SCIENTIFIC RELEASE

SPF Palatability
N°1 WORLDWIDE

# DOG KIBBLE ODOR: WHAT CAN WE LEARN FROM THE HUMAN NOSE?

DIANA
Performance from nature
PET FOOD
Member of the Symrise Group

# DOG KIBBLE ODOR: WHAT CAN WE LEARN FROM THE HUMAN NOSE?

Maiken THOMSEN, Laurence CALLEJON - DIANA Pet Food
Pascal TOURNAYRE, Erwan ENGEL, INRA-QuaPA MASS group

Smell is one of the most critical attributes impacting food palatability in dogs. To attract the animals and induce consumption, food needs to have a pleasant odor, rich in aroma molecules that dogs particularly appreciate, and free from the ones they dislike. In order to identify the compounds that are attractive or repulsive to an animal, it is thus crucial to get an accurate picture of food odor profile.

Classic aroma analysis methods such as gas chromatography detect most of the volatile molecules present in a product. However, there is no existing method to identify, among these molecules, the ones that are really perceived by the animal and that impact palatability performance. An innovative study was conducted by DIANA Pet food and INRA Clermont-Ferrand-Theix to evaluate the interest of using human olfactometry in the characterization of dog kibble odor.

## OLFACTOMETRY, ONE STEP FORWARD IN AROMA ANALYSIS

The Gas Chromatography Mass Spectrometry technique (GC-MS) is commonly used to characterize the odor of a food. This method gives a global picture of the main volatile components present in a product. However, it doesn't distinguish the compounds that actually contribute to the odor – the odor-active compounds - from those that don't. Moreover, this methodology lacks in sensitivity towards aroma compounds present at very low concentrations. Yet it is well known that some volatile compounds can have a big impact on the global odor of a product even at trace amounts.

In order to identify odor-active molecules in a product, including those present at low levels, gas chromatography can be coupled with olfactometry. Widely applied in the field of humans, the Gas Chromatography – Olfactometry technique (GC-O) combines classic gas chromatography with sensory evaluation by humans. In this method, volatile extracts of a food are prepared and separated by chromatography, and then presented successively to different judges who describe the odors they perceive and give them an intensity score. This interesting method allows to determine precisely which molecules are really responsible for the global odor of a product.

One improvement of the GC-O method consists in doing a simultaneous detection of the odor-active molecules of the same volatile extract by 8 trained judges. This 8W-GC-O method reduces the analysis time, avoids the risk of sample extract variability, and takes into account the judges' individual variability (Berdagué and Tournayre, (2005).

The study below evaluated the interest of combining classic GC-MS with 8W-GC-O as a first step to characterize the odor of dog diets with different palatability levels.

## COMBINING INSTRUMENTAL AND SENSORY ANALYSIS

### 2 Diets, 2 Odors, 2 Palatability levels

Two dry dog diets were manufactured using a same super premium kibble base, coated with two different dog palatants known to have significantly different palatability levels:

- Diet A was coated with 2% of a premium dog liquid palatability enhancer
- Diet B was coated with 2% of a super premium dog liquid palatability enhancer

Palatability trials conducted in Panelis, DIANA Pet Food's expert center in palatability measurement, using a 2-bowl preference test for dogs, confirmed Diet B's

superior palatability compared to Diet A's. This difference was observed for both intake ratio and 1st choice, substantiating the importance of the food odor in food selection by the dogs (Figure 1). The odor of Diet B was thus considered different and more attractive to dogs than the odor of Diet A.

**Experimental Diets relative palatability performance**

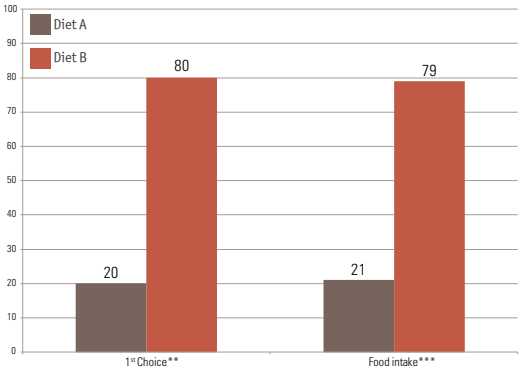


Figure 1: Palatability performance of Diet A and Diet B in dogs, 2- bowl preference test

## Aroma analysis protocol

The following aroma analysis protocol combining instrumental and sensory analysis was applied to both diets (Figure 2):

- A volatile extract of the diet was obtained by applying Purge and Trap dynamic head-space to kibbles placed whole in glass vials.
- The extract was then injected into a gas-chromatograph in order to separate the molecules according to their polarity and evaporation temperatures.
- The separated molecules were led to an olfactory port to be characterized by 8 sensory panelists, and to an MS detector to allow identification of odor-active compounds.
- The 8 judges, placed in individual booths, evaluated the perceived odors of the extracts at the olfactory port for 36 minutes by attributing an intensity score and an odor quality to the eluting molecules (Videosniff® method - Berdagué and Tournayre, 2002). The qualitative sensory results were then categorized into different odor-types and the mean intensity scores were calculated as a function of time (AcquiSniff® Software, Tournayre and Berdagué, 2003-2012). An aromagram was established for each extract, and Students t tests were applied in order to determine the significantly different odorants between the two extracts.
- The chromatogram obtained with the MS was used to identify the compounds corresponding to odor zones perceived by the judges.

