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Procedia Engineering 37 (2012) 240 – 249

Procedia Engineering

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The Second SREE Conference on Engineering Modelling and Simulation (CEMS 2012)

Analysis of Volatile Components from *Dictyophora rubrovolota Zang, ji et liou*

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Abstract

Volatile compounds extracted by simultaneous distillation and extraction (SDE) from *Dictyophora rubrovolota Zang, ji et liou* were analyzed by gas chromatography-mass spectrometry (GC-MS), and the aroma-active volatiles were identified by aroma extract dilution analysis (AEDA) method with gas chromatography-olfactometry (GC-O). 82 volatile components were identified by GC-MS, including 11 aldehydes, 10 ketones, 6 alcohols, 2 hydroxybenzenes, 9 esters, 19 acids, 14 hydrocarbons, and 11 other compounds. By GC-O analysis, 22 aroma-active compounds were identified, among which seven key flavor volatiles with high flavour dilution factor (FD) ranging from 27 to 3 included 2,3-pentanedione (FD 27，0.074 mg/kg , yogurt flavor), acetic acid (FD 27, 12.72 mg/kg, sharp acidity), 2-methylbutanoic acid (FD 27, 1.039 mg/kg, smelly socks smell, aldehyde taste), (E)-2-octenal (FD 9, 0.066 mg/kg, pine oil odour), 2-phenyl-2-butenal (FD 9, 0.12 mg/kg, astringent taste, aldehyde flavor, fragrant beans), benzaldehyde (FD 3, 0.136mg/kg, formaldehyde smell, resin taste), 3,5-diethyl-2-methyl-pyrazine (FD 3, 0.082 mg/kg, musty, bark corrupt taste, smelly).

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Keywords: *Dictyophora rubrovolota Zang, ji et liou*; volatile components; GC-MS; GC-O; AEDA

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doi:10.1016/j.proeng.2012.04.234

1. Introduction

Dictyophora rubrovolota Zang, ji et liou is a genus of *dictyophora indusiata* [1,2], which in China are also named as "Zhu sun". The wild *Dictyophora rubrovolota Zang, ji et liou* mainly grows in humus of bitter bamboo forest in Guizhou province, Yunnan province and Sichuan province [1]. There are eleven kinds of *dictyophora indusiata* reported in the world, and seven are growing in China. Meanwhile, in china there are only four *dictyophora indusiatas* in market which are often used as a food for its delicious taste, rich nutrition and unique flavor[3,4], for they has been cultivated successfully and made cheaper and more available. They are *Dictyophora indusiata* Fisch, *Dictyophora duplicata (Bosc) E. Fish*, *Dictyophora Echinovolvata Zang, Zheng et Hu*, *Dictyophora rubrovolota Zang, ji et liou*. Before, we have researched *Dictyophora Echinovolvata Zang, Zheng et Hu*, now the flavor of *Zheng et Hu*, *Dictyophora rubrovolota Zang, ji et liou* would be researched.

At present, the researches about *Dictyophora rubrovolota* are mainly involved in the aspects of cultivation [5-7], nutrient components and its efficacy [8, 9], culture preservation & distribution characteristics [1, 2], polysaccharides extraction & the antitumor effect of poilsaccharides [10,11]. However, the aroma-active compounds of extracts of *Dictyophora rubrovolota* have not been reported. It interests us to investigate the aroma-active components of *Dictyophora rubrovolota* since those dishes made of them usually present a very specific and delicate flavor. Herein, the volatile components of *Dictyophora rubrovolota* were extracted by SDE and analyzed by GC-MS, the aroma-active compounds among which were identified by AEDA and GC-O.

2. Materials and Methods

2.1. Materials and Chemicals

Dried embryo of *Dictyophora rubrovolota Zang, ji et liou* were purchased from the local market of Guiyang city, Guizhou province, and was identified by Kunming Institute of Botany, Chinese Academy of Science, Kunming. The voucher specimen number was Huang Mingquan 2011-02.

Dichloromethane (anhydrous, 99.5%), diethyl ether (99.8%) and sodium carbonate (anhydrous) were purchased from Sinopharm Chemical Reagent Co. Ltd (Beijing, China). n-alkanes (C_7-C_{30}) and the reference compounds listed in Table 1 were purchased from Sigma and Aldrich (Steinheim, germany).

