r]	buttery	MS, RI	2	2
1030	thiophene[*,T]	garlic bologna, sulfury	RIL	8	128
1038	ethyl butanoate	fruity, banana	MS, RI	4	
1053	ethyl 2-methylbutanoate[r]	fruity	MS, RI	4	
1065	ethyl 3-methylbutanoate	fruit, sweet, banana	RI		64
1082	butyl acetate	fruity, juicy	MS, RI	2	
1103	dimethyl disulfide[*]	vegetal	MS, RI	2	
1106	hexanal[r]	green, fresh	MS, RI		4
1161	**unk**	plastic, fatty, waxy		32	
1191	2-heptanone[r]	fruity, banana, sweet, floral	MS, RI	16	32
1202	methyl hexanoate[r]	fruity, green, sweet	MS, RI	8	4
1251	ethyl hexanoate[r]	fruity, floral	MS, RI	8	
1326	1-octen-3-one[*,r]	mushroom, earthy	RI	8	16
1333	2-heptanol[r]	woody, earthy, vegetal, minty	MS, RI	4	512
1369	hexanol[r,T]	floral, spice	MS, RIL	4	
1383	dimethyl trisulfide[*]	vegetal, garlic	MS, RI	2	16
1402	nonanal[r]	floral, fruity	MS, RI	2	
1467	acetic acid[r]	acid, sour	MS, RI	16	32
1476	1-octen-3-ol	mushroom	RI	4	
1490	methional[*]	potato, earthy, onion	RI	32	512
1491	octyl acetate	floral, sweet	MS, RI	4	
1508	theaspirane A[*,r,T]	floral, earthy, tea, green	MS, RIL	4	
1550	theaspirane B[*,r,T]	earthy, fruity, sweet	MS, RIL	4	
1560	linalool[r]	sweet, floral, berry, green	MS, RI	4	128
1574	octanol[r]	waxy, fruity	MS, RI	2	
1622	2-undecanone[r]	floral, grn, pine, citrus	MS, RI	8	128
1634	**unk**	roasted peanuts		2	
1650	butanoic acid[*,r]	rancid cheese, sour, pungent	MS, RI	32	
1693	2-methylbutanoic acid[*,r]	rancid cheese, sour, acid	MS, RI	32	128
1724	**unk**	plastic curtain, waxy			32
1749	*l*-carvone	peppermint, fresh leaf	MS, RI	8	4
1794	*p*-methylacetophenone	fresh, green, floral, fruity	MS, RI		32
1851	β-damascenone[r,T]	sweet, floral, grape, blackberry	MS, RIL	4	32
1868	hexanoic acid[*,r]	pungent, sour	MS, RI	2	128
1875	**unk**	waxy citrus, lemon, woody			32
1905	benzyl alcohol[r,T]	sweet, citrus, grass	MS, RIL	2	2
1952	phenethyl alcohol[r]	floral, perfume, peach	MS, RI	8	64
1967	**unk**	grass, pungent, green, floral			8
2017	cinnamic aldehyde[T]	sweet, spice, cinnamon	MS, RIL	4	
2018	**unk**	floral, grass, green			8
2053	2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone[*,r]	fruity, sweet, caramel	RI	8	1024
2078	2-ethyl-4-hydroxy-5-methyl-3-(2*H*)-furanone[*,T]	cooked bramble, sweet caramel	RIL	32	16
2114	4-hydroxy-5-methyl-3-(2*H*)-furanone[*,T]	caramel, strawberry, cooked bramble	RIL	32	8
2211	4,5-dimethyl-3-hydroxy-2-(5*H*)-furanone[*,T]	spice, curry, fruity	RIL	4	128
2246	5-ethyl-3-hydroxy-4-methyl-2-(5*H*)-furanone[*,T]	roasted meat, cumin, maple syrup	RIL	4	256
2294	cinnamic alcohol[r,T]	floral, tea, sweet, fruity	RIL	8	32

