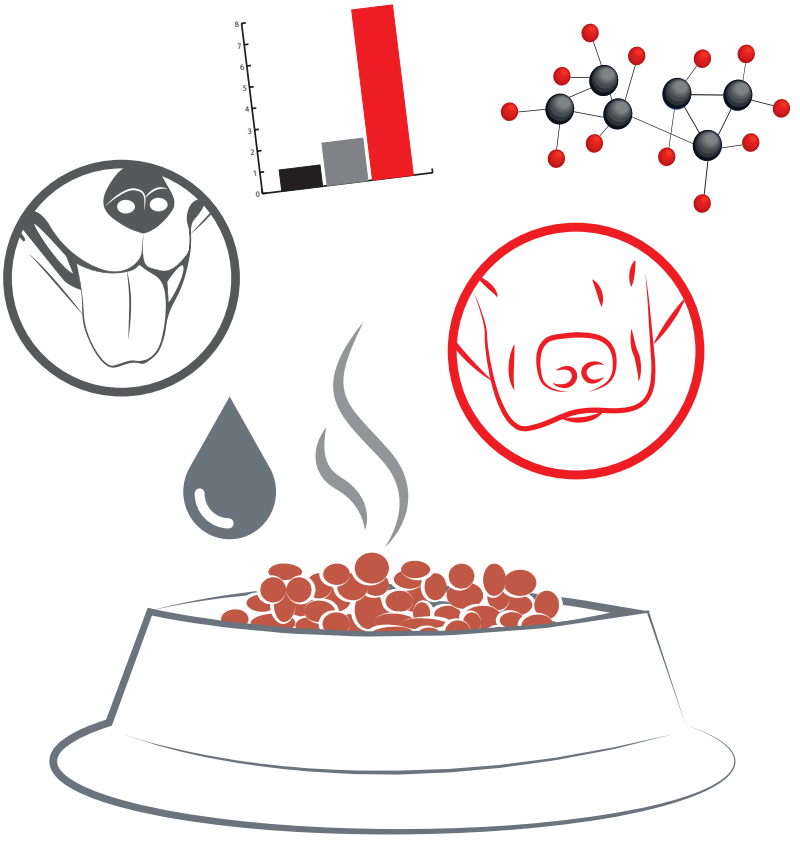
SPF Palatability
N°1 WORLDWIDE

SCIENTIFIC RELEASE

DOG PALATABILITY DRIVERS



DIANA
Performance from nature
PET FOOD
Member of the Symrise Group

INTRODUCTION

Dogs are often considered easy to satisfy, however the wide diversity in breeds and size of dog population alone is sufficient to make dog palatability research a challenge.

Knowing dogs' physiology, understanding feeding behavior and being able to distinguish fine preferences is one of Diana Pet Food field of expertise.

In a growing and changing market, innovation as well as fundamental research and breakthrough technologies will be necessary to create the most adapted and high performing palatability solutions.

DOGS BEHAVIORAL AND PHYSIOLOGICAL SPECIFICITIES

> FEEDING BEHAVIOR

Dogs are not strict carnivorous. Their wild ancestors ate flesh meat when available but were used to complete their meals with vegetable. They are known to eat preferably during daylight, and generally have only one or two large meals.

> SENSES

SMELL is dog's most developed sense; it plays a major role in food search and food selection. Before eating, dogs will usually start by sniffing their food.

Nose anatomy obviously plays a role in dog's sense of smell. When a dog sniffs, he inhales volatile molecules in his nasal cavities where they are trapped by mucus and processed by the olfactory cells receptors. These receptors will, in turn, deliver the information to the olfactory bulb and then to the brain. Once in the olfactory bulb, scents are transported to the frontal cortex for recognition as well as to other regions of the brain including centers for emotions, memory and pleasure.

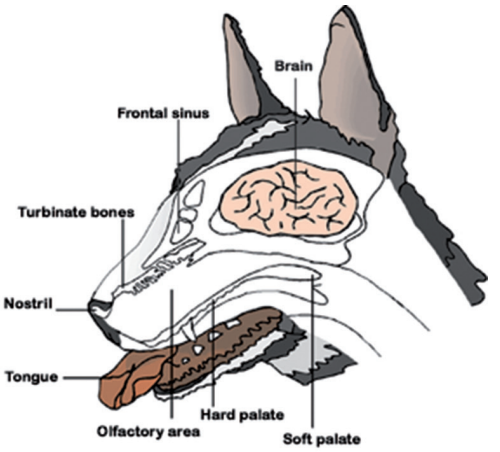


Figure 1: Anatomy of a dog's nose (Correa, J. 2005)

Olfactory epithelium surface is an indicator of animals' olfactory capabilities. With a surface 10 to 100 times bigger than in human beings, twice more olfactory receptors (757 intact olfactory receptors in dogs, and 382 in humans), and 300 millions olfactory neurons (5 millions in human), dogs are able to detect some substances 1000 to 1 million times less concentrated than human beings.