2.2. Isolation of Volatiles by SDE

The Likens-Nickerson apparatus was used to extract the dried embryo of *Dictyophora rubrovolota Zang, ji et liou*. 35 g of dried *Dictyophora rubrovolota Zang, ji et liou* were placed in 2000mL round bottom flask mixed with 1L distilled water. The distillation was performed for two times using the same solvent, each time for 3h (an experience value) after refluxing with fresh solvent of 90mL. Dichloromethane and diethyl ether were used respectively in this experiment as extraction solvent. Then the two extracts were combined, dried over by anhydrous sodium sulfate (25g) for 12h, then filtrated by common glass filler and concentrated to approximately 0.5 mL by a revolving evaporator (not heated), then the sample was preserved for analysis in refrigerator with the temperature of -20℃.

2.3. Analysis by GC-MS

Mass spectra data were obtained with an Agilent 6890N-5973i and Thermo Trace GC Ultra-DSQII. The corresponding analysis conditions were as the followings.

Agilent 6890N-5973i: electron ionization source, 70eV, ion source temperature 230℃; electric quadrupole 150℃; transfer line temperature 250℃; full scan, mass range 29-450; capillary column DB-Wax (30m×0.25mm×0.25μm); oven temperature 35℃ for 1min, with 18℃/min to 53℃, retaining 12min, then 5℃/min to 57℃, retaining 3min, then 4℃/min to 100℃, retaining 6min, and then 5℃/min to 150℃, retaining 6min, 7℃/min to 220℃, retaining 13.5℃, final 6℃/min to 240℃, retaining 15min; carrier gas, helium (99.999%); constant flow rate 1.0mL/min; injector temperature, 250℃; splitless; injector volumn,1μL; solvent delay,5.6min.

Thermo Trace GC Ultra-DSQII: ion source temperature 250℃; capillary column RTX-5 (30m×0.25mm×0.25μm); oven temperature 35℃ for 1min, with 18℃/min to 53℃, retaining 5min, then 2℃/min to 100℃, then 6℃/min to 186℃, retaining 6min, final 8℃/min to 280℃, retaining 5min; injector temperature, 260℃; solvent delay, 3.3min; the others as the same above.

Compounds were identified by first comparing their mass spectra with NIST 2008 mass spectra database and then comparing the RI (retention index) values relative to C_7-C_{30} n-alkanes with the published. When accessible, identifications were further confirmed with authentic chemicals.

The amounts of some compounds which had gained authentic chemicals were calculated by external standard methods in GC, the others were getted in GC by relating peak areas to that of pentadecane (2.32 mg/mL, diethyl ether as solvent) internal standard using a response factor of 1. Every amount was the average obtained by three analyses.

2.4. Odor Analysis by GC-O

GC-O analysis was carried out on an Agilent 6890N GC coupled with a capillary column DB-Wax (30m×0.25mm×0.25μm).The SDE concentrates (1μL) were injected at 250℃ in splitless mode; Oven temperature and carrier gas were identical to those of GC-MS analysis. The column effluent was divided (1:1) between the flame ionization detector (FID) and the olfactometer (Sniffer 9000, Brechbuhler Scientific Analytical Solutions INC, Switzerland) by one 'Y'shape glass splitter. The effluent to the odor port was enclosed with a stream of humidified air of 10mL/min and transferred to the glass detection cone by one length of capillary at the temperature of 220℃.

Five trained assessors, including two flavourists, were selected to smell and record the odor descriptions for the GC-O experiment following the way of Guillard, et al [12]. Retention times of the odor responses were converted into retention index values using the retention time of a series of n-alkanes (C_7-C_{30}).

3. Results and Discussion

3.1. SDE/GC-MS Analysis

Simultaneous distillation and extraction apparatus was used to extract the volatiles from *Dictyophora rubrovolota Zang, ji et liou* for the reason that the method of simultaneous distillation and extraction (SDE) is an exhaustive extraction method, and a 'total volatile analysis' result can be obtained[13,14]. Moreover, the method of SDE can simulate the cooking process of delicacies in which *dictyophora indusiata* is an indispensible ingredient, and ensures that the volatiles generated and extracted are the natural flavors from delicacies made of *dictyophora indusiata*. With GC-MS, identification and quantitative analysis of volatile compounds extracted by SDE from *Dictyophora rubrovolota Zang, ji et liou* were present in table **1**.