**Aroma analysis experimental set-up**

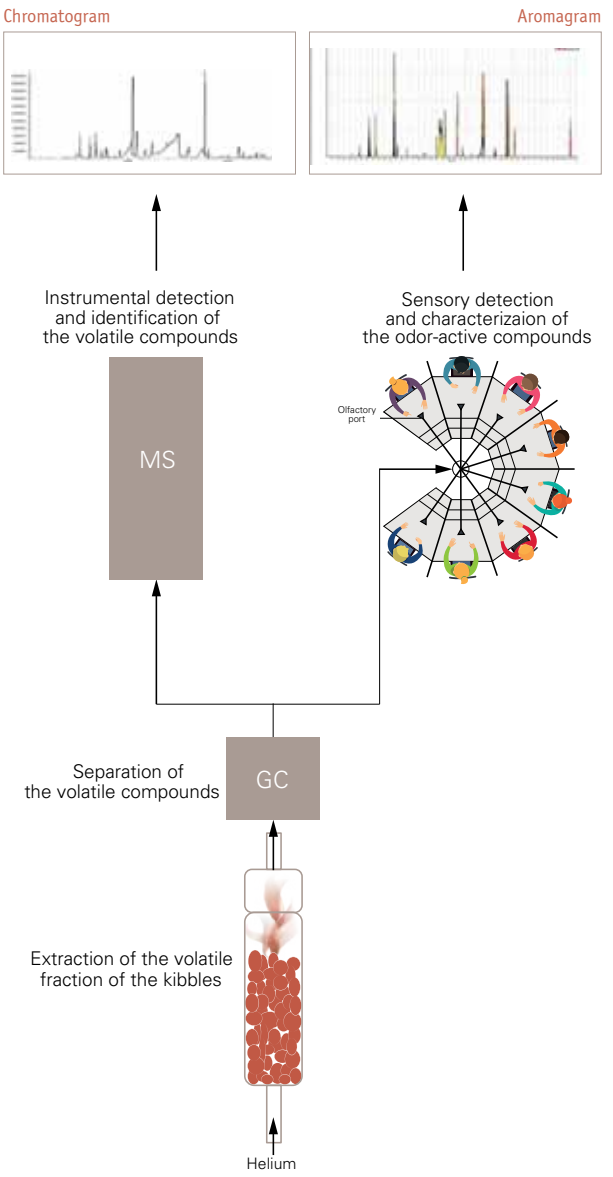


Figure 2: Protocol for kibbles aroma analysis using GC-MS and 8W-GC-O

## FROM VOLATILE COMPOUNDS TO ODOR-ACTIVE MOLECULES

The analysis obtained from the GC-MS led to the detection of more than 100 volatile compounds in the extracts. The 8W-GC-O allowed to detect a total of 22 odor zones corresponding to 22 odor-active compounds. The odor zones identified expressed various odor classes, such as meaty, chemical, fishy, fruity, etc. They also showed different intensities depending on the extract, as a result of different concentrations in the sample.

Among the 22 odor-active compounds identified with 8W-GC-O:

- 7 compounds perceived by the judges were not detected by GC-MS because of their presence in trace amounts in the products
- 17 compounds were perceived by judges in both extracts with a similar odor quality and intensity
- 4 compounds were perceived by judges in both extracts with a similar quality but with different intensity
- 1 compound was perceived by judges only in extracts from Diet A

Table 1 summarizes the results obtained from GC-MS and from 8W-GC-O on the 22 odor-active compounds identified.

The analysis of the chromatograms and aromagrams of both extracts confirmed that the compounds present in the highest amounts were not necessarily those perceived most intensely.

Figure 3 shows that in the particular case of Diet B, the compound circled in red was for instance intensively perceived but not detected with MS. On the contrary, the compound circled in black was detected by MS but was not associated to any odor-active zone.

