velp®

ANALYTICAL GUIDE

A Complete Overview of Protein, Fiber, Fat, and Oxidation Stability in Pet Food

The analysis of protein, fiber, fat, and oxidation stability is a core part of pet food evaluation, supporting accurate labelling, consistent quality control, and the development of balanced formulations. These parameters describe both the composition and the functional behavior of raw materials and finished products, helping ensure compliance with regulatory requirements and consistency across production batches. In dry pet food such as kibbles, processing and storage conditions can influence product characteristics over time, making reliable analytical data necessary not only for nutritional assessment but also for evaluating shelf life and stability. As formulations become more sophisticated and ingredient selection more diverse, analytical methods support the characterization of these systems and the verification of their performance.

Protein Determination (pag. 3 - 5)

Protein content is calculated from nitrogen analysis using a nitrogen-to-protein conversion factor, following two internationally validated methods.

The **Kjeldahl method**, developed in 1883, involves the catalytic digestion of organic material in concentrated sulfuric acid, converting organically bound nitrogen into ammonium sulfate. The ammonia is then released by alkalization, distilled, and quantified, allowing the calculation of nitrogen and protein content. This method is widely used due to its robustness and broad regulatory acceptance, particularly for complex matrices such as kibbles containing mixed ingredients, including cereals, animal proteins, and fiber-rich components.

The **Dumas method**, introduced in 1831 and later modernized through automated elemental analyzers, is based on high-temperature combustion of the sample, converting nitrogen compounds into nitrogen gas for detection. Unlike Kjeldahl, Dumas measures both protein and non-protein nitrogen, often resulting in slightly higher values. Its main advantages are speed, safety, and automation: analyses are completed within minutes without the use of hazardous chemicals.

Both methods provide nitrogen recoveries above 99.5% when properly executed, with modern systems ensuring reproducibility and limited operator intervention.

Fiber Determination (pag. 6 – 8)

Fiber is a component of plant-based ingredients used in pet food and influences digestibility, intestinal function, and nutritional balance. From a nutritional perspective, it is defined as the hydrolytically indigestible and partially fermentable fraction, while chemically it includes cellulose, hemicellulose, lignin, and soluble compounds such as pectins. In formulations, fiber contributes to product structure and supports digestive regulation.

Two different methods are applied.

Crude Fiber analysis (Weende method) is based on sequential acid and alkaline digestion to remove non-fibrous components, followed by gravimetric determination of the remaining residue. Crude Fiber is the standard parameter typically required for pet food labeling. **Detergent fiber analysis** (Van Soest method) provides a more detailed characterization, making it useful for evaluating ingredient composition and digestibility. Neutral Detergent Fiber (NDF) measures total cell wall components, while Acid Detergent Fiber (ADF) quantifies the less digestible fraction, primarily cellulose and lignin.

Modern fiber analyzers automate digestion, filtration, and washing steps, improving repeatability and reducing manual handling.

Fat Determination (pag. 8 – 10)

Fat content contributes to the energy value, palatability, and physical characteristics of pet food. Its determination supports formulation control, nutritional labeling, and batch-to-batch consistency.

The most commonly used technique is solvent extraction, where lipids are separated using organic solvents such as petroleum ether. The extracted fraction is then measured gravimetrically to determine total fat content. In pet food analysis, this approach is often implemented through automated systems based on Soxhlet or Randall principles, which reduce extraction time and improve reproducibility.

Automation enables precise control of parameters such as temperature, solvent volume, and extraction cycles, allowing consistent results even for complex matrices.

Oxidation Stability Determination (pag. 11 – 12)

Oxidation stability describes how pet food reacts over time to the presence of oxygen, particularly in products containing lipids. Lipid oxidation can lead to rancidity and changes in product quality, making its evaluation part of routine analysis. This parameter is typically assessed using accelerated conditions, where samples are exposed to elevated temperature and oxygen pressure. Under these conditions, oxidation processes occur more rapidly, allowing shorter time frame measurements. The main parameter obtained is the Induction Period (IP), which represents the time required for the onset of oxidation. Longer IP values indicate greater oxidation resistance. This approach allows comparison between formulations, raw materials, packaging solutions, and storage conditions, providing insight into the effects of processing and ageing, supporting shelf-life estimation and formulation adjustments.

