Chocarom Pyrazine

A pyrazine for flavors and fragrances

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Pyrazines are materials obtained in Maillard reactions as by-products of the browning reaction of sugars and proteins or amino acids. These reactions occur during roasting, cooking, baking, and so forth of different food products. Pyrazines are the most widely distributed heterocyclic compounds in nature, having functional groups, e.g. alkyl, acetyl, alkoxy and thioalkoxy. Pyrazines containing these chemical functions are present mainly in roasted products, generally thermally treated, including cocoa, coffee, barley, popcorn, nuts, bread, potato and beef. Within all these pyrazines, over 70 pyrazines containing only alkyl groups have been identified in heated foods.[1]

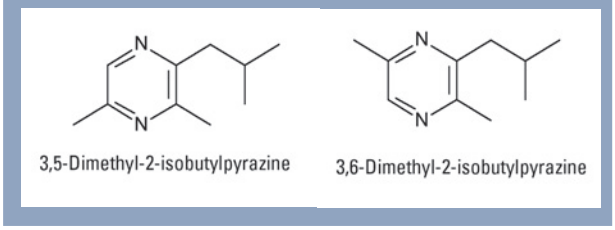
Alkylpyrazines, which are key to the flavor of chocolate, are formed via the Maillard reaction during the roasting process of cocoa production.[2] Many of the pyrazines have a nut-like aroma similar to that of peanuts.[3] The importance of these materials has historically motivated organic chemists to synthesize them for use as ingredients in flavor formulations for roasted nuts, chocolate, meat flavors, and so forth. By 1970, the first pyrazines obtained GRAS status in the United States for use as flavoring substances. Today, many pyrazines have commercial use in food applications.

Results and Discussion

Chocarom pyrazine is a colorless transparent liquid consisting of a 1:1 isomeric mixture of 3,5-dimethyl-2-isobutylpyrazine and 3,6-dimethyl-2-isobutylpyrazine, as shown in F-1.[4]

Chocarom pyrazine isomers were isolated from the skin and flesh of potato (*Solanum tuberosum* L.) cultivars after baking.[5] 3,5-Dimethyl-2-isobutylpyrazine [2,5-dimethyl-3-(2-methylpropyl)-pyrazine] was isolated by Oruna-Concha et al. from the following potato cultivars: Cara, Nadine, Flanna and Marfona. 3,6-Dimethyl-2-isobutyl-pyrazine [3,5-dimethyl-2-(2-methylpropyl)pyrazine], was found by the same team in Cara and Marfona potato cultivars. 2,5-Dimethyl-3-isobutylpyrazine was also detected by Welty et al. in chocolate ice cream prepared from cocoa flavor.[6] Both pyrazines were also found as key odorant compounds in dark chocolate by Counet et al.[7] The role of amino acids in alkyl-substituted pyrazines formation in model systems containing pyruvaldehyde was examined by Mea.[8] 2,5-Dimethyl-3-isobutylpyrazine was formed in the model system with valine. Both isomers were prepared synthetically by Chen by reacting acetol, isobutyraldehyde and ammonium acetate, with low yield of 22.3 percent.[9]

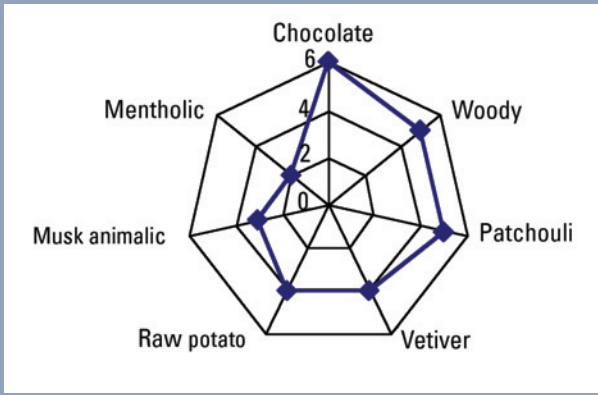
Chocarom pyrazine consists of a 1:1 isomeric mixture of 3,5-dimethyl-2-isobutylpyrazine and 3,6-dimethyl-2-isobutylpyrazine **F-1**



The odor threshold of 3,5-dimethyl-2-isobutylpyrazine and 3,6-dimethyl-2-isobutyl-pyrazine was published by Wagner et al. to be >2000 ng/l air, which indicates a relatively low φ value of only ca. 82,000.[10,11]

The odor and flavor of Chocarom pyrazine is reminiscent of the warmth of cocoa and hazelnut; it is slightly musky and animalic, with patchouli and vetiver tones, and a mentholic note (see F-2).