Table 1: **Odor-active compounds detection and characterization with GC-MS and 8W-GC-O**

| Compound | INSTRUMENTAL DETECTION with GC-MS | SENSORY CHARACTERIZATION WITH 8W-GC-O | | | |
|---|---|---|---|---|---|
| | | Intensity score in Diet A | Intensity score in Diet B | | |
| 1 | ✓ | + | + | Compounds with Same odor type Same intensity | |
| 2 | ✓ | + | + | | |
| 3 | ✓ | + | + | | |
| 4 | ✗ | + | + | | |
| 5 | ✓ | + + | + + | | |
| 6 | ✓ | + | + | | |
| 7 | ✗ | + + + | + + + | | |
| 8 | ✗ | + + + | + + + | | |
| 9 | ✓ | + | + | | |
| 10 | ✓ | + | + | | |
| 11 | ✓ | + | + | | |
| 12 | ✗ | + + | + + | | |
| 13 | ✓ | + | + | | |
| 14 | ✗ | + | + | | |
| 15 | ✗ | + + | + + | | |
| 16 | ✓ | + + | + + | | |
| 17 | ✓ | + | + | | |
| 18 | ✓ | + | + + | Compounds with Same odor type Different intensity | Compounds supposed to be responsible for odor difference |
| 19 | ✓ | + + + | + + + + | | |
| 20 | ✓ | + | + + | | |
| 21 | ✓ | + | + + | | |
| 22 | ✗ | + + | − | Compound perceived in one product only | |

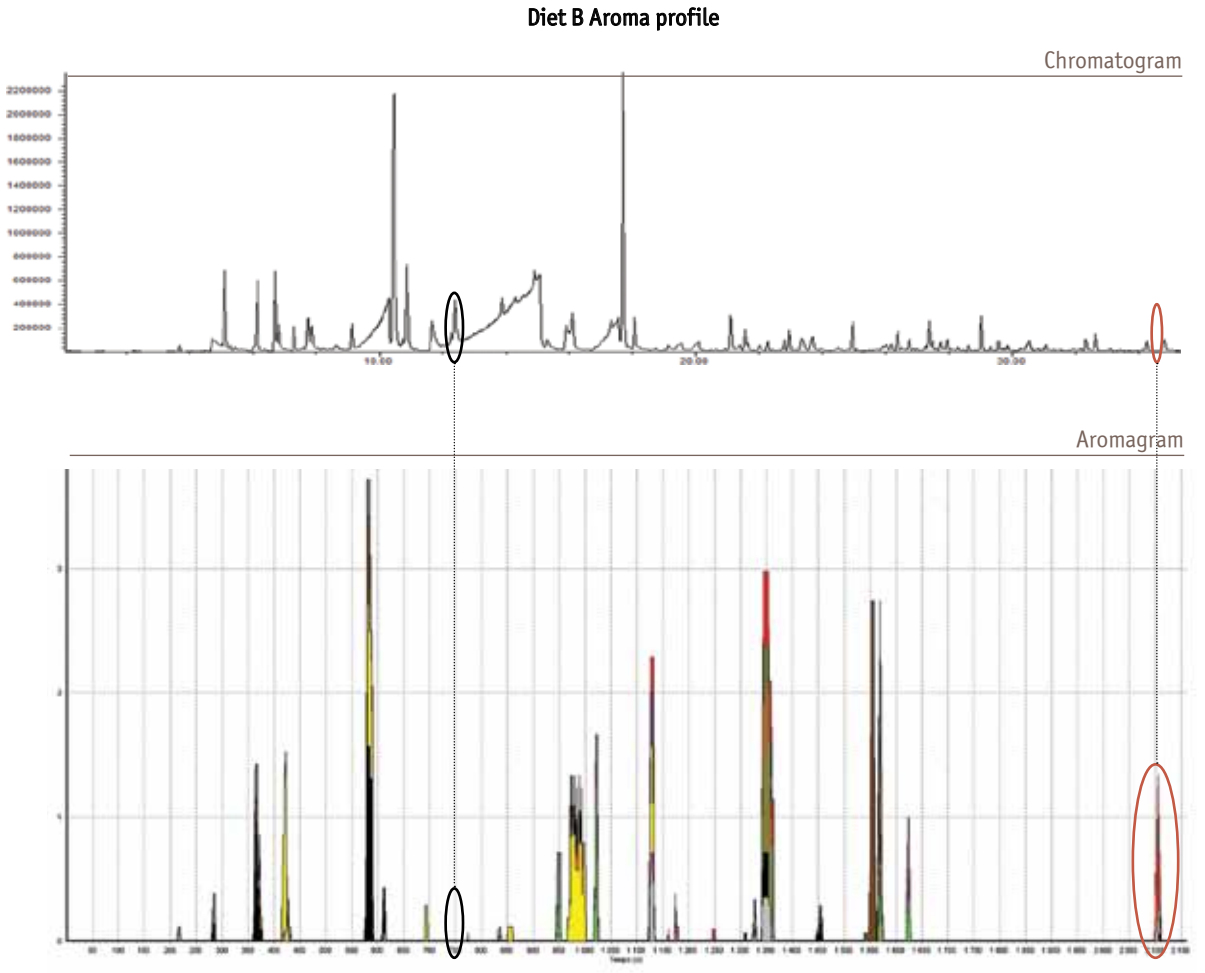


Figure 3: Comparison of Diet B chromatogram and aromagram

## FROM HUMAN SENSORY ANALYSIS TO DOG PALATABILITY

The above results confirmed the interest of coupling olfactory perception with instrumental analysis to assess kibble aroma profiles. Human olfactometry acted here as a filter since it allowed to select, among all the volatiles present in the diets, only the compounds having a role in the product odor. Olfactometry data limited the study to 22 out of more than 100 volatiles.