Protein Determination in Pet Food

Introduction

Protein determination in pet food can be performed using two internationally recognized analytical approaches: the Kjeldahl method and the Dumas method. Both are accepted by major standardization bodies such as AOAC, AACC, and ISO, and are routinely applied for labelling, quality control, and research activities across the pet food sector. The **Dumas method**, introduced in 1831, predates the Kjeldahl technique (1883). For many years, however, it was more difficult to apply consistently because it requires tightly controlled combustion conditions that early instrumentation could not always reproduce. As a result, the **Kjeldahl method** became the most widely adopted procedure and remained the standard approach for nitrogen and protein determination for a long time, especially in routine and regulatory laboratories. Advances in instrumentation have progressively changed this scenario. Modern combustion analyzers allow the Dumas method to be performed rapidly, cleanly, and with full automation, reducing manual handling and increasing laboratory throughput. The technique detects all nitrogen-containing compounds (such as nitrates, nitrites, and certain heterocyclic molecules), so results may be slightly higher than those obtained with Kjeldahl.

In comparison, Kjeldahl relies on the conversion of nitrogen into ammonium, and some compounds may be only partially converted, leading to lower measured values. Occasional deviations may also occur, as the Kjeldahl workflow includes several procedural variants involving different catalysts, digestion conditions, acid volumes, and sample sizes.

These variables can influence the final result if the method is not applied consistently. When properly optimized, both approaches reach nitrogen recovery levels of ≥ 99.5%.

The detection limit is generally lower for Dumas (around 0.001 mg N absolute) compared with Kjeldahl (≥ 0.1 mg N absolute), making the two techniques suitable for different laboratory workflows and sample types.

This allows analysts to select the approach that best aligns with throughput requirements, matrix characteristics, regulatory expectations, and laboratory setup.

Nitrogen and protein determination in pet food samples are compared using:

- The NDA 702 Dumas Nitrogen Analyzer,
- The Kjeldahl system, composed of:
  - DKL 20 Automatic Digestion Unit,
  - KS 1000 Scrubber,
  - UDK 159 Automatic Distillation Unit.

Kjeldahl Method

The modern Kjeldahl method consists in a procedure of catalytically supported mineralization of organic material in a boiling mixture of sulfuric acid and sulfate salt at digestion temperatures higher than 400 °C.

During the process, the organically bonded nitrogen is converted into ammonium sulfate. Alkalizing the digested solution liberates ammonia, which is quantitatively steam distilled and determined by titration.

1) Sample Digestion

Grind the sample using a grinder (1 mm particle size). Weigh 0.5 - 1 g of the sample to an accuracy of 0.1 mg into a 250 ml test tube. For each sample, add to the test tube:

- 1 catalyst tablet VW (code A00000282; 4.875g K_2SO_4, 0.075 g $CuSO_4$ x $5H_2O$, 0.050 g Se, 5 g) 13 ml concentrated sulphuric acid (96-98%),
- 2 antifoam tablets VS (Code A00000283),
- 20 ml concentrated sulfuric acid (96-98%).

Prepare some blanks with all chemicals and without a sample.

Connect the Digestion Unit to the KS 1000 Scrubber (code F307A0660) to neutralize the acid fumes created during the digestion phase.

Digest the samples for 60 min. at 420 °C according to the method “cereals and animal feed” (n° 7 on DKL 20).

2) Distillation and Titration

Let the test tubes cool down to 50-60 °C.

Condition the UDK 159 unit by performing the Automatic Check-up in the Menu-System and a Washdown.

Distill the samples according to the following parameters (pre-defined method n° 7):

- H_2O (dilution water): 50 ml
- HCl (0.2N) as titrant solution
- NaOH (32 %): 50 ml
- Protein factor: 6.25
- H_3BO_3 (4 % with indicators): 30 ml

Distillation & Titration analysis time: from 4 minutes for one test.

Dumas Method

The modern Dumas method consists of the complete combustion of the sample in an oxygen-rich environment at temperatures typically above 1000 °C.

During the process, all nitrogen-containing compounds are converted into nitrogen gas, which is then purified from other combustion products and quantitatively measured by a thermal conductivity detector.

1) Sample Preparation

Grind the test samples using a grinder to a suitable fineness (particle size ≤ 0.5 mm) to obtain ≤ 2,0 % relative standard deviation (RSD) for 10 successive nitrogen determinations.[1]

Using a spatula, put the sample directly into the tin foil. Close the tin foil, obtaining a capsule, and load the capsule into the autosampler.

2) Analysis

Fill the following fields in the database:

- Sample name,
- Weight, Method,
- Sample type,
- Calibration curve.

