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Article

Validation of an Analytical Method by Headspace Gas Chromatography with Flame Ionization and Evaluation of Matrix Effect of Volatile Compounds in Cat Food

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This study describes the validation of a headspace gas chromatography with flame ionization (HS-GC-FID) method for the determination of propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal originated by lipid oxidation from cat food samples, as well as the evaluation of the matrix effect in the results. This method was applied to the analysis of commercial cat food and proved to be suitable for the determination of these volatile compounds in different samples. Mean recoveries between 88 and 109% were obtained and repeatability expressed as relative standard deviation was always lower than 6.95%. The intra- and inter-assay precisions ranged from 0.44 to 20.88% and from 0.45 to 20.52%, respectively. In addition, the matrix effect of cat food samples was determined by comparing the slopes of the standard addition method, and the external calibration curve and its influences were verified. These results highlight the high potential of this method, which allows the determination of lipid oxidation products in cat food samples directly, without requiring prior sample preparation techniques.

Keywords: aldehydes, lipid oxidation, cat food, degradation products, GC-FID

Introduction

The animal feed industry, particularly for dogs and cats, has been responsible for an important economic movement over the past few years. Rations should be nutritionally suitable for the proper development, health, and welfare of pets.[1,2] The main nutrients necessary in animal food include proteins, fats, carbohydrates, fibers, vitamins and minerals.[3] The source of lipids in animal food has a function of energy supplementation and influences palatability, digestibility, and fatty acid profile.[4] Lipids are complex mixtures comprising a wide range of compounds, such as, triacylglycerols, diacylglycerols, free fatty acids, phospholipids, and other minor components.[3,5] However, the presence of this portion of fat may be sensitive to lipid oxidation resulting in deterioration of the food.[4]

Lipid oxidation is a chemical reaction that results in the production of undesirable compounds, odors, and off-flavors, besides it is responsible for decreasing the nutritional value, deterioration of the texture, and reducing the shelf life of food items.[6-8] The primary oxidation products are those that can be produced through the reaction of oxygen in a free radical process with unsaturated fatty acids, triacylglycerols, saturated fatty acids, monounsaturated fatty acids, and even esters.[9] However, they are highly reactive compounds and decompose to produce volatile and non-volatile secondary products, including aldehydes, which are normally secondary products used to indicate the oxidation level.[6,8,9] Hexanal is the main product of fats oxidation used to monitor the flavor and odor deterioration of pet food. In this regard, the identification and quantification of volatile compounds such as hexanal and their octanal, propionaldehyde, *trans*-2-nonenal equivalents, among others, are useful chemical indicators of oxidative deterioration.[9]

Volatile compounds from lipid oxidation are routinely analyzed by gas chromatography with flame ionization (GC-FID) detection.[10] Ally GC-FID with the headspace (HS) technique is an opportunity to improve the quality of the chromatographic analysis, which consists of collecting the gas phase containing analytes above a solid or liquid

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matrix in a closed vial to be sequentially analyzed by GC.[10,11] Such association is an effective measurement technique of volatile chemicals in complex matrix samples and requires minimal sample treatment resulting in shorter time analysis and offering high throughput performance.[10,12]

Peña *et al.*[13] used this HS-GC technique to quantify linear aldehydes, from pentanal to decanal. The authors chose to use this injector, which consists of pre-concentrating the analytes. The authors compare the results obtained by this method with those obtained by the traditional HS method, where it was found that the first, by promoting the pre-concentration of the analytes, tends to provide more analytical sensitivity, thus allowing the linear range to be wider at lower analyte concentration values.

Optimizing the parameters that influence an HS-GC-FID analysis is a critical step in the development of a method. Several factors exert influence over an HS-GC-FID method, such as injection volume, incubation temperature, sample mass, among others.[14] Therefore, consideration of these factors is essential to optimizing the process to achieve higher responses. Optimization of analytical procedures can be achieved through the use of multivariate statistical techniques using response surface methodology (RSM), which is based on the adjustment of a polynomial equation and symmetrical models to the experimental data to describe the behavior of the independent variables. Furthermore, by using RSM, it is possible to reduce the number of experiments as well as obtain more information about the characteristics of the variables.[15,16]

Once a method of analysis has been developed, it must be tested and approved to be considered adequate for the proposed purposes, normally assessing parameters such as selectivity, linearity, precision, accuracy, robustness, and matrix effect (ME).[17] ME is a validation parameter that aims to assess the interference of the matrix components in the analyte signal. These interferences can increase or decrease the chromatographic response of the analyte, leading to quantification errors.[18] However, a method to compensate the matrix effects in headspace analysis is the use of internal standards.[18]

In this context, the present study developed and validated a headspace-GC-FID method to determine propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal in cat food samples.