Eighty two volatile components were identified in total, including 11 aldehydes, 10 ketones, 6 alcohols, 2 hydroxybenzenes, 9 esters, 19 acids, 14 hydrocarbons, and 11 other compounds. Among these

identifided volatiles, the prominent components were (Z,Z)-9,12- octadecadienoicacid (132.3 mg/kg), n-hexadecanoic acid (42.10 mg/kg), pentadecanoic acid (36.20 mg/kg), acetic acid (12.72 mg/kg), and so on.

19 acids were found in this experiment and the total content of the acids was 241.5 mg/kg, representing 88.10% of 82 volatiles and ranking the predominant. Among the volatile acids, (Z, Z)-9, 12-octadecadienoic acid (132.3 mg/kg) was the largest followed by n-hexadecanoic acid (42.1 mg/kg), pentadecanoic acid (36.2 mg/kg), et al. These short carbon-chain acids and long carbon-chain acids were in possible respectively derived from the oxidation of corresponding aldehydes and hydrolysis of corresponding lipids during the extraction.

Ten ketones were identified and the total amount was 16.034mg/kg, accounting 5.85% of the all identified compounds. Acetion was the largest (12.20 mg/kg) followed by natural musk ambrette (2.337 mg/kg), 4-hydroxy-2-butanone (0.823 mg/kg), and coffee furanone (0.235 mg/kg), et al. Albaflavenone, a sesquiterpene antibiotic, is secondary found in *dictyophora indusiata* after we have firstly seperated and identified it from *Dictyophora indusiata (Vent:Pers.) Fischer,* which will be reported in the other journal. The other ketones may be formed by the oxidative degradation of unsaturated fatty acid, for instance, the oxidative decomposition of linoleic acid[15]. Acetion may be come from the degradation of glycogen [16]. Natural musk ambrette (oxacycloheptadec-8-en- 2-one) was a marcolactone , it may be produced by the lactonization of ambrettolic acid or derived from the acid of 6, 16-dihydroxyhexadecanoic acid which could dehydrate into the isoambrettolic aic[17]. Coffee furanone was probably generated by Maillard reaction during the SDE [18].

Eleven aldehyde compounds whose total content was 4.267 mg/kg were identified from the SDE extract, representing 1.56% of the identified volatiles. The furfural (1.815 mg/kg) was the largest followed benzeneacetaldehyde (0.925 mg/kg), hexanal (0.479 mg/kg) , heptanal (0.365 mg/kg), et al. Aroma aldehydes (benzeneacetaldehyde, benzaldehyde and 2-phenyl-2-butenal) are possibly connect to amino acid degradation [19], e.g benzeneacetaldehyde that was a Strecker aldehyde from 2-phenylalanine degradation and had also been detected in many SDE extract[4] and was produced by heating [20]. The aliphalic aldehydes including saturated and unsaturated aldehydes may be arise from the oxidation degradation of unsaturated fatty acids. For example, hexanal may come from the oxidative decomposition of linoleic acid [15, 21].

Six alcohols were found, representing 0.77% of the identified volatiles with the total content of 2.111 mg/kg. n-Bisabolol (0.829 mg/kg) was the largest followed by 2,3-butanediol (0.396 mg/kg), phenylethyl alcohol (0.378 mg/kg), nerolidol (0.203 mg/kg), et al. n-Bisabolol and nerolidol might be characteristic products of the metabolism of *Dictyophora rubrovolota Zang, ji et liou* during growing-up, and n-bisabolol did not be found in *Dictyophora indusiata Fisch* [4] and *Dictyophora echinovolvata Zang, Zheng et Hu* [22-24].

Nine esters were identified from the SDE extract in total, the content of esters was 2.156 mg/kg and the proportion was 0.79%, with 1-methoxy-2-propyl acetate (0.431 mg/kg) as the largest followed 9,12-hexadecadienoicacid methyl ester (0.361 mg/kg), 9,12-octadecadienoicacid ethyl ester (0.319 mg/kg), ethyl 9-hexadecenoate (0.282 mg/kg), et al. These esters can be come from the interaction of alcohols and free carboxylic acids which can be derived from decomposition of lipids.