Radar organoleptic description of Chocarom pyrazine **F-2**



The φ value of a single molecule **F-3**

$$\varphi = \frac{MM \times 10^3}{\text{Threshold (pm)}}$$

The acceptable daily[1] intake for Chocarom pyrazine **T-1**

Food category	Mean consumption, g/day*	Anticipated usual use level (ppm)	Anticipated maximum use level (ppm)	PADI mg/person/d**
Baked goods	137.2	1.5	2.0	0.21
Breakfast cereals	20.0	1.5	2.0	0.03
Milk products	39.5	2.0	3.0	0.08
Processed vegetables	85.0	0.5	1.5	0.04
Seasonings and flavors	0.01	5000	10000	0.05
Soups	31.7	0.5	1.5	0.02
Sweet sauce	6.8	2.0	3.0	0.01

* Based on MRCA mean frequency of eating and USDA mean portion size
**PADI, mg/person/day calculated by: (Mean consumption, grams/day) x (Anticipated Usual Use Level, ppm) x 0.001
Reference: Market Research Corporation of America (MRCA), in conjunction with the food intake and nutritive value of the diets of men, women, and children in the United States, Spring 1965, a preliminary report by the consumer and Food Economics Research Division, Agricultural Research Service, United States Department of Agriculture(References)

The main food applications of Chocarom pyrazine are chocolate, cocoa, baked goods, breakfast cereals, milk products, roasted and processed vegetables, soups, baked potatoes, sweet sauces and mint flavors.

T-1 shows the acceptable daily intake for Chocarom pyrazine.[12]

One particular flavorist application of Chocarom pyrazine is as a rich chocolate building block for bakery, dairy products confectionery and ice-cream flavors. In this instance, the recommended dosage is 500 to 3000 ppm in the final product.

In fragrance applications, the material imparts a strong and natural, original and long-lasting effect. Chocarom pyrazine may find use in both women's and men's creations, in which it boosts woody, chypre, oriental and fougere notes. It is also appropriate for floral accords, in which it provides depth and warmth.

One particular perfumery creation employing Chocarom pyrazine is Tonkinarom. The fragrance base possesses the following olfactive profile: animalic, macrocyclic, polycyclic-free musky. In this base, Chocarom pyrazine provides a typical effect of the musk absolute from the pouches of the musk deer. Tonkinarom was formulated in two variations, the second being free of animalic derivated components and polycyclic musks.

φ Value of Chocarom Pyrazine

φ Value of flavor and fragrance ingredients provides an interpreter for the odor intensity of a single molecule — taking into consideration its molecular mass (MM) — analogous to the ε value in UV/VIS data of particular molecules. The φ value of a single molecule is defined in F-3.

T-2 shows the φ values for some commercially important pyrazines.

As seen in T-2, the isomers that comprise Chocarom pyrazine (3,6-dimethyl-2-isobutylpyrazine

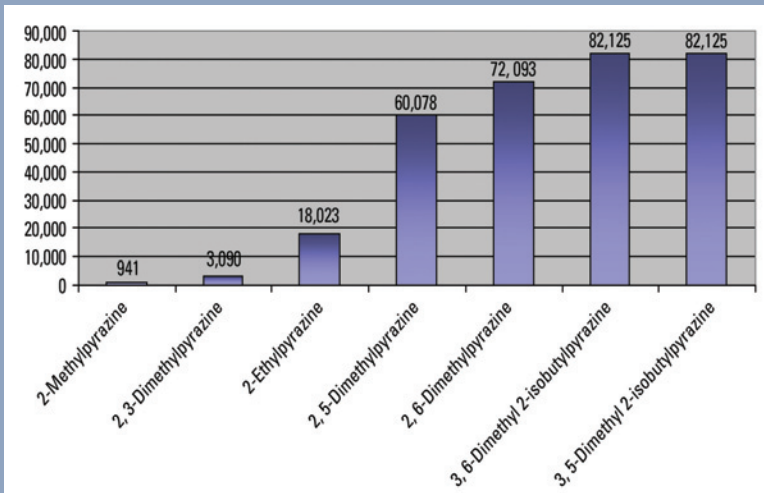
φ Value for some commercially important pyrazines **T-2**