Experimental

Chemicals

Propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal were purchased from Millipore Sigma (St. Louis, MO, USA). 2-Propanol was purchased from Merck (Darmstadt, Germany). All reagents were of analytical grade and all reference standards had purities greater than 95%. Ultrapure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

Sample

Cat food samples were acquired at pet shops in Maringá (Paraná, Brazil) (23°25'31"S, 51°57'19"W). Afterward, they were grounded, sieved (80 mesh) to ensure homogeneity, and stored in polyethylene containers.

Standard solutions

Propanal (499.10 μg mL^{-1}), pentanal (498.96 μg mL^{-1}), hexanal (499.19 μg mL^{-1}), octanal (500.00 μg mL^{-1}), and *trans*-2-nonenal (499.14 μg mL^{-1}) individual stock solutions were prepared in 2-propanol solvent and stored at −18 °C. A working standard mixture solution was prepared by appropriate dilution of the stock solutions in 2-propanol. The spiked sample solution was prepared by adding 800 μL of working standard mixture solution into 20.0 mL headspace vial containing 1.0 g of sample and 4.2 mL of ultrapure water. Moreover, 5.0 mL of ultrapure water was added to a 20.0 mL headspace vial containing 1.0 g of sample, which was used as non-spiked sample. To construct the standard addition curves, solutions with six concentration levels were selected according to the detectability of each analyte. An external calibration in the same concentrations was also performed by the dilution of the working standard solution in ultrapure water.

Experimental design

Different parameters were evaluated for optimization of the HS-GC-FID method. A central composite design (CCD) was developed to assess the influence of the factors: incubation temperature (X_1, ºC), incubation time (X_2, s), salting out (X_3, mol L^{-1}) and water volume (X_4, mL). The CCD, associated with the surface response methodology (SRM), was developed by the Design Expert software.[19] The four factors were coded at five levels, $-\alpha$, −1, 0, +1 and $+\alpha$ (Table 1) which resulted in an experimental design of 30 experimental points, including five central points. Analysis of variance (ANOVA) was used to estimate the statistical parameters. The fitting quality of the polynomial model was evaluated by way of the determination coefficient (R^2). The results were expressed as sum of chromatographic peak area.

Table 1. Factors and their coded and actual values used for the central composite design

Factor	Level				
	−α	−1	0	+1	+α
Incubation temperature, A / °C	75	80	85	90	95
Incubation time, B / s	10	20	30	40	50
Salting out, C / (mol L^{-1})	0.50	1	1.50	2	2.50
Water volume, D / mL	3.50	4	4.50	5	5.50

Level of variables: factorial points (±1), central points (0), axial points (±α).

Headspace gas chromatography with flame ionization detector analysis

Chromatographic analysis was performed on GC-Shimadzu GC-2010 Plus with flame ionization detection (FID). Chromatographic separation was performed using a capillary column of polyethylene glycol (PEG)-G16, 30 m × 0.32 mm × 0.5 μm. The extraction was performed using a headspace auto sampler (CTC Analytics). 1.0 g of powder sample was weighed into a headspace vial with 5.0 mL of ultrapure water, and sealed with silicone septum and an aluminum cap. Then, it was incubated at 80 ºC for 20 min and 1.0 mL of the vapor phase was injected by means of a heated syringe (105 °C) in split mode with a 1:5 ratio. The oven program was as follows: start at 40 °C, hold for 6 min and heat to 100 °C at 10 °C min^{-1}. After this, heat at 50 °C min^{-1} until 250 °C hold for 5 min. The inlet and detector temperatures were held at 240 and 250 °C, respectively. Gas flows were 1.5 mL min^{-1} for carrier gas (N_2), 8.5 mL min^{-1} for make-up gas (N_2), and in the FID were 40.0 and 400.0 mL min^{-1} of gas (H_2) and synthetic air, respectively. The identification of the aldehydes was performed by comparing the retention times of the samples to those of the standard.

Analytical performance

The International Conference of Harmonization (ICH) guideline[20] and AOAC Official Methods of Analysis were used for the evaluation of linear range, linearity, precision, accuracy, selectivity, robustness, stability, and limit of quantification (LOQ). Linearity was performed by means of the standard addition method. ME was evaluated by comparing the slopes of the external calibration curves and standard addition calibration. Accuracy was verified through nine determinations covering the levels, low, medium, and high (0.5, 5.0, and 20.0 μg g^{-1}), comprising the range of the analytical method and precision was verified at the same three levels, wherein the analysis was performed on one day (triplicate), and repeated on another day. The lower level of linearity is the lower limit of quantification.