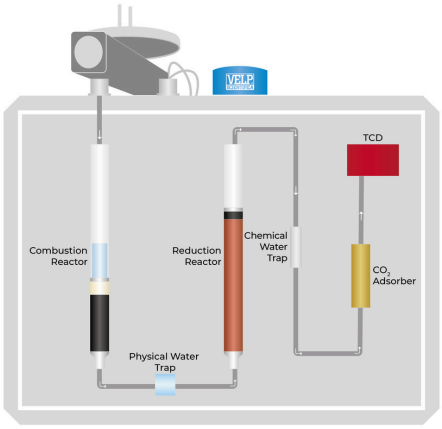
The “FEED FOR ANIMALS, DRY” method shows the following parameters:

- Protein factor: 6.25
- O_2 flow rate: 400 ml/min
- O_2 factor: 1.6 ml/mg.

Press “start” to begin the analysis. Analysis time: from 3 minutes for one run.

The analysis is carried out using the NDA 702 Dumas Nitrogen Analyzer, based on high-temperature catalytic combustion technology, which has proved to deliver precise, reliable, and matrix-independent results in a few minutes.

The samples are introduced into the combustion reactor via the electronic autosampler after purging with carrier gas. During combustion, the solid sample is converted into a gas mixture containing NO_2, H_2O, CO_2, and residual O_2. After combustion of the sample, the gases produced are carried by a helium flow to the DriStep™ electronic physical trap, able to condense more than 99 % of water.



Flow diagram - NDA 702 Dumas Nitrogen Analyzer

The gas stream then reaches the reduction furnace, where a formulation of highly active copper powder Vcopper™ helps the reduction of NOx into molecular nitrogen N_2. Before detection, the gas passes through a chemical trap for fine removal of water and moisture.

The sample gas is led to the chemical-free auto-regenerative CO_2 absorbers, which let pass only the elemental nitrogen that is detected by the innovative LoGas™ Thermal Conductivity Detector (TCD) with no requirement for a reference gas. The signals from the detectors are transferred to the PC for further calculation by the software package DumaSoft™, and the results are displayed in 3 – 5 minutes.

[1]AOAC 990.03 - Protein (Crude) in Animal Feed: Combustion Method

Method Comparison

The Dumas method offers several advantages, including simplicity, speed, safety, and a high level of automation, which allows for largely unattended operation in the laboratory. Unlike the Kjeldahl method, the Dumas method does not rely on concentrated sulfuric acid or chemical catalysts, eliminating exposure to hazardous reagents. Numerous comparative studies have been published, and the Dumas method has been recognized in a series of international standards.

Advantages of the Dumas combustion method include:

- Ease of operation – Samples are simply enclosed in tin foil and loaded directly into the analyzer.
- Short analysis time – From 2 hours with Kjeldahl to as little as four minutes per sample with Dumas.
- Operator safety – No hazardous chemicals required.
- No chemical waste – Minimal environmental impact.

At the same time, the Kjeldahl method remains widely used and valued for its robustness, long-standing regulatory acceptance, and compatibility with a broad range of sample matrices. Its procedural flexibility—including different catalysts, digestion conditions, and sample sizes—allows laboratories to optimize workflows according to specific feed types and analytical requirements. For many routine quality control applications, the Kjeldahl method continues to provide reliable and reproducible results, especially when standard operating procedures are carefully validated.

Conclusion | Protein Determination

Nitrogen values obtained with the two methods are closely aligned, confirming good agreement between Kjeldahl and Dumas for pet food samples. As expected, slightly lower recoveries are observed with the Kjeldahl method, since Total Kjeldahl Nitrogen accounts mainly for organic nitrogen and ammonia. In contrast, the Dumas method typically provides slightly higher values, as it also detects nitrogen from compounds such as nitrates and nitrites.[2]

Both approaches provide consistent and reproducible results when properly applied, making them suitable for routine protein determination in processed matrices such as kibbles. The choice of the method depends on laboratory workflow, throughput requirements, and preferred level of automation.