However, all dogs are not equal in regards to smelling ability. Dog's size and breed have a direct influence on the epithelium surface and thus on the number of receptors. Furthermore, dog nose anatomy also impacts smell capability. A narrow and long nose will increase the air flow and therefore the contact with receptors, whereas brachicephale will experience the opposite effect (Vadurel and Gogny, 1997).

TASTE, the gustatory perception, is the sensation in mouth produced when a substance reacts chemically with taste buds receptors. The sensation of taste is categorized in five basic tastes: sweet, sour, salty, bitter and umami.

Dogs can detect all 5 basic tastes, but only have 1700 gustative buds while humans have 9000 (Leibetseder, 1980). Therefore, their taste will be less developed than their smell.

VISION, HEARING and TOUCH should not be forgotten as dogs are as well sensitive to the size, shape and texture of a food.

DOG PALATABILITY DRIVERS

By Laurence Callejon, R&D Dog Platform Manager

FROM PRODUCT ATTRIBUTES & DOG INDIVIDUAL FOOT PRINT TO PALATABILY

It is important to consider palatability as a two dimensions system involving the product and the animal. Feeding behavior and preferences are guided both by the food's attributes as well as by the dog's individual characteristics.

All product attributes do not equally contribute to the overall product palatability. Figure 2 shows a representation of the relative importance of each attribute as drivers of palatability.

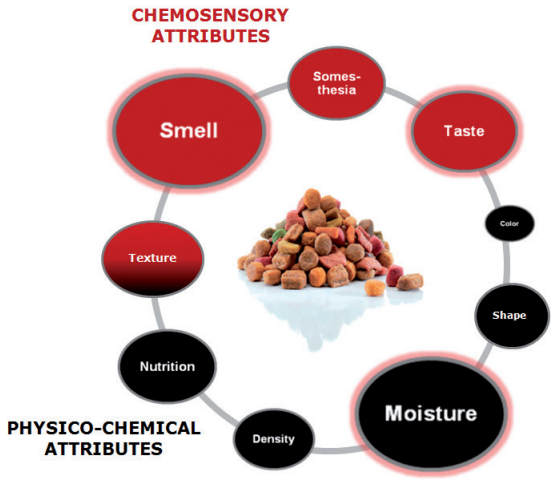


Figure 2: Main food attributes driving dog palatability

Meal time is a very complex sensorial cascade involving all the attributes listed above:

- Smell and moisture (impacting flavor release) are key for food selection,
- Size and shape are crucial for taking,
- Texture, taste and somesthesia are decisive for chewing,
- Nutrition and density are very important for digestion.

The stimuli perception and neurological treatment by the animal, affected by its feeding memory and sensorial make-up, will help him decide whether he wants to continue or stop eating.

SMELL: THE N°1 ATTRIBUTE

Smell, being dog's most developed sense, is crucial for palatability. Diana Pet Food clearly demonstrated it by analyzing palatability answers of dog expert panels over 2 years. Data of 7000 versus tests performed in Panelis (Diana Pet Food expert palatability center) Brazilian and French kennels were statistically processed. Results highlighted that 80% of 1st choice answer and consumption ratio were correlated (Figure 3), clearly establishing a strong link between pet food product smell and its consumption.

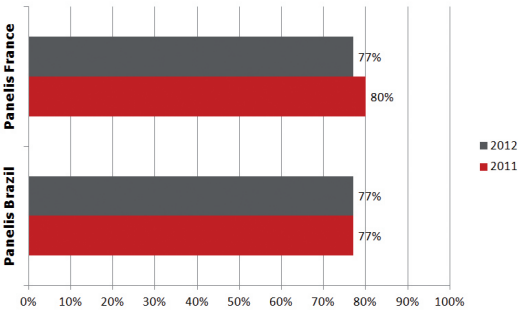


Figure 3: Correlation between 1st choice and consumption ratio at Panelis

Consequently, research on smell characterization and pet food volatile compounds is a critical investigation field for which different tools can be used.

