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On the Role of (–)-2-Methylisoborneol for the Aroma of Robusta Coffee

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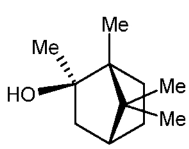
The role of 2-methylisoborneol (MIB) in coffee aroma is controversially discussed in the literature. MIB is known as an off-flavor compound in drinking water and food, but it has also been suggested as a key flavor component of Robusta coffee, discriminating Robusta from Arabica coffee. To check this hypothesis the role of MIB in coffee brews was studied. Two reference samples containing pure Arabica and Robusta coffee brews were compared with five samples of Arabica coffee brews containing increasing amounts of MIB. The sensory panel consisting of 12 assessors perceived a distinct difference in the Arabica coffee odor and flavor in the presence of 10−25 ng/kg MIB, which is close to its threshold value in water. The sensory impression was described as musty, mold-like, and earthy. The intensity increased with increasing concentration of MIB. The panelists agreed that there was no similarity with the Robusta reference sample. The Arabica coffee brew spiked with MIB was no longer palatable due to the odor and flavor defect formed.

KEYWORDS: Coffee; Robusta; aroma; flavor; (−)-2-methylisoborneol; off-flavor

INTRODUCTION

(1*R*-*exo*)-1,2,7,7-Tetramethylbicyclo[2.2.1]heptan-2-ol, also named (−)-2-methylisoborneol (MIB), was first reported by Zelinsky in 1901 (*1*). Since then, this odorant has frequently been associated with off-flavors in water and food (reviews in *2* and *3*). For example, MIB has been found to be responsible for the off-flavor of canned mushrooms (*4*), musty wheat grains (*5*), and fish (*6*). MIB is known as a secondary metabolite of aquatic and soil organisms such as Cyanobacteria (*7*), Actinomycetes (*8*), and fungi (*9*).



(-)-2-Methylisoborneol

Despite the controversy about the odor quality of MIB (*10*−*12*), it is generally reported to elicit a musty, moldy, earthy note, particularly at low concentrations. The odor threshold of MIB in water was found to be very low, varying from 1.4 ng/L (*13*) to 100 ng/L (*14*). The threshold value of MIB in water was reported to depend on water temperature and residual chlorine concentration (*15*).

Interestingly, MIB has also been suggested by Vitzthum et al. (*16*) as a key aroma compound in Robusta coffee. The authors found 185−430 ppt of MIB in roasted Robusta coffee, but less than 20 ppt of MIB in roasted Arabica coffee on a dry matter basis (*17*). Rouge et al. (*18*) confirmed the predominance of MIB in Robusta coffee, but found that it is degraded during the roasting process. This is in line with the results of Blank et al. (*19*) who could not confirm the key role of MIB for roasted Robusta coffee aroma using the method of aroma extract dilution analysis. In addition, Grosch et al. (*20*) found 740−1280 ppt of MIB in green Robusta coffee beans, but also up to 420 ppt of MIB in green Arabica coffee beans. These results questioned the key role of MIB in Robusta coffee aroma and called for a clarification.

The purpose of this study was to evaluate the role of MIB in coffee flavor and in particular to check the hypothesis of Vitzthum and co-workers that MIB significantly contributes to the characteristic Robusta note.

EXPERIMENTAL PROCEDURES

Materials. (−)-2-Methylisoborneol (MIB, 98%) was from Aldrich (Steinheim, Germany) and (*E*)-2-decenal (99%) was from Alfa (Karlsruhe, Germany). Ethanol for sensory evaluation was from Merck (Darmstadt, Germany). Diethyl ether obtained from Merck was freshly distilled prior to use on a Vigreux column (50 × 1 cm). Arabica (Colombia) and Robusta (Indonesia) coffees were roasted to a medium degree and stored under nitrogen at −20 °C prior to use.

Odor Threshold of MIB in Air. The odor threshold of MIB was determined by gas chromatography−olfactometry (GC−O) (*21*). (*E*)-2-Decenal was used as internal standard for the sensory evaluation. The sample containing MIB (8.1 μg/mL) and (*E*)-2-decenal (110 μg/mL) in diethyl ether was diluted with the solvent in 1:1 steps until none of the compounds could be perceived by GC−O. The odor

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threshold of MIB was calculated based on the following formula:

$$O_X = \frac{O_I \times D_I}{C_I} \times \frac{C_X}{D_X}\ [\text{ng/L air}]$$

where C_I and C_X represent the concentration, and D_I and D_X represent the dilution value, of the internal standard and the odorant, respectively (*22*). The term O_I is the odor threshold of the internal standard, (*E*)-2-decenal, which has previously been determined, i.e., 2.7 ng/L air (*23*).

Odor Threshold of MIB in Water. The odor threshold of MIB was determined in glass vials (45 mL, 65 × 40 mm), with glass lids, containing 20 mL of an aqueous solution of MIB. The sample was prepared by dissolving MIB (10.0 mg) in tap water (98 mL) and ethanol (2 mL). The solution containing 100 ppm of MIB was diluted with tap water to obtain MIB solutions of 100 ppb, 1 ppb, and 100 ppt concentrations. Threshold determination was carried out with coded solutions containing 0, 5, 10, 20, 50, 100, 200, and 500 ng/kg (ppt) of MIB. The sensory panel was asked to (i) indicate the sample different from water and (ii) describe the odor using one or more words. Replications were conducted on the following day. In all samples, the ethanol concentration was adjusted to 0.1%. The detection threshold is given as the concentration range representing the average of the individual threshold values.