[a] *, not previously reported in blackberry; r, reported in red raspberry; T, tentative identification. [b] MS, mass spectral data; RIL, retention index from literature; RI, retention index from standards.

high speed. Calcium chloride was added to inhibit enzyme activity as described by Buttery and others (*21*). The puréed fruit was passed through a commercial stainless steel food mill to remove seeds. The seed pulp was batch-extracted three times with freshly distilled pentane: diethyl ether (1:1 v/v) while the seedless purée was extracted three times in a separatory funnel. The extracts were combined to yield a total volume of 880 mL. Nonvolatiles were removed from the organic extract using solvent-assisted flavor extraction (SAFE) at 50 °C under vacuum according to the method proposed by Engel and others (*22*). The organic SAFE extract was dried with anhydrous Na_2SO_4, concentrated to 1 mL by solvent distillation, and reduced to its final volume of 0.1 mL with a flow of nitrogen.

GC/O Analysis. The analysis was performed using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector (FID) and an olfactometer. Samples were analyzed on a Stabilwax column (30 m × 0.32 mm i.d. cross-linked poly(ethylene glycol), 1 μm film thickness, Restek Corp., Bellefonte, PA) and a DB-5 column (30 m × 0.32 mm i.d., cross-linked phenyl-methyl polysiloxane, 1 μm film thickness, J&W Scientific, Folsom, CA). The column effluent was split 1:1 (by volume) into the FID and a heated sniffing port with a fused silica outlet splitter (Alltech Associates, Inc., Deerfield, IL). Injector and detector temperatures were 250 °C. The helium column flow rate was 2.0 mL/min, and the 2 μL sample injections were splitless. The oven temperature was programmed for a 2 min hold at 40 °C,

Table 2. AEDA of Cv. Marion and Evergreen Blackberries (DB5 Column)