> SENSORY ANALYSIS

It is one of the most important tools to characterize product flavor and highlight preferences. Working with both human and dogs, with expert and consumer panels, will bring research teams' complementary information.

Several protocols are used in Panelis to assess a product palatability performance. Some have specific criteria to establish the influence of a pet food smell, of which:

- Preference test: 2 bowls are presented; first choice and consumption ratio are measured. First choice will be a key indicator of the appreciation of product smell by the dog.
- Treats test: 2 treats are presented, 1st taken, 1st consumed and 1st finished are monitored. First taken is also a key indicator of the appreciation of product smell by the dog.

> ANIMAL BEHAVIOR MEASUREMENT

Study of animal behavior also gives very useful information on product appreciation. Animal video observation can help establish links between dog's behavior and product acceptance and palatability. Data such as latency (time it takes a dog to come to his bowl and start eating) will also provide information on product attractivity.

> ANALYTICAL CHARACTERIZATION

The most common analytical technique to characterize smell is gas chromatography. Recently we highlighted the need to work with a more precise tool than GC/MS* analysis because some aromatic differences that were perceived by dogs were not observed with this technique (A. Lagadec Marquez et al. 2010).

GCxGC/TOF-MS* is an innovative method providing more precise volatile profiles. GCxGC separates molecules in two dimensions leading to less co-elutions and TOF-MS is a more sensitive detector than MS-Quad. The aromatic profile obtained when combining these tools is richer (higher number of peaks) and thus probably more representative of what dogs perceive.

> PALATABILITY ENHANCER DEVELOPMENT

Statistical treatment of experimental design combines results from all sensorial, behavioral and analytical studies (Figure 4). It allows identification of compounds having positive, neutral or negative impact on palatability. Palatability enhancer performance can then be improved by:

- Integrating or synthesizing aroma having positive impact (or decreasing synthesis of molecules having a negative one),
- Selecting new raw materials or ingredients that provide the relevant precursors for positive impact molecule synthesis,
- Orienting the process in order to reach the desired olfactive/taste profile.

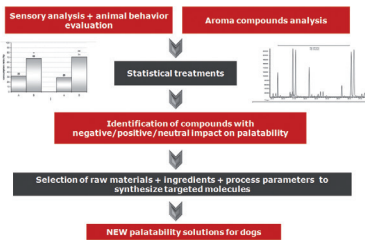


Figure 4: Dog palatability drivers integration on PE development

The omission method* can be used to identify molecules having positive, negative or neutral impact on palatability. In the example below, several compounds were selected according to their odorant activity values (concentration/detection threshold). Prototypes were formulated omitting groups of compounds and then tested in palatability versus the control containing all the compounds (control). Figure 5 presents the palatability results (treats versus protocol) of 2 prototypes (011 and 012).

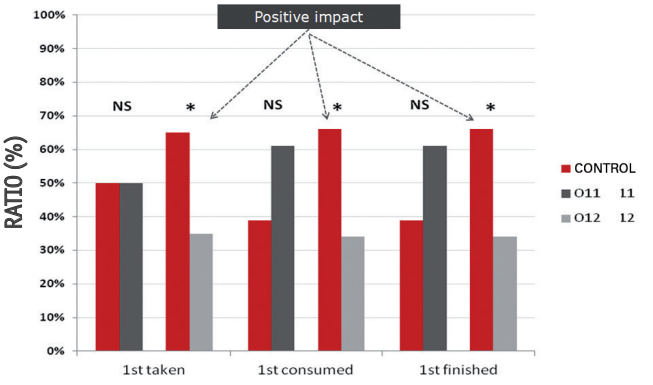


Figure 5: Palatability assessment of recombined palatant with different omissions

Prototype 011 is NS (not statistically significant*) to the control and thus neutral in palatability while prototype 012 is significantly less consumed than the control. The group of compounds removed in the prototype 012 must have a positive impact on palatability.

Olfaction is a key driver in dog palatability and innovative tools are available to understand and characterize aroma and its expression. Therefore, olfaction drives palatability enhancers' development, but other drivers need to be taken into consideration as well.

Moisture

MOISTURE: WHY DO DOGS PREFER MOISTER KIBBLES?