Six nitrogen compounds including 5 pyrazines and 1 pyrrole were found from the SDE extract, they were tetramethyl pyrazine (1.893 mg/kg), trimethyl pyrazine (0.501 mg/kg), 2-acetylpyrrole (0.472 mg/kg), 2,3,5-trimethyl-6-ethylpyrazine (0.085 mg/kg), 3,5-diethyl-2-methylpyrazine (0.082 mg/kg) and 2,3-dimethylpyrazine (0.075 mg/kg), and these six nitrogen compounds accounts for 1.13% of 82 volatiles. Nitrogen compounds such as 2-acetylpyrrole might result from Maillard reaction [18] and it have been reported that *Dictyophora rubrovolota* riches in amino acid, protein, polysaccharide, vitamine [9] which are the critical ingredients for Maillard reaction.

Above all, One sulfur compound (dibenzothiophene. 0.766 mg/kg) was found and this sulfur compound may be derived from sulfur-containing amino acid, such as cysteine [25]. 11 aliphalic hydrocarbons and 3 aromatic hydrocarbons, fourteen hydrocarbons were found in total by SDE/GC-MS, accounting for 0.80% of the identified compounds with the content of 2.197mg/kg. Four oxygen compounds were found, among these, 1,1-diethoxy ethane (1.211 mg/kg) was the largest. Two hydroxybenzenes were found, including phenol (0.111 mg/kg) and 2, 4-bis (1, 1-dimethyl)-phenol (0.027 mg/kg), representing 0.05% of the 82 volatiles.

Table 1 Volatile components identified in Dictyophora rubrovolota Zang, ji et liou extracted by SDE and analyzed by GC-MS

Retention Index		Compounds	Amount (mg/kg)	[b]Identification method
DB-Wax RI/[a]RI	RTX-5 RI/RT(Refer.)			
Aldehydes	Subtotal		4.267	
1049/1064		hexanal	0.479	MS,RI,S
1149/1182	908/901[26]	heptanal	0.365	MS,RI
1381/1377	1062/1063[27]	(E)-2-octenal	0.066	MS,RI,S
1406/1447	848/848[28]	furfural	1.815	MS,RI,S
1453/1496		benzaldehyde	0.136	MS,RI,S
1511	971.3/972[29]	5-methyl-furfural	0.144	MS,RI
1580/1623	1052/1051[30]	benzeneacetaldehyde	0.925	MS,RI,S
1711/1710	1327/1325 [31]	(E,E)-2,4-decadienal	0.113	MS,RI,S
1871	1281/1281[28]	2-phenyl-2-butenal	0.120	MS, RI
	1106/1108[32]	nonanal	0.046	MS,RI,S
	1299	2,4-nonadienal	0.058	MS
Ketones	Subtotal		16.03	
1028/1056		2,3-pentanedione	0.074	MS,RI,S
1230		coffee furanone	0.235	MS,S
1253/1286	749	acetoin	12.20	MS,RI,S
1440/1497		2-acetyl furan	0.046	MS,RI
1531		4-hydroxy-2-butanone	0.823	MS
1560		butyrolactone	0.100	MS
1980		dihydro-5-pentyl-2(3H)-furanone	0.111	MS
	1465	2,3-dimethylchromone	0.057	MS
2173	1713	albaflavenone	0.051	MS, S
2982	2060	natural musk ambrette	2.337	MS
Alcohols	Subtotal		2.111	
1262		acetone alcohol	0.122	MS
1457		2,3-butanediol	0.396	MS
1607/1573		2-furanmethanol	0.183	MS,RI,S
1852	1123/1120[33]	phenylethyl alcohol	0.378	MS,RI,S