RI	compd[a]	aroma descriptors this study	basis of identification[b]	FD factors Marion	FD factors Eve
<500	acetaldehyde*,r	grass, green	MS, RI	1	8
516	dimethyl sulfide*,T	garlic, onion	RIL	16	8
557	2-methylpropanal*,T	wood, grass	MS, RIL	1	
579	2,3-butanedione (diacetyl)*,r	buttery	MS, RI	2	2
599	acetic acidr	acetic acid, vinegar	RI		1
609	methylethyl sulfide*,T	alliaceous, pungent	RIL	16	8
610	ethyl acetater	fruity	RI	1	
621	**unk**	pungent		8	4
649	3-methylbutanal	vegetal, earthy	MS, RI	1	1
661	thiophene*,T	sour, green, earthy, onion	RIL	4	2
727	dimethyl disulfide*	pungent, garlic, sulfury	MS, RI	32	2
753	ethyl 2-methylpropanoate*	fruity	RI		2
758	2-methylthiophene*,T	earthy, pungent	RIL	32	5
770	ethyl butanoate	fruity	MS, RI	2	
791	hexanalr	green, fresh	MS, RI	64	1
808	butanoic acid*,r	cheesy, pungent	RI	1	2
848	ethyl 2-methyl/3-methylbutanoater	fruity, sweet, berry, banana	RI	128	1
854	2-methylbutanoic acid*,r,T	cheesy, sour, smelly	RIL	32	1
874	*t*-2-hexenalr	fruity, orange, green	MS, RI	1	4
897	methional*	baked potato	RI	256	2
906	2-heptanolr	peppermint, green, woody	MS, RI	8	2
957	**unk**	woody, floral, green			1
959	benzaldehyder,T	fruity, berry, juicy	MS, RIL	64	2
971	1-octen-3-one*,r	mushroom, earthy	MS, RI	2	1
979	dimethyl trisulfide*	green veggie, garlic	RI	16	
980	1-octen-3-ol	woody, earthy, mushroom	RI		6
999	hexyl acetater	fruity	MS, RI	1	1
1002	ethyl hexanoater	floral, fruity	MS, RI	32	1
1033	limonenor	overripe melon, green, tea	MS, RI	4	2
1042	*t*-β-ocimene*,T	sweet, floral, woody, perfume	RIL	8	2
1045	benzyl alcoholr,T	floral, fruity, rose	MS, RIL	1	2
1072	2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone*,r	caramel, strawberry	RI	32	
1087	4-hydroxy-5-methyl-3-(2*H*)-furanone*,T	cotton candy, sweet	RIL	4	
1096	linaloolr	fruity, green, sweet, watermelon	MS, RI	16	8
1099	α-terpinolenor,T	woody, sweet, earthy	MS, RIL	1	6
1100	nonanalr	watermelon, citrus, floral	MS, RI	8	8
1104	octyl formate*	fruity	RI	1	
1112	phenethyl alcoholr	fruity, floral, rose, sweet	MS, RI		3
1131	4,5-dimethyl-3-hydroxy-2-(5*H*)-furanone*,T	roasted vegetables, sweet, caramel	RIL	32	4
1136	2-ethyl-4-hydroxy-5-methyl-3-(2*H*)-furanone*,T	floral, sweet, caramel	RIL	2	2
1149	neo-allo-ocimene*,T	citrus, vegetal, cucumber	MS, RIL	16	4
1161	*t*-2-nonenal*	watermelon, fresh vegetable, green	MS, RI	1	6
1179	*p*-methylacetophenone	floral, hot candy, sweet	RI	2	8
1234	*l*-carvone	anise, fennel	RI	2	
1255	5-ethyl-3-hydroxy-4-methyl-2-(5*H*)-furanone*,T	caramel, smoky	RIL	1	
1290	2-undecanoner	wet grass, tea, floral, green	MS, RI	4	1
1305	theaspirane A*,r,T	warm spices, vegetal, pungent	MS, RIL	2	1
1321	**unk**	caramel, fruity, tea, green			1
1324	4-vinylguaiacol*,r,T	BBQ rub, spicy	RIL	1	
1370	eugenolr	woody, citrus, spicy	RI	4	4
1392	β-damascenoner,T	floral, berry, sweet, grape	MS, RIL	8	4
1433	**unk**	sweet, fruity, herbal, tea		2	
1451	**unk**	floral, spice, perfume, fruit, juicy		4	
1496	β-iononer	floral, perfume, woody, spicy	MS, RI	2	1
1543	elemicinT	green tea, spicy, perfume	RIL	1	

[a] *, not previously reported in blackberry; r, reported in red raspberry; T, tentative identification. [b] MS, mass spectral data; RIL, retention index from lite retention index from standards.