Standard References

Kjeldahl	• AOAC 2001.11 • AOAC 984.13 • REG CE 152/2009 • EN ISO 5983-2:2009
Dumas	• AOAC 992.23 • AOAC 990.03 • ISO 16634-1:2008

Sample name	Method	Sample quantity	Results (% N)	Results (% P)	Average ± SD% (% N)	Average ± SD% (% P)
Kibbles	**Kjeldahl**	1.060 g	3.81	23.84	**3.83 ± 0.04**	**23.95 ± 0.21**
		1.052 g	3.82	23.87		
		1.041 g	3.81	23.80		
		1.011 g	3.83	23.92		
		1.067 g	3.89	24.31		
	Dumas	103.10 mg	4.08	25.47	**3.93 ± 0.1**	**24.57 ± 0.62**
		104.29 mg	3.95	24.69		
		107.64 mg	3.94	24.64		
		100.27 mg	3.81	23.81		
		103.39 mg	3.88	24.22		

[2]Etheridge, R.D. et al. (1998) — "A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies on samples typical of an animal nutrition analytical laboratory"

Fiber Extraction in Pet Food

Introduction

Fiber is a structural component of plant-derived ingredients used in pet food, contributing to the physical structure of kibbles and influencing digestive processes. From a nutritional perspective, it is defined as the hydrolytically indigestible, partially fermentable fraction of the diet. Chemically, fiber is a variable mixture of cellulose, hemicellulose, lignin, and soluble dietary fibers such as pectins. The determination of fiber content in pet food is routinely performed to support product characterization, formulation control, and compliance with labeling requirements. In dry pet food, fiber plays a role in regulating intestinal transit, stool quality, and overall digestive balance.

As stated by the Association of American Feed Control Officials (AAFCO), “Because there is no guarantee of direct correspondence between chemical solubility and nutritional availability, in reality, fiber is defined by the method used to isolate it. The actual definition of fiber becomes method-dependent.” This highlights the need to strictly follow official methods to ensure reproducible and comparable results.

Crude Fiber (CF)

This method relies on the digestion of non-cellulosic compounds with sulfuric acid and potassium hydroxide. The remaining residue, after drying and loss-on-ignition, is weighed to determine crude fiber content.

Crude Fiber is commonly applied to processed pet food matrices such as kibbles and to their raw material components, including:

- Cereals (corn, wheat, barley)
- Protein meals and flours: soybean meal, wheat bran
- Formulated blends where fat has been removed before analysis

CF provides an estimate of the indigestible fraction of pet food, mainly cellulose and lignin, and is suitable for routine quality control and labeling purposes, where it remains the reference parameter. However, its main limitation is that it underestimates total fiber content, as hemicellulose and part of the soluble fraction are lost during chemical treatment. For this reason, it provides limited information on the full fiber composition of more complex formulations.[3]

Determination of Crude Fiber

The CF determination can be summed up in 5 steps:

During the **acid digestion** phase (boiling and filtering), the feed sample is boiled in a 1.25% sulfuric acid solution. This treatment solubilizes and removes the acid-digestible components of the sample, primarily sugars and starches. The acid digestion targets the easily hydrolysable fractions, allowing the structural components of fiber to remain in the residue.

After this step, the sample undergoes a **washing** stage, where the crucibles are thoroughly rinsed with hot water. This washing removes residual acid and brings the pH back toward neutrality, ensuring that no acid remains that could interfere with the next phase of digestion. The process then continues with the **alkaline digestion**, using a 1.25% sodium or potassium hydroxide solution.

This treatment removes proteins, part of the hemicellulose, and a portion of the lignin. The alkaline digestion isolates the more resilient fiber components by eliminating additional non-fiber materials that were not solubilized during the acid phase. Another **washing** step follows, again using hot water, to completely remove alkali residues from the crucibles and neutralize the pH. Proper washing is essential to prevent chemical carryover and ensure accurate gravimetric determination. The final phase is the **defatting** step, where the residues are rinsed with acetone. This step extracts remaining fats and helps promote rapid drying by removing moisture. Once the acetone evaporates, the crucibles can be dried and weighed to determine the crude fiber content accurately.

[3]de-Oliveira, L.D. et al. (2012) — "Fibre analysis and fibre digestibility in pet foods – a comparison of total dietary fibre, neutral and acid detergent fibre and crude fibre", Journal of Animal Physiology and Animal Nutrition, 96: 895–906.

Chemicals and Equipment Required

- Analytical balance, 4 decimals.
- Glass Crucibles P2, 6pcs (A00000140).
- Sulfuric acid (H_2SO4) 1.25%.
- Potassium hydroxide (KOH) 1.25%.
- n-octanol as an antifoaming agent.
- Acetone, technical grade.
- FIWE Advance – Fully Automatic Fiber Analyzer
- COEX – Cold Extractor

Crucibles Preparation

Connect the optional Velp barcode reader to the FIWE Advance. Select Analysis/Details, scan the crucible, weigh 1 g of the sample portion into each crucible (M_{sample}), and transfer the M_{sample} value from the balance to FIWE Advance. This operation is repeated sequentially for the remaining positions.