Substance	Molecular mass	φ Value
2-methylpyrazine	94.11	941
2,3-dimethylpyrazine	108.14	3,090
2,5-dimethylpyrazine	108.14	60,078
2,6-dimethylpyrazine	108.14	72,093
2-ethylpyrazine	108.14	18,023
2-ethyl-5-methylpyrazine	122.16	732,989
2-ethyl-6-methylpyrazine	122.16	1,221,600
2,3,5-trimethylpyrazine	122.16	305,400
2-methoxy-3-methylpyrazine	124.14	24,828,000
2-methoxy-3-isobutylpyrazine	166.22	831,100,000
2-acetylpyrazine	122.13	1,969,839
3,6-dimethyl-2-isobutylpyrazine	164.25	82,125
3,5-dimethyl-2-isobutylpyrazine	164.25	82,125

and 3,5-dimethyl-2-isobutylpyrazine) have φ values of ca. 82,000, thus belonging to the low φ value pyrazines group.

This characteristic shows more clearly the pyrazines' odor impact, as displayed in the graphs of low, medium and high φ value pyrazines in F-4, F-5 and F-6, respectively. Low odor impact pyrazines are defined as having φ values up to 250,000, e.g. 2,5-dimethylpyrazine. Medium odor impact pyrazines have φ values of 250,000 to 5,000,000, e.g. 2-acetylpyrazine. High impact odor pyrazines, e.g. 2-methoxy-3-isobutylpyrazine, have a φ value of 24,828,000.

Low φ value pyrazines **F-4**



Physical Data of Chocarom Pyrazine

Chocarom pyrazine is a colorless transparent liquid, insoluble in water. In ethyl alcohol 50 percent it is soluble from 1:1 to 1:10. The material's boiling point is 73°C to 75°C at 2 mm Hg. Its flash point is 75°C (cc); specific gravity $D^{20/20}$: 0.924 to 0.934; refractive index n_D^{20}: 1.489 to 1.494.

Stability Data of Chocarom Pyrazine

Chocarom pyrazine was kept at 15°C for 55 days in order to measure changes in its physical parameters and odor profile. The color changed to 70 APHA (40 APHA start point); no change was seen in other physical parameters and odor profile. In another experiment Chocarom pyrazine was kept at 40°C for 22 days. The color changed to 70 APHA (40 APHA start point); no change in other physical parameters and odor profile.

The GC of Chocarom pyrazine, MS of dimethylisobutylpyrazine and NMR analyses of Chocarom pyrazine are presented in F-7, F-8 and F-9, respectively.

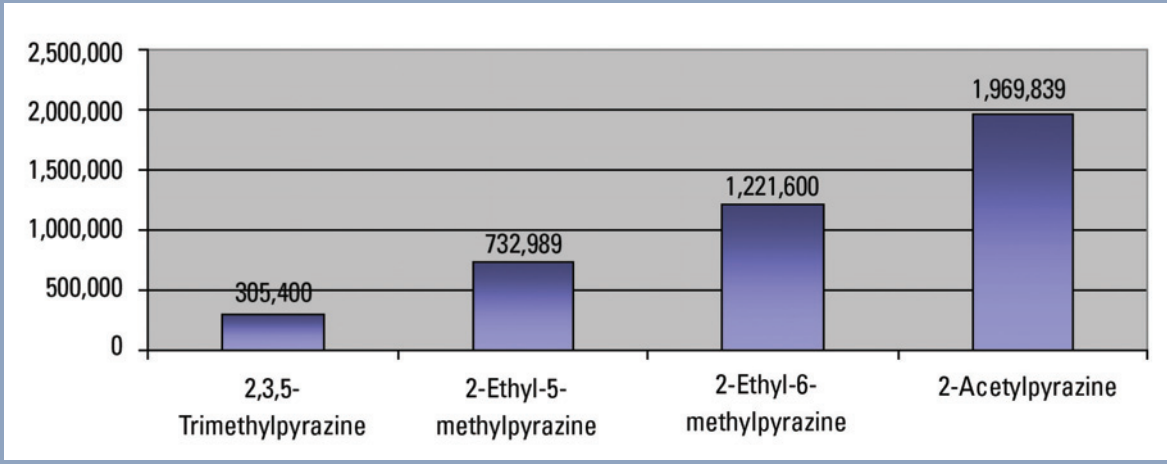
The substitution pattern of 3,5-dimethyl-2-isobutylpyrazine and 3,6-dimethyl-2-isobutylpyrazine was elucidated by a combination of NMR methods, especially in mixtures by gradient selected ^{1}H-,^{15}N-HMBC experiments at natural abundance level.[13]

F-10 shows the ^{1}H- and ^{13}C-NMR data for both isomers.