The precise analysis of aromagrams and chromatograms of the selected volatiles then highlighted the odor compounds that were typical of each product: only 5 odor-active compounds out of the 22 identified were significantly different between the 2 diets and might explain odor difference between Diet A and Diet B.

However, differences between dog and human olfactory systems and odor perception are huge. The above study allowed to identify 5 odor-active molecules in kibbles... as perceived by humans! If human olfactometry is a very interesting tool, it has to be considered as a first step to studying dog food aroma profiles. It is indeed essential to evaluate the impact of selected molecules on dogs to get relevant information on their role in food palatability performance.

The use of false-bottom-bowls would be the second step to determine the odorant impact of candidate molecules on dog palatability. With this method, it is possible to stimulate the dog's olfactory system by releasing volatile compounds from under kibbles (Figure 4). The attractivity potential of each molecule, or mix of them, can thus be evaluated through preference tests using these special bowls.

**Principle of False Bottom Bowl**

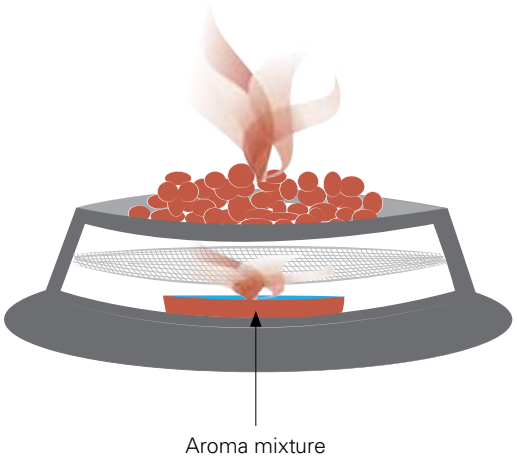


Figure 4: Schematic representation of the false-bottom-bowl used to study odor-active molecules in dogs

This final identification of odor-active and palatable aroma compounds, validated with animals, gives solid input to designing foods with improved attractivity. Palatability enhancers used in top coating on kibbles are for instance known to play a key role in a diet's final odor.

The rich information about the impact of odor-active compounds on palatability performance, obtained from the combined instrumental, human olfactometry and animal sensory analyses will serve as a guide to design high-performing dog palatants. Product formulation and process will be orientated accordingly to produce the right balance of identified attractive molecules.

## CONCLUSION

Food odor is critical in dog palatability performance. Classical instrumental aroma analysis methods such as Gas Chromatography are convenient but limited to fully characterize dog diet aroma profiles.

Combined with olfactory oriented palatability evaluations conducted with dogs, human olfactometry can successfully be used in a first approach to help identify the molecules that contribute to the global odor of kibbles.

## REFERENCES

-Berdagué, J.L. and Tournayre, P. (2005). GAS CHROMATOGRAPHY-OLFACTION ANALYSIS DEVICE AND METHOD. International patent number WO2005001470.

- Berdagué J.L., Tournayre P., 2002. «The VIDEO-Sniff® method, a new approach for the «Vocabulary-Intensity-Duration» study of «Elementary Odours» perceived by gas chromatography-olfaction» in «Flavour research at the dawn of the twenty-first century". TEC & DOC Editors, 11 rue Lavoisier - Paris, p 514-517.

- Tournayre, P. & Berdagué, J. L. (2003-2012). AcquiSniff® (Version 6.5.5) [Computer software]. IDDN.FR.001.210006.002.RP.2003.000.30000: France: Institut National de la Recherche Agronomique.

## IF YOU NEED FURTHER INFORMATION, DO NOT HESITATE TO CONTACT THE AUTHORS

LAURENCE CALLEJON
DIANA Pet Food
R&D Dog Platform Manager
lcallejon@diana-petfood.com

MAIKEN THOMSEN
DIANA Pet Food
PhD - R&D Dog Project Manager
mthomsen@diana-petfood.com

PASCAL TOURNAYRE
INRA-QuaPA MASS group
PhD - Engineer of electronics

ERWAN ENGEL
INRA-QuaPA MASS group
PhD - Research director
Group leader