then 40−100 °C at 5 °C/min, then 100−230 °C at 4 °C/min (10 min

GC-MS Analysis. Analysis of the original concentrate

Case No.: IPR2023-00208
U.S. Patent No. 11,013,250

Table 3. AEDA Summary of Cv. Marion and Evergreen Blackberries

cultivar	compd[a]	cultivar	compd[a]
	Acids		
both	acetic acid[r]	both	hexanoic acid[*,r]
both	butanoic acid[*,r]	both	2-methylbutanoic acid[*,r]
	Alcohols		
both	benzyl alcohol[r]	both	linalool[r]
both	cinnamic alcohol[r]	Marion	octanol[r]
Evergreen	heptanol[r]	both	1-octen-3-ol
both	2-heptanol[r]	both	phenethyl alcohol[r]
Marion	hexanol[r]		
	Aldehydes		
both	acetaldehyde[*,r]	both	methional[*]
both	benzaldehyde[r]	both	3-methylbutanal
Marion	cinnamic aldehyde	Marion	2-methylpropanal[*]
both	hexanal[r]	both	nonanal[r]
both	*t*-2-hexenal[r]	both	*t*-2-nonenal[*]
	Esters		
Marion	butyl acetate	Evergreen	ethyl 2-methylpropanoate[*]
both	ethyl acetate[r]	both	hexyl acetate[r]
Marion	ethyl butanoate	Marion	methyl butanoate
both	ethyl hexanoate[r]	both	methyl hexanoate[r]
both	ethyl 2-methylbutanoate[r]	Marion	octyl acetate
Evergreen	ethyl 3-methylbutanoate	Marion	octyl formate[*]
	Furanones		
both	2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone[*,r]	both	5-ethyl-3-hydroxy-4-methyl-2-(5*H*)-furanone[*]
both	4,5-dimethyl-3-hydroxy-2-(5*H*)-furanone[*]	both	4-hydroxy-5-methyl-3-(2*H*)-furanone[*]
both	2-ethyl-4-hydroxy-5-methyl-3-(2*H*)-furanone[*]		
	Hydrocarbons		
both	limonene[r]	both	*t*-β-ocimene[*]
both	neo-allo-ocimene[*]	both	α-terpinolene[r]
	Ketones		
both	2,3-butanedione (diacetyl)[*,r]	both	β-ionone[r]
both	*l*-carvone	both	*p*-methylacetophenone
both	β-damascenone[r]	both	1-octen-3-one[*,r]
both	2-heptanone[r]	both	2-undecanone[r]
	Phenols		
Marion	elemicin	Marion	4-vinylguaiacol[*,r]
both	eugenol[r]		
	Sulfur		
both	dimethyl disulfide[*]	both	methylethyl sulfide[*]
both	dimethyl sulfide[*]	both	2-methylthiophene[*]
both	dimethyl trisulfide[*]	both	thiophene[*]
	Theaspiranes		
both	theaspirane A[*,r]	Marion	theaspirane B[*,r]

[a] *, not previously reported in blackberry; r, reported in red raspberry.

film thickness (Restek) and the other a 30 m × 0.25 mm i.d. DB-5 (cross-linked phenyl-methyl polysiloxane) column with a 0.25 μm film thickness (J&W Scientific). The helium column flow rate was 2.0 mL/min, and the 2 μL sample injections were splitless. The oven temperature was programmed as for the GC/O analysis. Injector, detector transfer line, and ion source temperatures were 250, 280, and 230 °C, respectively. Electron impact mass spectrometric data from *m/z* 35−300 was collected at 5.27 scans/s, at an ionization voltage of 70 eV. RI were estimated in accordance with a modified Kovats method (*23*). Compound identifications were made by comparing aromas with authentic standards and Kovats RI, RI reported in the literature (*25*, among others), and/or mass spectral data from the Wiley 275.L (G1035) Database (Agilent Technologies, Inc.).

Further Identification of Some Aroma Compounds by GC-MS Analysis. To further clarify AEDA volatile composition, for each cultivar, 5 kg of IQF blackberries was thawed at room temperature in a single layer for 3 h. The berries were blended by pulsing for a total of 3 min at high speed in a commercial blender, and the purée was poured into a stainless steel pan. Concentrated pectolytic enzyme (Vinozym FCE G, Novo Nordisk, Franklinton, NC) was prepared and thoroughly mixed into the purée. A total of 0.15 g of enzyme were added to the Marion purée, and 1.0 g was added to the more viscous Evergreen purée. The mixture was covered with aluminum foil and left to stand at room temperature overnight (15 h). Five hundred grams of NaCl was blended in, and the mixture was strained and extracted as for the GC/O analysis using CH_2Cl_2 (total volume 2400 mL). The extraction produced an emulsion that was broken with centrifugation for 20 min (1800 rpm, approximately 1000 g). The organic extract was then further prepared as for the GC/O analysis and reduced to its final volume of 0.2 mL with a flow of nitrogen. Analysis conditions and methods were identical to those used for the 1 kg samples, except that 5 μL of sample was injected, and the oven temperature was programmed for a 2 min hold at 40 °C, then 40−230 °C at 1 °C/min (2 min hold).