Descriptive Sensory Analysis with Coffee Brews. Standard coffee brews (7 g/130 mL) were prepared in a coffee machine using roasted and ground coffee (which had been stored in closed glass vials at −20 °C), tap water, and a coffee filter. The freshly prepared coffee brew was allowed to cool to 60 °C in a thermostat before adding defined amounts of MIB. This temperature was kept constant until the sensory evaluation. During the procedure of sample preparation the vials were closed to minimize aroma losses. The time between sample preparation and sensory testing was typically less than 30 min. The samples (20 mL) for odor description were served in glass vials (45 mL, 65 × 40 mm), and the samples for taste testing (5 mL) were served in reaction tubes (10 mL). Each panelist was presented with seven samples: two reference samples based on pure Arabica and Robusta coffee and five coded samples prepared with Arabica coffee containing defined amounts of MIB, i.e., 5, 10, 25, 50, and 100 ng/kg (ppt). The sensory panel was asked to (i) indicate the sample(s) different from the reference Arabica coffee brew, (ii) describe the difference using one or more words, and (iii) judge the Robusta likeness. Odor and flavor evaluations were performed in separate sessions, with each repeated twice.

Sensory Panel. This sensor evaluation group was composed of 12 trained panelists who were familiar with describing odor and flavor of reference compounds and food products. They also participated in the coffee flavor research program. Training was performed on a regular basis using molecules with defined descriptors. The sensory evaluation was performed in daylight in a special room with 12 boxes dedicated for sensory testing.

RESULTS

In this study the term odor is defined as the sensory impression obtained by orthonasal perception (*24*). This may proceed by sniffing the effluent from GC−O or the headspace above the coffee brew. Aroma is defined as the impression obtained by retronasal perception, i.e., by introducing the sample in the oral cavity. Flavor is the combined effect of aroma and taste perception.

Threshold Values of MIB. The odor threshold of MIB was determined as 0.006−0.012 ng/L air using the GC−O method (**Table 1**). As described in the Experimental Section, this value is related to the odor threshold of (*E*)-2-decenal. In absolute terms, the sensory GC threshold corresponds to 0.002−0.005 ng of MIB injected onto the GC capillary, which is close to the value of 0.005 ng published by Sävenhed et al. (*25*).

The orthonasal detection threshold in water was found to be in the range of 20−30 ng/L (**Table 1**), which is in good agreement with the data reported by Persson (*26*), i.e., 18−42 ng/L with an average of 29 ± 19 ng/L. The retronasally determined value was lower by a factor of 3−4, i.e., 5−10 ng/L water (**Table 1**), which is close to the value of 2.5 ppt reported by Vitzthum et al. (*16*). However, in the case of MIB in water, Ito et al. (*15*) found similar values for the orthonasal and retronasal thresholds, i.e., 5−24 ng/L and 7−27 ng/L, respectively. These authors also claimed that residual chlorine increases the threshold concentration of MIB. In general, the variation in the literature data (*3*) stems from the fact that the approach of threshold determination, i.e., ortho- or retronasal, is not taken into account when comparing threshold values. It should be mentioned that the sensory purity of the MIB reference sample was checked by GC−O, ruling out any minor byproduct that may interfere with the odor of MIB.

Table 1. Odor and Flavor Qualities and Detection Thresholds of (–)-2-Methylisoborneol in Air and Water

sensory qualities and detection thresholds		
orthonasal (ng/L air)	orthonasal (ng/L water)	retronasal (ng/L water)
0.006–0.012 musty, mold-like, earthy	20–30[a] musty, mold-like, earthy	5–10[b] musty, mold-like, earthy, green, grassy

[a] The sensory panel ($n = 11$) indicated the following samples as threshold: mainly 20 mL (6), 10 mL (2), and 50 mL (3). [b] The sensory panel ($n = 10$) indicated the following samples as threshold: mainly 5 mL (5), 10 mL (4), and 20 mL (1).

In this study, the odor quality was described as musty, mold-like, and earthy (**Table 1**). At higher concentration (500 ppt), the retronasally perceived note was in addition green and grassy. In general, the odor quality of MIB has been mostly associated with musty (*27*), which was different from the earthy note of geosmine, but usually difficult to distinguish (*28*). Solutions with the high concentration of 1000 ppm MIB still show the musty/earthy note, but in addition a camphor-like character (*29*) that has also been claimed by Tyler et al. (*10*).

Coffee Brews Spiked with MIB. The sensory panel was asked to describe the odor and flavor changes as compared to the reference sample prepared from pure Arabica coffee. Special attention was paid to the concentration where the odor or flavor deviated from the Arabica note. Furthermore, the panelists were asked to compare the samples having a modified odor or flavor with the reference sample prepared from pure Robusta coffee.