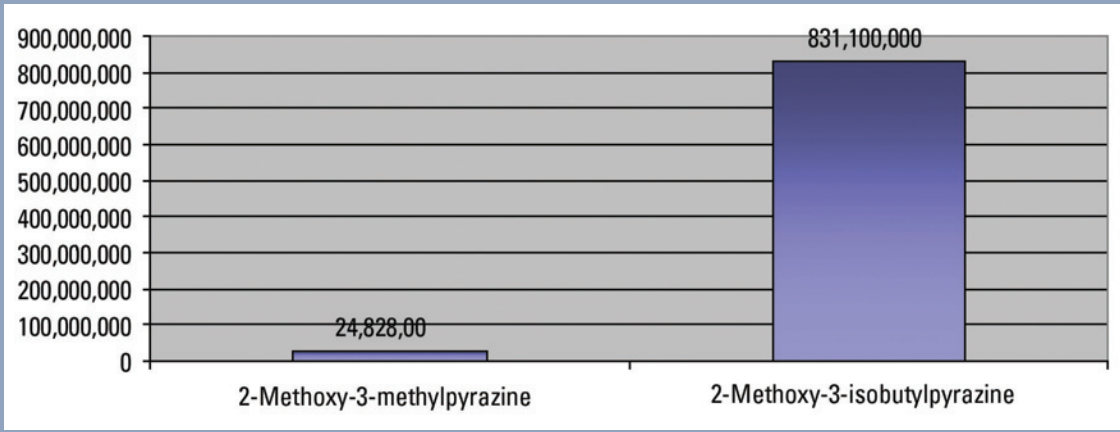
Since ^{15}N-NMR spectrum of pyrazines has proven to be a powerful tool for their structure elucidation, it is used to study this type of molecules.[14,15] F-11 shows the ^{15}N-NMR chemical shift of both nitrogen atoms of each isomer.

F-12 shows the two dimensional ^{1}H-, ^{15}N-HMBC correlations of each isomer.