RESULTS AND DISCUSSION

Tables 1 and **2** list Marion and Evergreen blackberry volatiles separated with polar and nonpolar columns. On the polar

Case No.: IPR2023-00208
U.S. Patent No. 11,013,250

column, a total of 51 aroma compounds were detected, with 45 of them identified. On the nonpolar column, 55 aromas were detected, and 51 of them were identified. Among these identified aromas, 12 were detected on the polar column only, while 17 were detected on the nonpolar column only. Combined data (**Table 3**) show that 63 odor active volatiles were detected and 48 were common to both cultivars. Marion contained 60 of 63 volatiles, and Evergreen contained 51.

The most significant (FD ≥ 16) odor active volatiles in Marion determined on the nonpolar (DB-5) column were methional (FD = 256); ethyl 2-methylbutanoate (FD = 128); benzaldehyde and hexanal (FD = 64); 2-methylbutanoic acid, 2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone, 4,5-dimethyl-3-hydroxy-2-(5*H*)-furanone, ethyl hexanoate, dimethyl disulfide, and 2-methylthiophene (FD = 32); and linalool, neo-allo-ocimene, dimethyl sulfide, dimethyl trisulfide, and methylethyl sulfide (FD = 16). In addition, 2-ethyl-4-hydroxy-5-methyl-3-(2*H*)-furanone, 4-hydroxy-5-methyl-3-(2*H*)-furanone, and butanoic acid (FD = 32), ethyl acetate, acetic acid, and 2-heptanone (FD = 16) may also be important to Marion blackberry flavor, as they had high FD factors as determined on the polar (Stabilwax) column.

Many significant (FD ≥ 16) odor active volatiles in Evergreen were identified on the DB-5 column. The most important aroma compounds included methional, ethyl 2-methylpropanoate, thiophene, and dimethyl disulfide (FD = 2048); hexanal and ethyl 2-methylbutanoate (FD = 1024); 2-methylthiophene (FD = 512); benzaldehyde (FD = 256); heptanol (FD = 128); 1-octen-3-ol, *t*-2-nonenal, and α-terpinolene (FD = 64); phenethyl alcohol (FD = 32); and 1-octen-3-one, 2-undecanone, acetic acid, 2-methylbutanoic acid, and β-ionone (FD = 16).

In addition, as determined on the polar (Stabilwax) column, 2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone (FD = 1024); 2-heptanol (FD = 512); ethyl 2-methylpropanoate and 5-ethyl-3-hydroxy-4-methyl-2-(5*H*)-furanone (FD = 256); thiophene, linalool, 2-undecanone, hexanoic acid, 4,5-dimethyl-3-hydroxy-2-(5*H*)-furanone and dimethyl sulfide (FD = 128); ethyl 3-methylbutanonate (FD = 64); cinnamic alcohol, 2-heptanone, *p*-methylacetophenone, and β-damascenone (FD = 32); and 2-ethyl-4-hydroxy-5-methyl-3-(2*H*)-furanone, 1-octen-3-one, and dimethyl trisulfide (FD = 16) may also be important to Evergreen blackberry flavor.

The cultivars have comparable compound types and numbers but with widely differing aroma impacts, as measured by FD factors. Fresh Marion blackberry aroma has been described as floral, fruity, sweet, caramel-fruity, and woody, while fresh Evergreen aroma is spicy, green, herbaceous, fruity, and sweet. However, there are no prominent corresponding compositional differences between the cultivars within a volatile class. Both cultivars contain the same numbers of odor active acids, furanones, hydrocarbons, ketones, and sulfur compounds. The Marion contains one more theaspirane (theaspirane B); two more alcohols (hexanol, octanol), aldehydes (cinnamic, 2-methylpropanal), and phenols (elemicin, 4-vinylquaiacol); and five more esters (methyl butanoate, ethyl butanoate, butyl acetate, octyl acetate, and octyl formate) than Evergreen. The Evergreen has one alcohol (heptanol) and two esters (ethyl 2-methylpropanoate, ethyl 3-methylbutanoate) not present in Marion. Of 27 newly reported volatiles, three organic acids, two aldehydes, five furanones, two hydrocarbons, two ketones, six sulfur compounds, and one theaspirane are shared by the cultivars. This relatively large number of new volatiles is probably due to the extraction and analytical methods used. It is thought that some portion of Marion aroma is due to its hybrid pedigree, which contains at least five bramble species, including raspberry (*20*). However, although 35 volatiles in this study have been previously reported in red raspberry (*12*, *18*, *26*, *27*), 30 of them are common to both Marion and Evergreen; only four are unique to Marion. Five volatiles out of 63 were described with aroma descriptors specific to bramble fruit (berry, blackberry); no single compound was unanimously described as characteristically blackberry.