2014/2050		nerolidol	0.203	MS,RI,S
2192	1700	nbisabolol	0.829	MS
Esters	Subtotal		2.156	
1342	900	2,3-butanediol diacetate	0.092	MS
1516	940	1-methoxy-2-propyl acetate	0.431	MS
1565	1057	2-methyl-propanoic acid, ethyl ester	0.100	MS
2203	1900/1901[34]	hexadecanoic acid, methyl ester	0.053	MS,RI
2277		ethyl 9-hexadecenoate	0.282	MS
2477	2113	(E,E)-9,12-octadecadienoic acid, methyl ester	0.274	MS
	946	3-hydroxy-butanoic acid ethyl ester	0.244	MS
	2094	9,12-hexadecadienoic acid, methyl ester	0.361	MS
	2178	9,12-octadecadienoic acid, ethyl ester	0.319	MS
Acids	Subtotal		241.5	
1385/1435	711/660[35]	acetic acid	12.72	MS,RI,S
1513		2-methyl-propanoic acid	0.288	MS
1576		butanoic acid	0.114	MS
1617		2-methyl-butanoic acid	1.039	MS,S
1784		hexanoic acid	0.217	MS,S
2027/2075	1208/1201[28]	octanoic acid	0.357	MS,RI,S
2139/2177	1297/1297[32]	nonanoic acid	0.118	MS,RI
2232		3-nonenoic acid	0.040	MS
2240		n-decanoic acid	0.081	MS
2401		undecylenic acid	0.138	MS
2450	1580/1580[27]	dodecanoic acid	0.385	MS,RI
2498		cis-5-dodecenoic acid	0.277	MS
2556	1674/1678[27]	tridecanoic acid	0.278	MS,RI
2672	1778/1777[36]	tetradecanoic acid	9.144	MS,RI
2795	1900	pentadecanoic acid	36.20	MS
2838		14-pentadecenoic acid	4.803	MS
2909	2054	n-hexadecanoic acid	42.10	MS,S
3000		oleic acid	0.864	MS,S
	2228/2236[37]	(Z,Z)-9,12-octadecadienoic acid	132.3	MS,RI
Hydrocarbons	Subtotal		2.197	
1091/1122	860	ethylbenzene	0.150	MS,RI
1105	871	p-xylene	0.161	MS
2685	1821	phenanthrene	1.021	MS
	894.6[38]	nonane	0.117	MS,RI,S
	992[38]	decane	0.100	MS,RI,S

	1192[38]	dodecane	0.166	MS,RI,S
	1203/1213[39]	2,6-dimethy undecane	0.022	MS,RI
	1292	tridecane	0.047	MS,RI,S
	1415	6-dodecene	0.018	MS
	1428/1422[40]	(+)-β-cedrene	0.007	MS,RI
	1448	calarene	0.097	MS
	1540	cis-α-bisabolene	0.092	MS
	1655	1,E-11,Z-13-octadecatriene	0.137	MS
	1690	heptadecane	0.062	MS,RI,S
Nitrogen compounds		Subtotal	3.108	
1302/1335		2,3-dimethyl-pyrazine	0.075	MS,RI,S
1362/1391	1006/1005[41]	trimethyl-pyrazine	0.501	MS,RI
1422/1457	1087/1089[42]	tetramethyl-pyrazine	1.893	MS,RI,S
1460	1175	3,5-diethyl-2-methyl-pyrazine	0.082	MS
	1157/1163[42]	2,3,5-trimethyl-6-ethylpyrazine	0.085	MS,RI
1920/1952		2-acetylpyrrole	0.472	MS,RI
Others	Subtotal		2.757	
	730/725[44]	1,1-diethoxy ethane	1.211	MS,RI
	839	2-ethyl-1,3-dioxolane	0.110	MS
1398	996	1,3-dioxolane,2-methoxymethyl-2,4,5-trimethyl-	0.205	MS
	1515	2,4-bis(1,1-dimethyl)-phenol	0.027	MS
1962/1996		phenol	0.111	MS,RI,S
2116	1666	α-bisabololoxide B	0.327	MS
2628	1794/1780[43]	dibenzothiophene	0.766	MS,RI

[a]DB-Wax RI: Reference RI, which were all attained from the the web site of http://www.odour.org.uk

[b]Identification method: MS, compared with Nist08 Mass Spectral Database; RI, agree with retention index of literatures; S, compared with retention indexes and mass spectra of the authentic chemicals.

3.2. Gas Chromatography-Olfactometry Analysis

GC-O analysis was done to find out the aroma-active compounds which played the key role on the distinctive flavor of *Dictyophora rubrovolota Zang, ji et liou*. The results was presented in Table **2**.