Analysis with FIWE Advance

Select “Analysis” and then method “CF - Crude fiber (Weende method)”, including the following parameters:

- Crucibles porosity P2
- Preheat: Yes
- Enzyme: No
- H_2SO4: 150 ml
- N-octanol: Yes
- Digestion time: 30 minutes
- Washing: 3 x 50 ml of distilled water
- KOH: 150 ml
- Digestion time: 30 minutes
- Washing: 3 x 50 ml of distilled water

Lower the lever and position the heating shield, and press “Start” to begin the process.

At the end of analysis, remove the crucibles from the unit, place them in the COEX unit for defatting (25 ml acetone for 3 times), and dry the crucibles (130 °C ± 2 °C). Leave to cool in the desiccator.

In the Result menu, select the crucibles batch ID analyzed, press calculate, scan the crucibles with a barcode reader, and weigh to the nearest 0,0001 g (Mdry). Ignite the crucible with the residue in a muffle (525 ± 15 °C) for at least 3 h or until carbon-free. Leave to cool in a desiccator.

In the Result menu, select the crucibles batch ID analyzed, press calculate, scan the crucibles with a barcode reader, and weigh to the nearest 0,0001 g (M_{ash}).

Crude Fiber Calculation

To calculate this parameter, it is necessary to subtract the weight of the crucible after the dry process from the weight of the crucible after the incineration in the muffle.

Crude Fiber % = (M_{dry} - M_{ash}) *100/M_{sample}

Where

M_{dry} = sample weight after drying

M_{ash} = sample weight after ashing

M_{sample} = sample weight

Sample name	M_{sample} (g)	M_{dry} (g)	M_{ash} (g)	CF %
Kibbles	1.0800	31.079	31.058	1.94
	1.0749	31.788	31.768	1.94
	1.0670	31.767	31.747	1.92
			Average ± SD %	**1.93 ± 0.01**

Conclusion | Fiber Extraction

The fiber analyses performed delivered reliable and reproducible results, with values in line with the declared crude fiber content on the label (1.9%).

The use of an extraction system specifically designed for fiber determination, such as the FIWE Advance, simplifies the standardization of analytical conditions and improves overall laboratory workflow.

Compared with traditional manual procedures, analysis time is significantly reduced (2 hours with automated fiber analyzers versus up to 6 hours manually), while automated filtration supported by integrated pump and air pressure ensures consistent operation and ease of use. The results obtained show high reproducibility and compliance with official analytical procedures.

Standard References

- ISO 6865:2000
- AOAC 978.10
- AOAC 962.09
- REG CE 152/2009

Fat Extraction in Pet Food

Introduction

Fat content determination is a routine parameter in pet food analysis, supporting product characterization, labeling verification, and formulation control. Accurate fat measurement allows consistent evaluation of energy content and helps monitor variations between production batches, as well as changes occurring during storage. This study describes a reliable method for the determination of crude fat in pet food samples, based on solvent extraction according to the Randall principle. The method relies on the solubilization of lipids into an organic solvent, followed by gravimetric quantification of the extracted fraction after solvent removal.

Compared to traditional Soxhlet extraction, the Randall approach operates through a sequence of immersion, washing, and solvent recovery phases, significantly reducing analysis time while maintaining comparable extraction efficiency.

The methodology is suitable for processed matrices such as dry pet food (kibbles), which often contain a combination of animal and vegetable lipid sources distributed within a complex structure.

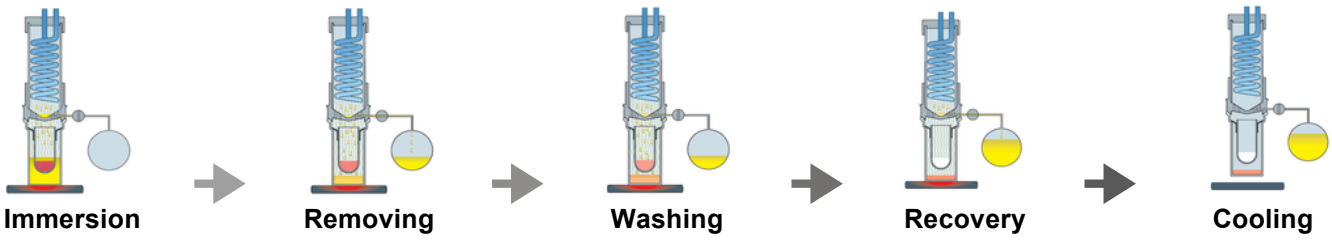
In this study, fat extraction was performed on two different kibble formulations, allowing the evaluation of inter-formulation variability in lipid content and composition.