Moisture content strongly impacts dog palatability. Generally, dogs can start detecting a moisture difference starting at 0.5 point whereas human detection threshold starts at 1.4pt or 2pt (depending on the level of moisture and products) (Figure 6). This difference is observed whatever the palability enhancer form used (liquid, dry, liquid & dry).

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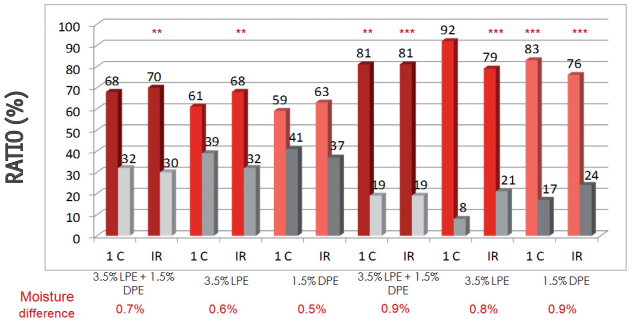

Figure 6: Impact of moisture on dog palatability

In another experiment, 2 kibbles differing only by their moisture content (3% vs 6%) were studied. First a versus palatability test was performed, obviously concluding that dogs preferred the moister kibble (Figure 7). More interestingly it was noticed that the preference started right at food selection (1st choice), highlighting that moisture variation impacts kibble aromatic perception. A complementary analysis conducted with GCxGC/Tof-MS showed that the number of volatiles compounds (furans, nitrogen and sulfur compounds) increased with kibble moisture.

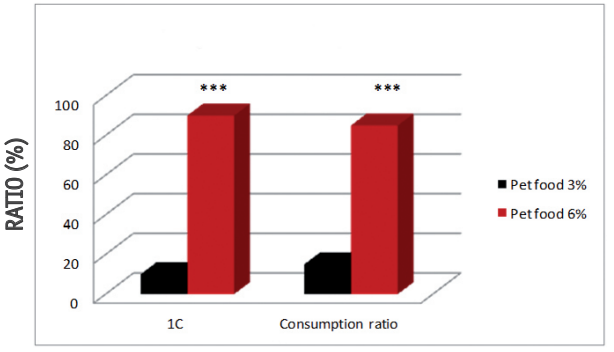

Figure 7: Palatability results (versus test) when increasing kibble moisture from 3 to 6%

Moisture impacts kibble palatability by affecting, not only food smell, but also texture. Indeed, water content will obviously modify the rigidity and the structure of the kibble.

Taste TASTE ALSO COUNTS

Taste is not as developed as smell in dogs; nevertheless it also plays an important role not in food slection, but in its consumption. Sensory and behavioral tools to measure pet food taste are similar to those used for smell. More specifically, the consumption ratios measured in the preference test as well as the assessment of acceptability monitored in the liking test (1 bowl presented) are more relevant to characterize product taste. For analytical characterization, HPLC* is classically used to identify and quantify sapid compounds such as amino acids or sugars, as well as protein or fat composition. The electronic tongue can also be used, it is especially effective to identify the taste of a known molecule in a simple system. Its interest in research and taste comprehension is therefore limited.

Palatant formulation can thus be "taste oriented". Using an experimental design (Doelhert plan*), the best combination of 2 taste-compounds leading to a 70%/30% consumption ratio was established. Some parameters were fixed (kibble, fat coating, moisture) whereas the 2 taste-compounds level were variable parameters. Results were statistically processed in order to obtain the targeted performance area through the response surface (Figure 8).

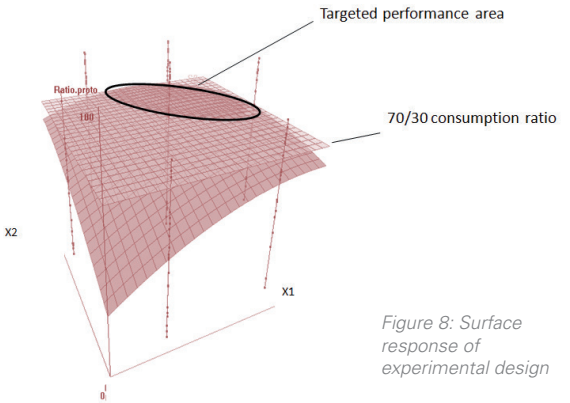

Figure 8: Surface response of experimental design

Process can also be "taste oriented". Indeed, managing protein hydrolysis and therefore the release of free amino acids and small peptides helps increase palatability performance.