AEDA is a suitable method to screen potent odorants in blackberry, and results indicate that characteristic blackberry aroma is apparently a complex formulation of volatiles. Marion and Evergreen blackberries have many potent odorants in common, but qualitative aroma comparisons consistently note the more floral, caramel-fruity, sweet aroma of Marion as compared to the spicy, herbaceous, less fruity aroma of Evergreen. Because a FD factor is the ratio of an odorant's concentration in an initial GC/O extract to its concentration in the most dilute extract that still allows detection, the value is a relative measure (*28*) and does not conclusively determine that one cultivar contains more of a given aroma compound than another. Because the aroma profile of a food is, among others, a function of volatile concentrations and odor thresholds, the next step in identifying specific aroma differences between Marion and Evergreen is the quantification of each aroma with a high FD factor and calculation of its odor activity value (OAV), the ratio of the aroma concentration to its odor threshold in air. OAVs are better measures of which aroma compounds contribute to a cultivar's aroma and of the differences in cultivar aroma profiles.

ACKNOWLEDGMENT

IQF Marion and Evergreen blackberries were donated by Townsend Farms (Fairview, OR).

LITERATURE CITED

(1) Mazza, G.; Miniati, E. Small Fruits. In *Anthocyanins in Fruits, Vegetables, and Grains*; Mazza, G., Miniati, E., Eds.; CRC Press: Boca Raton, Florida, 1993; p 85.

(2) Honkanen, E.; Hirvi, T. The Flavour of Berries. In *Food Flavours Part C. The Flavour of Fruits*; Morton, I. D., Macleod, A. J., Eds.; Elsevier Science Publishers B. V.: New York, New York, 1990; pp 147−158.

(3) Shamaila, M.; Skura, B.; Daubeny, H.; Anderson, A. Sensory, chemical and gas chromatographic evaluation of five raspberry cultivars. *Food Res. Int.* **1993**, *26*, 443−449.

(4) Zabetakis, I.; Holden, M. A. Strawberry flavor: analysis and biosynthesis. *J. Sci. Food Agric.* **1997**, *74*, 421−434.

(5) De Ancos, B.; Ibanez, E.; Reglero, G.; Pilar, C. M. Frozen storage effects on anthocyanins and volatile compounds of raspberry fruit. *J. Agric. Food Chem.* **2000**, *48*, 873−879.

(6) Hakala, M. A.; Lapvetelaeinen, A. T.; Kallio, H. P. Volatile compounds of selected strawberry varieties analyzed by purge-and-trap headspace GC-MS. *J. Agric. Food Chem.* **2002**, *50*, 1133−1142.

(7) Wrolstad, R. E.; Culbertson, J. D.; Nagaki, D. A.; Madero, C. F. Sugars and nonvolatile acids of blackberries. *J. Agric. Food Chem.* **1980**, *28*, 553−558.

(8) Plowman, J. E. Sugars and acids of raspberries, blackberries, and other brambles. *Lebensm.-Wiss. Technol.* **1991**, *24*, 113−115.

(9) Scanlan, R. A.; Bills, D. D.; Libbey, L. M. Blackberry flavor components of commercial essence. *J. Agric. Food Chem.* **1970**, *18*, 744.

Case No.: IPR2023-00208
U.S. Patent No. 11,013,250

(10) Houchen, M.; Scanlan, R. A.; Libbey, L. M.; Bills, D. D. Possible precursor for 1-methyl-4-isopropenylbenzene in commercial blackberry flavor essence. *J. Agric. Food Chem.* **1972**, *20*, 170.