The SER 158 Automatic Solvent Extractor, with its automated operation and controlled extraction parameters, enables consistent analytical conditions, reduces manual handling, and supports reproducible results.

Extraction Procedure

The SER 158 Series - available in 3 and 6 positions - automates the hot solvent extraction process, typically involving five steps:

1) Immersion: The sample, weighed in an extraction thimble, is immersed in the boiling solvent.
2) Removing: The solvent level is automatically lowered below the thimble.
3) Washing: Condensed solvent flows over the sample and through the thimble, extracting the target compound.
4) Recovery: Approximately 90% of the solvent is collected in the internal recovery tank for reuse.
5) Cooling: The extraction cups containing the extracted matter are raised and cooled to prevent burning. The cups are then transferred to a drying oven, followed by a desiccator, and weighed to calculate the extract percentage.



Extraction process with the Velp SER 158

Chemicals and Equipment Required

- Solvent: petroleum ether
- Analytical balance, min. 3 decimals
- Cellulose thimbles 33x80 mm (Code A00000295)
- Glass extraction cup STD Ø 56x120mm (Code A00000290)
- Extraction thimbles holder Ø33 mm (Code A00000312)
- Viton seals (Code A00000297)
- Grinder

Extraction Procedure

The extraction cups containing the thimble can now be placed on the heating plate of the SER 158. To perform the extraction, select “Analysis” on the ControlPad and then method “Crude fat in feed”, with these parameters:

- Immersion Time: 25 minutes
- Removing Time: 10 minutes
- Washing Time: 55 minutes
- Recovery Time: 30 minutes
- Cooling Time: 4 minutes
- Petroleum Ether 40-60 °C, 100 ml

Close the safety guard and press “Start” to begin the extraction process. Once complete, transfer the extraction cups containing the extract to a drying oven set at 105°C for one hour. Allow them to cool to room temperature in a desiccator, then carefully weigh and record the final mass.

Sample Preparation

Grind the sample, reducing particle size to 0.75 -1 mm. Fix the extraction thimbles using the extraction thimble holders. Then, weigh 3 g of the sample directly in the Velp extraction thimbles placed on the balance.
Place the empty cups in a drying oven (1 hour at 105 °C), then cool them in a desiccator to room temperature and record their weight for the tare. Load the thimbles containing the samples into the extraction cups and place them on the heating plate of the SER 158.

Results on Pet Food Samples

Results are automatically calculated and displayed by the ControlPad when weights are entered, either via a connected balance or manually.
The extract percentage calculation is performed by using the following formulas:

Extract (g) = (Total - Tare)
Extract (%) = Extract (g) x 100 / (Sample)

Where:
Sample = sample weight (g)
Tare = weight of the empty extraction cup (g)
Total = weight of the extraction cup + extract (g)
The following table reports the results of fat extraction for two different kibble formulas (dry dog food) with different fat contents.

Sample name	Batch	Tare (g)	Total (g)	Extract %	Average ± SD%
Kibbles	Formula 1	135.31	135.65	11.07	11.02 ± 0.95
		134.27	134.61	10.98	
		135.25	135.59	11.05	
		135.74	136.07	11.05	
		135.17	135.51	11.12	
		135.64	135.97	10.83	
	Formula 2	134.75	135.21	15.18	15.14 ± 0.87
		134.31	134.77	15.27	
		134.78	135.24	14.93	
		140.08	140.53	15.03	
		135.41	135.87	15.19	
		136.63	137.10	15.24	

Conclusion | Fat Extraction

The solvent extraction analyses performed on the two kibble formulas provided consistent and reproducible results, with a clear difference observed between the two formulations. Formula 1 showed an average fat content of 11.02 ± 0.95%, while Formula 2 resulted in a higher average value of 15.14 ± 0.87%. When compared with the declared nutritional values on the label, the results confirm that the method is capable of distinguishing between formulations with different fat contents, even in complex matrices such as pet food.

Across all replicates, the variability remained limited, confirming good repeatability of the method under the same operating conditions. The data also indicate that the extraction approach based on the Randall principle provides stable recovery of the lipid fraction, with gravimetric determination offering a straightforward and robust quantification step.