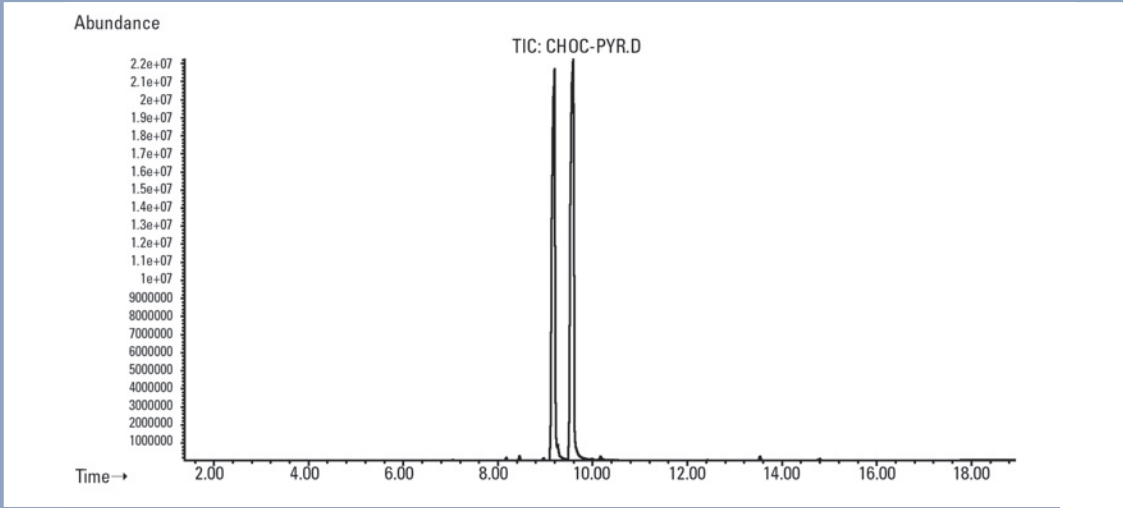
Medium φ value pyrazines **F-5**



High φ value pyrazines **F-6**

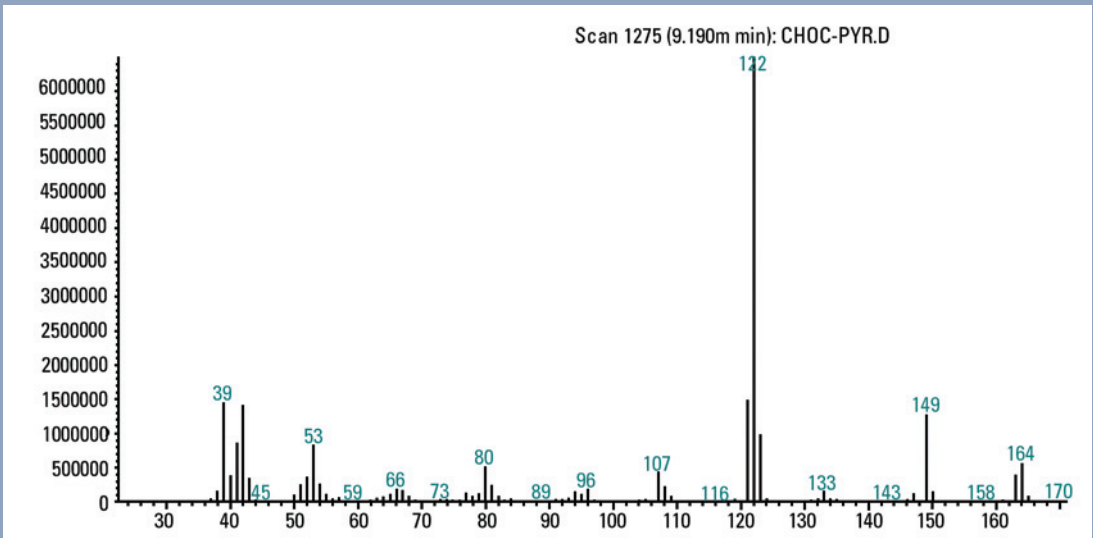


GC of Chocarom pyrazine **F-7**



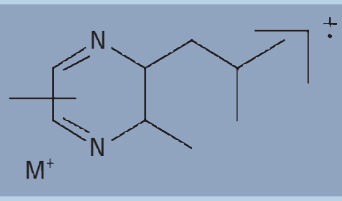
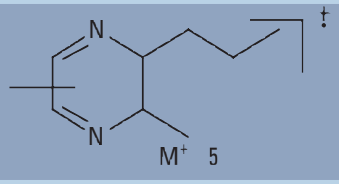
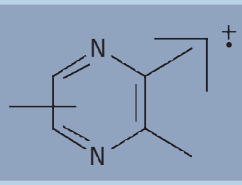
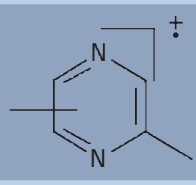
MS of dimethylisobutylpyrazine

F-8

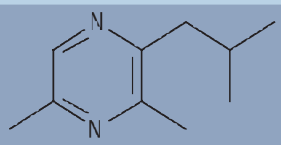
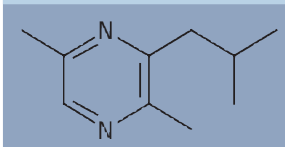


1. L.M. Nijssen, C.A. Visscher, H. Maarse, L.C. Willemsens and M.H. Boelens. TNO Nutrition and Food Research Inst., Zeist, The Nethelands, 7th Edn., 1996.
2. A. Arnoldi, C. Arnoldi, O. Baldi and A. Griffini, J. Agric. Food Chem., 36, 988, 1988.
3. P.G. Keeney, J. Am. Oil Chem. Soc., 49, 567, 1972.
4. Relevant CAS numbers are [38888-81-2] for Dimethyl-isobutylpyrazine (not defined positions), and [70303-42-3] for 3,5-Dimethyl-2-isobutylpyrazine.
5. M.J. Oruna-Concha, S. Craig Duckham and J. M. Ames. J. Agric. Food Chem. 49, 2414, 2001.

