Agri - Food

Chemicals

Geo - Environment

Energy

Application Note

Nitrogen/Protein analysis in PET food

Instrument: ECS 8024
Mode: N or CN

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The Guaranteed Analysis (GA)

Over the years, there has been an increase in the growth of the pet-food industry, driven by increasing pet ownership and a heightened awareness of pet nutrition. The growing population of adult pets is a key trend, increasing the sales of high-quality pet protein foods.

Common animal-based pet-food proteins are used in commercial pet food, such as chicken, turkey, beef, lamb, fish, etc. However, the animal-based ingredients used in the formulation of pet foods can cause an allergic reaction in a susceptible pet that can present as skin-related symptoms or as digestive and gastrointestinal symptoms.

All pet food is required to provide a Guaranteed Analysis (GA) on the product label. The GA provides product information to regulators who review each label for compliance with nutrient requirements and voluntary label claims. The GA also provides information to consumers to help them find the levels of at least four nutrients: protein, fat, fiber and moisture. Additional nutrients must be included in the GA if a product label makes a claim that mentions a specific nutrient, such as calcium for strong teeth and bones.

The term “crude” in a Guaranteed Analysis refers to the method used to determine the nutrient content of your pet’s food. Protein content is determined by analyzing levels of nitrogen, and fat content is determined by analyzing levels of lipids present.

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Understanding the Protein percentage

It is a legal requirement to show the protein percentage as well as fat, fibre, ash and moisture under Analytical Constituents on pet food labels.
However, it is not possible to compare the protein levels of different products unless we work out how much protein the animal is actually consuming (i.e. grams of protein per day), taking into account feeding amounts as well as the water content of the product.

To compare the percentage protein of a wet food and a dry food, we need to allow for the fact that a wet food contains much more water.

Any food containing high levels of water will declare relatively low percentages of proteins, fats, etc, compared to that of a dried food, but because you feed more of the wet product, your pet will receive the required nutrients.
Wet food is made of around 60-80% water and the rest is called 'dry matter' which includes all the nutrients. A direct comparison of nutrients in a wet and a dry product is not possible unless a dry matter comparison is conducted.

It is important to allow for the feeding guide to work out exactly how much protein an animal gets from its food. If you compare two dry dog foods, not only may they have differing protein percentages but they could also recommend different feeding amounts. In order to work out the actual amount of protein the dog is consuming on a wet or dry diet, you must do the following conversion:

	% protein of dry matter	Recommended feeding amount (g/day)	Protein consumed (g/day)
Diet A	20%	800	$\frac{800 \times 20}{100} = 160$
Diet B	30%	500	$\frac{500 \times 30}{100} = 150$

So we can see that even though diet A declares a lower protein percentage than diet B, because the feeding amounts of diet A are more, the protein consumed is also more than diet B.

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Elemental analysis

Element analysis methods have been actively developed during the recent decades. Generally, an element analysis can be considered as a two-step process: the first step, ie the preparation step, is aimed at eliminating organic matters and the second step at quantifying the elements.

The sample preparation
Simple preparation is of great importance for element analysis since it may dramatically influence the results. Many factors should be considered while choosing sample preparation such as the concentration range of the targeted elements, the detection technique, the sample homogeneity, the contamination level, the time and labor investment, and the reagent consumption.

The key to success
The homogeneity of the sample is the key to obtaining good results, regardless of which analytical technique is used.

The following are the steps to prepare an analysis:
- sample preparation
- sample weight
- calibration of the detector
- selecting measurement conditions correctly
- assessment and evaluation of the results

Why do we use tin foil?
Tin combusts strongly exothermic to tin(IV)oxide. The energy released during that process supports the full combustion of the sample.

Tin foil is used in sample weighing and sample tablet are made from this which are then kept in the sample tray until analysis.
The foil wrapped samples are placed in the sample tray and the autosampler is then closed with the cover plate before analysis.

Autosampler with purification system from atmospheric nitrogen.

Samples wrapped into tin foil

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The elemental analyser

While the acid digestion according to the Kjeldahl method destroys the sample in a rather rustic way, combustion provides a somewhat more elegant and time saving alternative. The sample is oxidised in a controlled way and the resulting gaseous products are subjected to further analysis. The objective is the complete conversion of the sample into the primary products carbon dioxide, water and nitrogen dioxide, without the secondary products carbon monoxide (CO) and nitrogen monoxide (NO) being produced by incomplete combustion.

As air has a nitrogen content far in excess of 70 %, the key to success here is effective sealing against the ambient air in combination with an efficient purging function. An efficient autosampler is needed so that interaction of the sample and air is ruled out.

ECS 8024 elemental analyser

The ECS 8024 elemental analyser is based on the classic principle of combustion analysis according to Jean Dumas , almost maintenance-free operation is possible with modern control software.