Overall, the SER 158 Automatic Solvent Extractor ensured controlled and consistent extraction conditions, reducing operator-dependent variability and supporting reliable fat determination in routine analysis.

The automated workflow, combined with efficient solvent handling and standardized extraction steps, contributes to improved laboratory efficiency while maintaining analytical consistency across different sample types and formulations.

Standard References

- ISO 11085:2015
- AOCS Ba 3-38

Oxidation Stability in Pet Food

Introduction

Lipid oxidation is one of the main mechanisms responsible for quality deterioration in pet food, leading to changes in flavour, nutritional value, and overall product acceptability. In dry dog food (kibbles), oxidation processes can progress during storage and may be influenced by formulation, raw materials, and packaging conditions.

Oxidation stability assessment provides a practical way to evaluate how resistant a product is to oxidative degradation over time. In this study, two batches of kibble (fresh and aged) were analysed to compare their behaviour under accelerated oxidation conditions and to assess possible differences related to storage time.

Oxitest Principle

The oxidation stability of the samples was evaluated using the Oxitest reactor, which accelerates lipid oxidation by exposing the sample to controlled temperature and a high-pressure oxygen environment.

The system monitors the decrease in oxygen pressure inside two sealed chambers. This pressure drop is used to calculate the Induction Period (IP), defined as the time required before a noticeable increase in oxidation rate occurs. A longer IP corresponds to higher resistance to oxidation and better lipid stability.

The method enables rapid comparison of different formulations, storage conditions, or product batches under identical experimental conditions. The Oxitest also allows different evaluation scenarios, including:

- Repeatability Test
- Freshness test
- Formulas comparison
- Packaging comparison
- IP during ageing
- Estimated shelf life

In the present study, two batches of dry dog food (kibbles) with different formulations were analysed.

The Formula Comparison test was applied to interpret the results from complementary perspectives.

Sample Preparation

Samples of dry dog food were stored at room temperature before analysis.

Each sample was ground to obtain a homogeneous particle size distribution, ensuring representative oxidation behaviour during testing. Approximately 25 g of the sample was then weighed and carefully distributed on the titanium sample holders.

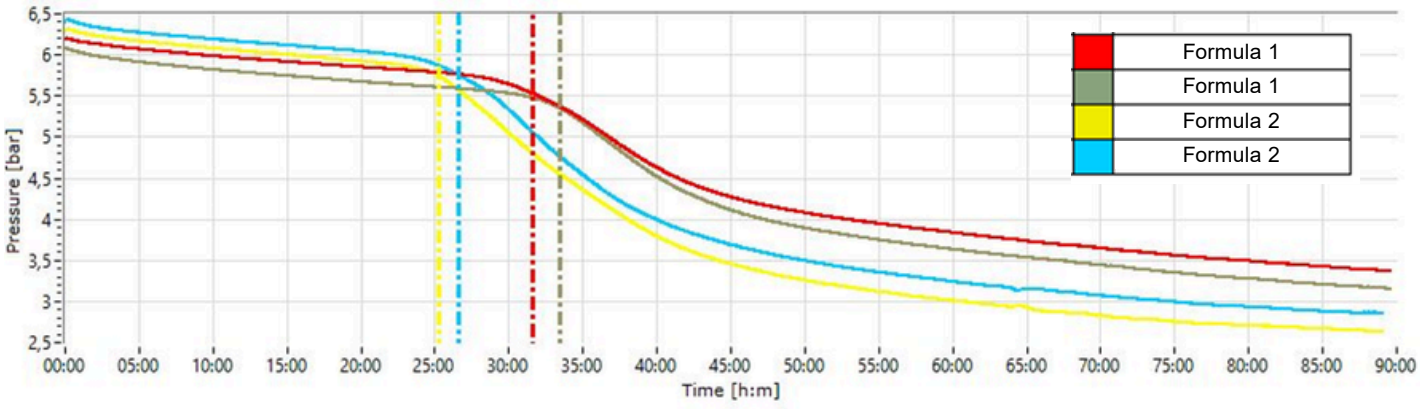
The same amount of material was prepared for each chamber to ensure comparable test conditions between the two reaction cells.

Analysis Procedure

Before starting the analysis, the O-rings of the reaction chambers were greased with silicone and properly positioned to ensure airtight sealing. The chambers were then closed using titanium covers, and the discharge valves were left open during the initial heating phase. The following experimental conditions were set using the OXISoft™ software:

- Temperature: 80 °C
- Oxygen pressure: 6 bar

Once the set temperature was reached inside the chambers, the discharge valves were closed, and oxygen was introduced into the system. Data acquisition started automatically, and the system recorded the oxygen pressure decrease over time until the end of the induction period.