NMR analyses of Chocarom pyrazine **F-9**

m/z	Species	Abundance
164.05		522432
149.05		976832
122.05		6334976
107.05		245312
57.00	$C_4H_9^{+\bullet}$	52808
43.00	$C_3H_7^{+\bullet}$	250880

The ^{1}H- and ^{13}C-NMR data for both isomers **F-10**

Pyrazine	^{1}HChemical shift δ[ppm],multiplicity, coupling constant(s) [Hz],integral, position,	^{13}CChemical shift δ[ppm],position
 3,5-Dimethyl-2-isobutyl-	8.211, brs, 1 H, H6; 2.660, d, 7.3, 2 H, CH_2; 2.532, d, 0.4, 3 H, C3 CH_3; 2.488, s, 3 H, C5-CH_3; 2.130, mc, 1 H; CH; 0.954, d, 6.6, 6 H, $CH(C\underline{H}_3)_2$	152.0 (C2); 151.1 (C3); 149.7 (C5); 140.9 (C6); 43.3 (CH_2); 28.5 (CH); 22.53 ($CH(\underline{C}H_3)_2$), 21.8 (C3-$\underline{C}H_3$); 21.0 (C5 $\underline{C}H_3$)
 3,6-Dimethyl-2-isobutyl-	8.148, brs, 1 H, H5; 2.660, d, 7.3, 2 H, CH_2; 2.521, d, 0.5, 3 H, C3 CH_3; 2.491, d/q, 0.6/0.6, 3 H, C6 CH_3; 2.130, mc, 1H; CH; 0.951, d, 6.6, 6 H, $CH(C\underline{H}_3)_2$	154.2 (C2); 150.0 (C6); 148.9 (C3); 140.6 (C5); 43.6 (CH_2); 28.6 (CH); 22.50 ($CH(\underline{C}H_3)_2$), 21.4 (C3-$\underline{C}H_3$); 21.1 (C6 $\underline{C}H_3$)

6. W.M. Welty, R.T. Marshall and I.U. Grun, J. Dairy Sci., 84, 21, 2001.

7. C. Counet, D. Callemien, C. Ouwerx and S. Collin, J. Agric. Food Chem., 50, 2385, 2002.

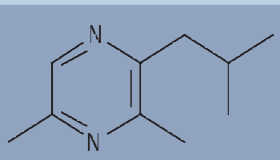
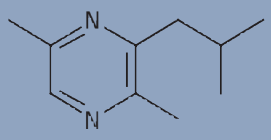
8. C.E. Mea, Zhongguo Nongye Huaxue Huizhi 32, 220, 1994.

9. T.K. Chen, EP 590296, assigned to Societe Des Produits Nestle S.A., 1993.

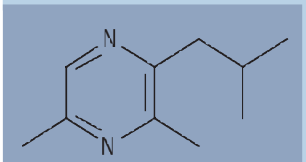
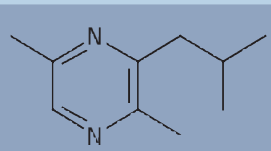
10. R. Wagner, M. Czerny, J. Bielohradski and W. Grosch, Z. Lebensm Unters Forsch A, 208, 308, 1999.

11. φ Value gives a better apprehension of the odor intensity of a single molecule, taking into consideration its molecular mass (MM); this value is discussed later.

The ^{15}N-NMR chemical shift of both nitrogen atoms of each isomer **F-11**

Pyrazine	N Chemical shift δ[ppm], position
 3,5-Dimethyl-2-isobutyl-	-44.8 (N1) -46.8 (N4)
 3,6-Dimethyl-2-isobutyl-	-45.1 (N1) -46.0 (N4)

The two dimensional ^{1}H-, ^{15}N-HMBC correlations of each isomer **F-12**

Pyrazine	1H, 15N HMBC Correlations (chemical shifts δ in ppm)
 3,5-Dimethyl-2-isobutyl-	N (-44.8) ↔ H(8.211); CH_2 (2.660) N(-46.8) ↔ CH_3 (2.532); CH_3 (2.488)
 3,6-Dimethyl-2-isobutyl-	N (-45.1) ↔ CH_2 (2.660); CH_3 (2.491) N (-46.0) ↔ H (8.148); CH_3 (2.521)

12. The table is taken from FEMA guidance lines for application to the FEMA Expert Panel for Consideration of GRAS Status.

13. H. Sommer, H. J. Bertram, G. E. Krammer, C. O. Schmidt, W. Stumpe, P. Werkhoff and M. Zviely, Magnetic Resonance in Chemistry, 38, 907, 2000.

14. M. Zviely, A. Kern, I. Gozlan and R. Frim, *Flavours and Fragrances*, Ed. by K.A.D Swift, Published by The Royal Society of Chemistry, 1997.

15. M. Zviely, A. Kern, I. Gozlan and R. Frim, Perfum. Flav., 23, 27, 1998. ■