With FD ranging from 1-27, 22 aroma-active compounds were identified, which may make the particular flavor of this fungi. Among these identified compounds, seven key flavor volatiles with high FD ranging from 27 to 3 included 2,3-pentanedione (FD 27, yogurt flavor), acetic acid (FD 27, sharp acidity), 2-methylbutanoic acid (FD 27, smelly socks smell, aldehyde taste), (E)-2-octenal (FD 9, pine oil odour), 2-phenyl-2-butenal (FD 9, aldehyde flavor, fragrant beans), benzaldehyde (FD 3, formaldehyde smell, resin taste), 3,5-diethyl-2-methyl-pyrazine (FD 3, musty, bark corrupt taste, smelly).There were seven unknown compounds which had odours, such as fragrant flavor, peanut candy, sweet (FD 3) and sour, fruit sweet, rice wine fragrant (FD 9), which mainly came out from the GC-O at the period of solvent delay in GC-MS, and so could not be identified. Odors such as mushroom flavor (FD 3) were not

identified by GC-MS for its too weak singal. Compounds with Log_3FD factor of <1 were considered to make only minor contributions to the overall aroma.

Comparing the results gained by SDE/GC-MS, thought the amount of (Z, Z)-9, 12- octadecadienoic acid (132.3 mg/kg) was the highest in the volatile, it had little contribution to peculiar flavor of *Dictyophora rubrovolota Zang, ji et liou*. The same were other long-chain fatty acid.

Table 2 Aroma-active compounds in the flavor of dried mushroom of *Dictyophora rubrovolota Zang, ji et liou* by AEDA

No.	Rentation index	Compound	Odour description	Log_3FD factor
1	921	unknown	fragrant flavor, peanut candy, sweet and sour	1
2	953	unknown	fruit sweet, rice wine fragrant	2
3	963-970	2,3-pentanedione	yogurt flavor	3
4	998	hexanal	green tea flavor, fresh leave flavor	<1
5	1048	heptanal	leafy	<1
6	1136	unknown	smells like the gas of heated gas in oven	<1
7	1263	unknown	smelly with sharp acidity odour	1
8	1273	unknown	mushroom	1
9	1328-1338	2,3-dimethylpyrazine	roasted rice flavor	<1
10	1372	(E)-2-octenal	pine oil odour	2
11	1382	acetic acid	sharp acidity odour	3
12	1398	unknown	pine oil odour, sour odour	1
13	1404	furfural	roasted potato or tomato	<1
14	1420	tetramethylpyrazine	dictyophora flavor	<1
15	1473	2-acetyl furan	greasy	<1
16	1480	benzaldehyde	formaldehyde smell, resin taste	1
17	1518	3,5-diethyl-2-methyl-pyrazine	musty, bark corrupt taste, smelly	1
18	1529	5-methyl-furfural	flower sweet	<1
19	1550	2-methyl-propanoic acid	baked sweet	<1
20	1609-1625	2-furanmethanol	sauce fragrant, flower	<1
21	1663-1684	2-methyl-butanoic acid	smelly socks smell, aldehyde taste	3
22	1715	unknown	earthy, wood scent	<1
23	1759	(E,E)-2,4-decadienal	licorice flavor, fatty	<1
24	1785	hexanoic acid	aldehyde, oil flavor	<1
25	1868-1891	2-phenyl-2-butenal	astringent taste, aldehyde flavor, fragrant beans	2
26	1981	2-acetylpyrrole	mugwort leafy	1
27	2020	2(3H)-furanone,dihydro-5-pentyl-	fatty	<1
28	2163	nonanoic acid	faint acidity flavor	<1
29	2179	isolongifolen-5-one	pleasant wood flavor	<1

FD: Flavour dilution factor. The concentrated sample was diluted by ethyl ether. The ratios were 1:3, 1:9, 1:27, 1:81, corresponding to FD factors of 3, 9, 27, 81, respectively.

Acknowledgement

Financial support from Beijing Natural Science Foundation Program and Scientific Research Key Program of Beijing Municipal Commission of Education (KZ201110011015), the National Natural Science Foundation of P. R. China (No. 31071610 and No. 31101350) and PHR (IHLB) (PHR20090504) is gratefully acknowledged.

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