(11) Gulan, M. P.; Veek, M. H.; Scanlan, R. A.; Libbey, L. M. Compounds identified in commercial blackberry essence. *J. Agric. Food Chem.* **1973**, *21*, 741.

(12) Georgilopoulos, D. N.; Gallois, A. N. Aroma compounds of fresh blackberries (*Rubus laciniata* L.). *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 374−380.

(13) Georgilopoulos, D. N.; Gallois, A. N. Volatile flavour compounds in heated blackberry juices. *Z. Lebensm. Unters. Forsch.* **1987**, *185*, 299−306.

(14) Georgilopoulos, D. N.; Gallois, A. N. Flavour compounds of a commercial concentrated blackberry juice. *Food Chem.* **1988**, *28*, 141−148.

(15) Humpf, H.; Schreier, P. Bound aroma compounds from the fruit and the leaves of blackberry (*Rubus laciniata* L.). *J. Agric. Food Chem.* **1991**, *39*, 1830−1832.

(16) Herrmann, K. The flavor compounds of fruit. V. berries (without strawberries). *Erwerbsobstbau* **1992**, *34*, 168−172.

(17) Li, W.; He, S.; Gu, Y.; Song, C. Study on volatile constituents in fruit of blackberry (*Rubus sp.*). *Zhongguo Yaoxue Zazhi* **1998**, *33*, 335−336 (in Chinese).

(18) Nijssen, L. M.; Visscher, C. A.; Maarse, H.; Willemsens, L. C.; Boelens, M. H. Fruits. In *Volatile Compounds in Food, Qualitative and Quantitative Data*, 7th ed.; Nijssen, L. M., Visscher, C. A., Maarse, H., Willemsens, L. C., Boelens, M. H., Eds.; TNO Nutrition and Food Research Institute: Zeist, The Netherlands, 1996; pp 15.1−15.16.

(19) Strik, B. C. Blackberry cultivars and production trends in the Pacific Northwest. *Fruit Var. J.* **1992**, *46*, 202−206.

(20) Finn, C.; Strik, B. C.; Lawrence, F. J. 'Marion' trailing blackberry. *Fruit Var. J.* **1997**, *51*, 130−133.

(21) Buttery, R. G.; Teranishi, R.; Ling, L. C. Fresh tomato aroma volatiles: a quantitative study. *J. Agric. Food Chem.* **1987**, *35*, 540−544.

(22) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation − a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrixes. *Z. Lebensm. Unters. Forsch.* **1999**, *209*, 237−241.

(23) Van den Dool, H.; Kratz, P. D. A generalization of the retention index system including linear temperature programmed gas−liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463−471.

(24) Schieberle, P.; Grosch, W. Evaluation of the flavour of wheat and rye bread crusts by aroma extract dilution analysis. *Z. Lebensm. Unters. Forsch.* **1987**, *185*, 111−113.

(25) Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; Deutsche Forschungsanstalt für Lebensmittelchemie and Institut für Lebensmittelchemie der Technischen Universität Munchen: Garching, Germany, 1998; pp 6−53.

(26) Fenaroli, G. Raspberry. In *Fenaroli's Handbook of Flavor Ingredients*, 3rd ed.; Burdock, G. A., Ed.; CRC Press: Boca Raton, Florida, 1995; Vol. II, pp 827−828.

(27) Roberts, D. D.; Acree, T. E. Effects of heating and cream addition on fresh raspberry aroma using a retronasal aroma simulator and gas chromatography olfactometry. *J. Agric. Food Chem.* **1996**, *44*, 3919−3925.

(28) Grosch, W. Review: Determination of potent odorants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values (OAVs). *Flavour Fragrance J.* **1994**, *9*, 147−158.

Received for review December 13, 2002. Revised manuscript received February 10, 2003. Accepted February 23, 2003. Research funding provided by a Small Fruit Research Grant from the Northwest Center for Small Fruits Research, through a USDA/CSREES Special Research Grant.

JF0262209