The odor of the sample containing 5 ppt of MIB could not be distinguished from the reference Arabica sample (**Table 2**). However, as little as 10 ppt of MIB led to a distinct deviation from the Arabica note, which was increased in the presence of 25 ppt MIB and was described as musty, mold-like, and earthy. The musty and mold-like odor increased with increasing concentration of MIB and was clearly perceived as an off-note. However, none of the panelists found a similarity with the Robusta reference sample.

A difference from the characteristic flavor of Arabica coffee brew was already perceived in the sample containing 10 ppt MIB, which was described as musty (**Table 2**). This note became more pronounced by increasing the MIB concentration. The mold-like off-note was already perceived in the sample containing 25 ppt MIB. This sample was no longer palatable. The samples containing higher amounts of MIB showed a strong off-flavor defect. Again, none of the samples was found to be Robusta-like. These data indicate that MIB does not play the role of a key aroma compound responsible for the typical Robusta flavor. Other groups also failed to increase the Robusta

Table 2. Sensory Description of Arabica Coffee Brews Spiked with Defined Amounts of (–)-2-Methylisoborneol (MIB)

MIB (ng/kg)	odor quality[a]	number of panelists[b]		flavor quality[a]	number of panelists[b]	
		yes	no		yes	no
5	(Arabica-like)	2	10	(Arabica-like)	3	9
10	(Arabica-like)	4	8	weak musty note	6	6
25	musty, mold-like, earthy	10	2	musty, mold-like, distinct off-note	10	2
50	musty, mold-like, distinct off-note	12	0	musty, mold-like, strong off-note	12	0
100	musty, mold-like, strong off-note	12	0	musty, mold-like, strong off-note	12	0

[a] Odor or flavor quality describing the difference from the reference sample (pure Arabica coffee brew). [b] Number of panelists ($n = 12$) who perceived a difference compared to the reference sample (pure Arabica coffee brew) or not.

note with MIB (*18*). These authors came to the conclusion that higher MIB levels are not necessarily specific to Robusta coffees. In the brew, the Robusta flavor character was not related to the presence of MIB.

These results are in contradiction to those of Vitzthum et al. (*16*) who claimed that "a concentration of 5.0 ppt in coffee beverage led to a significant Robusta taste impression being indicated by the highly significant increase of the descriptor score Robusta". However, we confirm the effect of MIB on the coffee brew flavor at concentrations close to its threshold. This modification effect is known for off-flavor compounds that are not intrinsic to the food, e.g., 2,4,6-trichloroanisol (*30*) and geosmin (*31*). They modify the balanced odor or flavor of the product and impart their character, which leads to off-notes.

DISCUSSION

The odor and flavor thresholds of MIB are very low and it shows sensory qualities that are not compatible with those of coffee. Indeed, the presence of MIB has been explained by microbial contamination. Typical examples are water and foodstuffs as reported by Maga (*3*) where very low amounts of MIB already led to off-notes. MIB has also been reported to occur in green coffee and to contribute, in combination with geosmin, to the earthy-moldy defect of Mexican green Arabica coffee (*31*).

This study also shows that addition of very low amounts of MIB to Arabica coffee brew changes the overall odor and flavor leading to a distinct musty, mold-like off-note in the concentration range of 10−25 ppt MIB. However, the modified odor and flavor do not resemble the characteristic Robusta note. Moreover, MIB is clearly shown to provoke an off-note that makes the coffee brew less palatable. Therefore, our results do not confirm the hypothesis that MIB might be a characteristic Robusta coffee compound (*16*). On the contrary, quantitative data of potent odorants indicate that 3-times higher levels of 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine, as well as 3 to 10 times higher levels of guaiacol, 4-ethylguaiacol, and 4-vinylguaiacol, in roasted Robusta coffee are most likely the cause for the aroma difference to Arabica coffee (*32*−*35*).

MIB is already present in the green coffee beans: amounts from 100 ppt (*31*) to 80−420 ppt in Arabica and 740−1280 ppt in Robusta green coffee (*20*) have been reported. In general, Robusta coffee beans are obtained by dry post-harvest treatment, in contrast to Arabica coffee to which the wet method is mainly applied. The post-harvest treatment of green coffee was thought to be critical to the levels of MIB (*31*). The Mexican Arabica coffee, reported by these authors, was obtained by the dry post-harvest method. Consequently, the coffee beans were exposed to contact with soil, which can contain high MIB levels. MIB has been reported to occur in garden soil in quantities of about 4 ppb (*36*), which is more than 100-fold above its threshold value. As MIB is known as a secondary metabolite of Actinomycetes and certain fungi present in the soil (*3*, *36*), MIB found in coffee is most likely of microbial origin.

In conclusion, we suggest that the concentration of MIB in coffee may depend on the conditions of post-harvest treatment, rather than on the coffee variety. The reason for the broad variation of MIB amounts in coffee seems to be the local soil microbial flora producing MIB that migrates into the green coffee beans. Depending on the conditions of coffee treatment, a certain portion of MIB may survive the roasting process and lead to off-notes in the coffee brew at very low concentration levels.

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