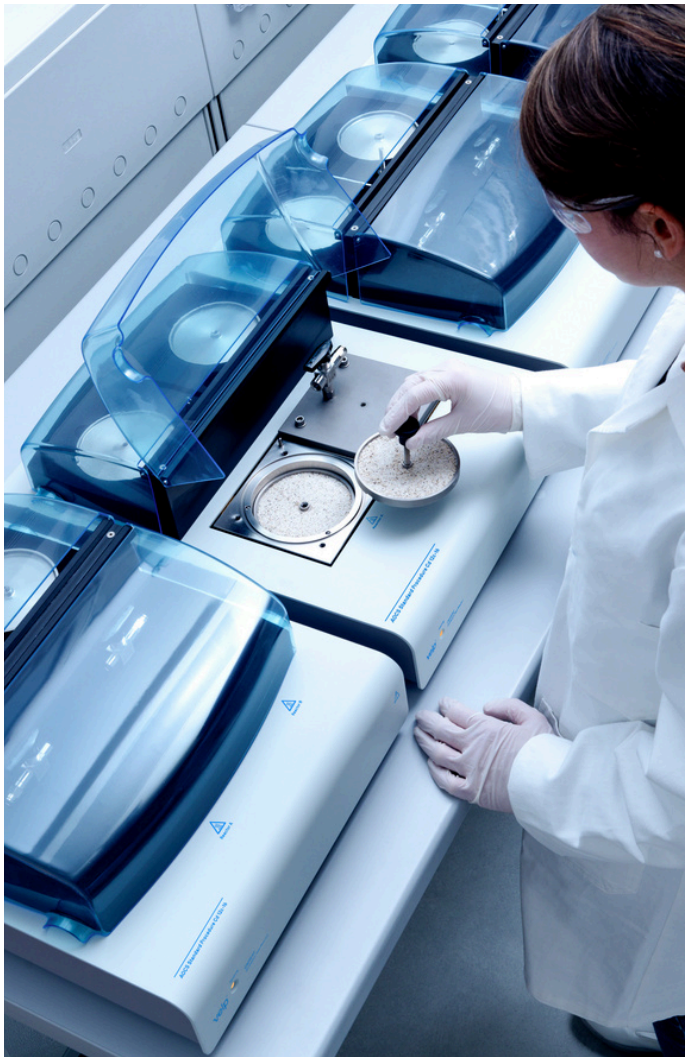
Results

The results obtained for the Induction Period (IP) are reported below:

- Formula 1: 31:38 h and 33:29 h
- Formula 2: 25:16 h and 26:38 h

Formula 1 showed a longer Induction Period compared to Formula 2, indicating a higher resistance to oxidation under accelerated conditions. These results confirm that the Oxitest is capable of discriminating between formulations of the same product, effectively differentiating their oxidative stability.

The Oxitest system also allows the evaluation of product stability through dedicated software functions such as freshness assessment and formulation comparison, enabling a broader interpretation of oxidative behaviour between different samples.



Sample name	M_{sample} (g)	Induction Period (h:mm)
Formula 1	25.0	31:38
	25.0	33:29
Formula 2	25.0	25:16
	25.0	26:38

Conclusion | Oxidation Stability

The oxidation stability assessment performed with the Oxitest reactor allowed a clear comparison between the two kibble formulas, highlighting differences in oxidative behaviour related to different compositions.

- Formula 1 showed longer induction periods, indicating a slower onset of oxidative processes under accelerated conditions.
- Formula 2 reached the oxidation transition earlier, suggesting a lower resistance to oxidation consistent with its higher fat content.

Beyond the numerical results, the study confirmed the usefulness of the Oxitest approach for rapidly differentiating formulations with different formulations under standardized conditions. The controlled environment of temperature and oxygen pressure ensured consistent measurement conditions, while the Induction Period provided a straightforward parameter to describe oxidation resistance.

The application of the Formula Comparison test further supported the interpretation of the data, allowing the two formulas to be evaluated not only in terms of oxidation resistance but also as products with potentially different stability profiles related to their composition.

Overall, the method proved well-suited for routine evaluation of lipid stability in pet food, offering reproducible results and a practical way to support product development, quality control, and shelf-life assessment."

Standard References

- AOCS Cd-12c-16

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