AND WHAT ABOUT FAT COATING?

Fat in coating will impact several product attributes: smell, taste and nutrition.

Poultry, pork and beef fat are most commonly used in the pet food industry. In order to investigate the role fat plays in dogs'

preference, 11 fat types of 3 origins were studied and analyzed (poultry: PL1 to PL3, pork: PK1 to PK6, beef: B1 to B2, a mix of beef and pork: M1).

For each fat, the following factors were monitored:

- Fat manufacturing process (time-temperature...)
- Raw material composition (viscera, bone, adipose tissue, rind...)
- Fatty acids composition (saturated, unsaturated, omega 3, omega 6)
- Volatiles composition
- Oxidation

In this protocol, all fats were coated at 6% with 1.5% of premium dog liquid palatant. They were tested against a control coated with 6% of poultry fat and 1,5% of the same premium dog liquid palatant.
A significant effect of the fat origin on the palatability is observed. We can clearly see in Figure 9, that beef tallow and the mix between beef and pork are the most palatable solutions. Pork fat seems to be more palatable than poultry fat as well.

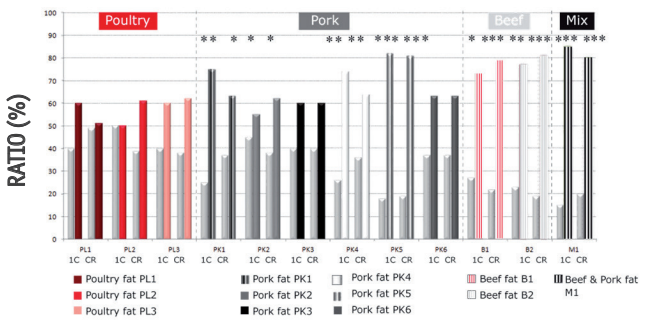

Figure 9: Palatability comparison of kibbles coated with different fat sources

We can also notice differences of dog appreciation between fats of the same origin (poultry or beef), confirming that there is an impact of fat manufacturing process and composition (volatiles, fatty acids...) on palatability. An overall approach is necessary in order to consider as well interactions occurring between fat and palatability enhancers.

Due to the high level of volatile compounds fat confers to the kibble, fat will highly impact smell. Then, the fat type and quantity used will change the overall nutrition profile and therefore affect the palatability. Furthermore, fat taste has been recently emphasized as a potential 6th taste providing in the mouth specific sensations that will have influence on the overall product taste.

TO SUM UP

Dogs have a very accurate olfaction system greatly influencing food selection and intake. Consequently, pet food smell and moisture play an important role in product acceptance. Other drivers such as taste, size and fat content will also influence the palatability and so will the interaction between palatant, fat and core kibble. For palatability experts, understanding and controlling these palatability drivers is essential and made possible thanks to innovative sensorial, behavioral and analytical tools. Sourcing innovative ingredients and precursors will help create the most adapted, high performing palatability enhancers satisfying to dogs' needs (Figure 10).

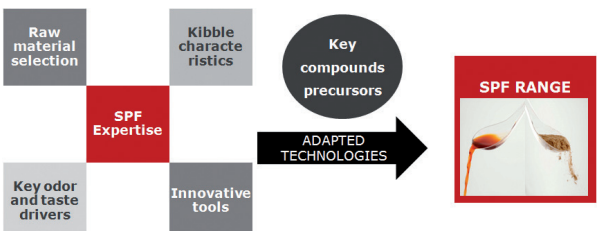

Figure 10: Management of palatability drivers to develop new products

**INDEX*
- *GC: Gas Chromatography*
- *MS: Mass spectrometry*
- *TOF: Time of flight*
- *Omission method: molecules are omitted by couples in order to see which ones have impact*
- *Student test on palatability results (levels of significance) :*
  - ****: at a 0.1% level of significance*
  - ***: at a 1% level of significance*
  - **: at a 5% level of significance*
  - *NS: Not statistically significant*
- *HPLC: High performance liquid chromatography*
- *Doehlert plan – 2 factors: Experimental points are allocated according to a regular hexagon with a central point*
- *IC: First choice during a versus palatability test*
- *IR: Intake Ratio during a versus palatability test*
- *DPE: Dry palatability enhancer*
- *LPE: Liquid palatability enhancer*

If you need further information, do not hesitate to contact the author.

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