The pneumatic autosampler feeds the solid or liquid samples packed in tin foil to the combustion furnace. At the same time, the helium gas flow is changed over to oxygen to initiate the combustion. The sample incinerates and the ash produced is collected in the ash insert. The gaseous combustion products react completely in the presence of catalysts to the required oxides and are transported through the machine by a helium flow after combustion. The nitrogen oxides are first reduced to elementary nitrogen in the reduction reactor while the secondary products, water and carbon dioxide, are separated in special traps. A gas flow consisting helium and nitrogen remains, from which the nitrogen can be measured using a thermal conductivity detector. Computer-control enables simultaneous evaluation of the analysis data.

Some examples

In these examples, we have reported some analyses carried out on different types of PET food.

In particular, we analyzed the nitrogen concentration in dog biscuits and other unknow samples provided by a customer (we can't say more due to confidential information).

The interesting connection between biomolecules and nitrogen analysis is that nitrogen in foodstuffs and feedstuffs is only incorporated into these amino acid chains in proteins.
Determining the nitrogen content therefore provides a qualitative inference of the quantity of protein in the product, if the amino acid chain only contains a restricted number of the 20 amino acids in total. What is known as the protein factor makes it possible to calculate the protein content from the nitrogen content.
These protein factors have been specified and standardized in international conventions:

Type of sample	Protein factor
Milk and dairy products	6,38
Meat, meat products	6,25
Cereals and cereal products	6,25
Egg and egg products	6,25
Soya and soy products	6,25
Feedstuffs	6,25
Wheat and wheat products	5,7

The analytical method therefore consists in determining the nitrogen content, from which the protein content is determined mathematically:

Protein content [%]
= nitrogen content [%] * protein factor

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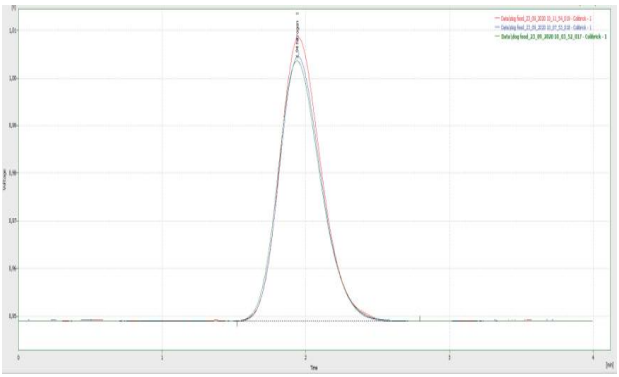
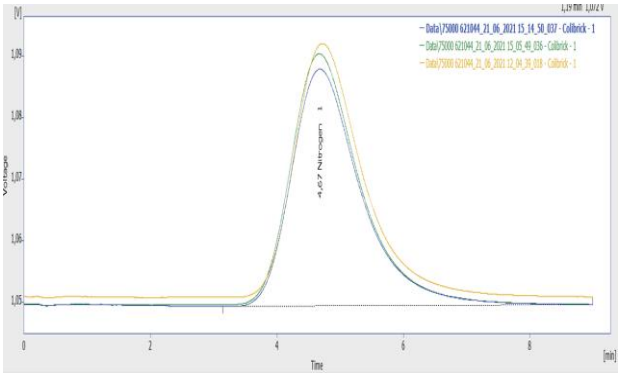
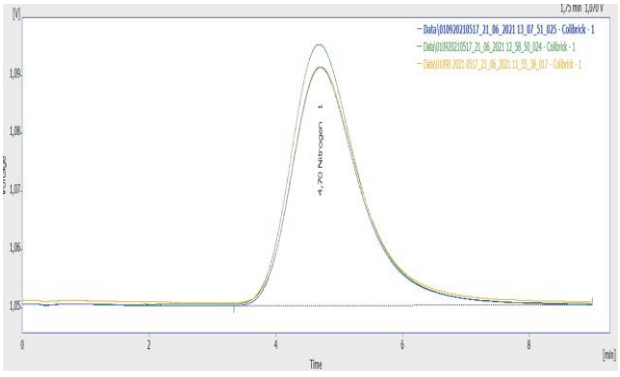
Sample	Weight (mg)	% N	Protein factor	%P
Dog biscuits	15,01	3,83	6,25	23,94
Dog biscuits	13,94	3,89	6,25	24,31
Dog biscuits	14,54	3,74	6,25	23,38
Average		3,82		23,88
Std Dev		0,08		0,47
RSD %		1,98		1,98



Sample	Weight (mg)	% N	Protein factor	%P
621044	18,62	3,82	6,25	23,88
621044	19,69	3,8	6,25	23,75
621044	19,87	3,78	6,25	23,63
Average		3,80		23,75
Std Dev		0,02		0,13
RSD %		0,53		0,53



Sample	Weight (mg)	% N	Protein factor	%P
210517	19,23	3,95	6,25	24,69
210517	20,6	3,97	6,25	24,81
210517	18,78	3,99	6,25	24,94
Average		3,97		24,81
Std Dev		0,02		0,13
RSD %		0,50		0,50



To send your samples: sales@nctechnologies.it

For analytical and technical questions: customerservice@nctechnologies.it

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