Results and Discussion

RSM experiments and model fitting experimental

Multiple regression analysis of the data was performed, and the cubic model was better adjusted to the experimental data. To explore the accuracy of the model and the interactions between the factors, the results were statistically analyzed through ANOVA calculations, as shown in Table S1 (Supplementary Information (SI) section). According to ANOVA analysis, the terms B, C, AB, AC, AD, BC, BD, CD, A^2, B^2, C^2, D^2, ABC, ACD, BCD, A^2B, A^2D, AB^2 were considered significant.

Method validation

The main goal of this research was to develop and validate a HS-GC-FID method to determine propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal in animal food samples. According to Agência Nacional de Vigilância Sanitária (ANVISA) RDC No. 166/2017,[18] validation of analytical methods must be demonstrated for the intended use, by submitting a validation study that produces documented evidence to prove the reliability of the results in the laboratory where the tests are conducted.

The analytical performance of the developed method was evaluated in terms of linearity, precision, accuracy, selectivity, robustness, stability, ME, and limit of quantitation (LOQ) for propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal.

Selectivity

The selectivity must be evidenced through the ability of the analytical method to detect and/or quantify the analyte of interest in the presence of other components of the matrix, i.e., the chromatographic peak is only consequential of the analyte of interest and not to the components that may be present in the sample, such as impurities, diluents, and matrix components.

Therefore, the first analytical parameter evaluated was the selectivity of the method. It was evaluated by comparing the retention times of each aldehyde and the matrix compounds. The analytical curves were obtained by the standard addition method and external standard method. Figure 1 shows the chromatogram of standards wherein the retention times were 2.52, 3.38, 4.91, 7.56,

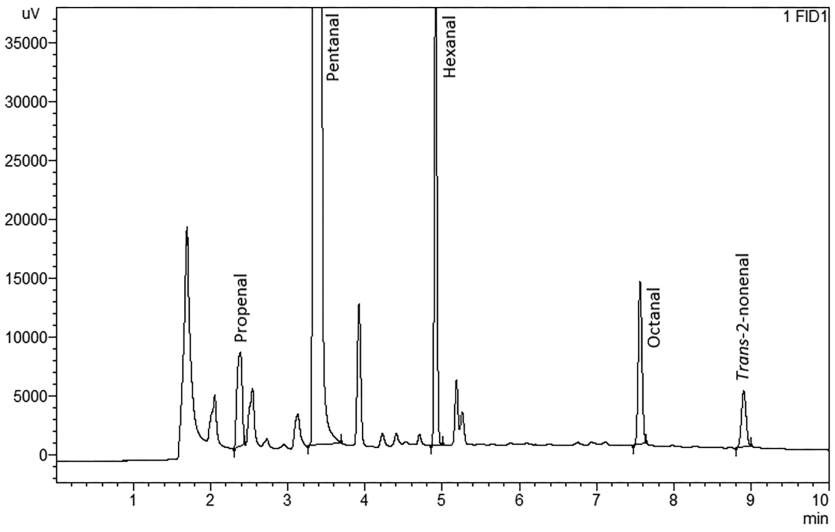

Figure 1. Chromatogram of propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal in cat food sample.

and 8.90 min for propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal, respectively. The first sign that appears in the chromatogram in greater prominence is related to the chromatographic method. No interfering peaks were found at the retention time of each compound and all were adequately resolved from each other.

Linearity and matrix effect

Linearity is the ability of an analytical method to provide responses directly proportional to the concentration of an analyte in a sample within a specific linear range, which is the interval between the highest to the lowest level of concentration. In this sense, linear range and linearity of propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal were established by means of the standard addition method and external standard method. Thus, from the working standard mixture solution, it was prepared six concentration levels (0.49, 0.98, 2.94, 4.89, 9.78, and 19.57 μg g^{-1}) for both analytical curves. Linear calibration curves were plotted by the least-squares regression of concentration *versus* the peak area of the calibration standards. As can be seen in Table 2, adequate linearity in the concentration range with correlation coefficients (R) higher than 0.990 was obtained for all selected compounds. The analysis demonstrated linearity between the peak areas of the analytes of interest and the concentration of the solution.[21,22]

Matrix interferences from other sample components can result in a decrease or increase in chromatographic response affecting quantification, resulting in a ME. Thus, through the ME test, it is possible to study those interferences that damage the instrument signal and interfere with the response of the final analysis. Therefore, the ME was determined by comparing the slopes of the built analytical curves with the standard solutions of propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal in the solvent, and with samples of animal food spiked with the standard solutions.

Hence, following the standard addition method and the construction of the external calibration curve described in the Experimental section (Table S2, SI section), the linear relationship of the two curves was verified. Statistical tests such as the parallelism test, equality of intercept, and coincidence test were carried out to verify the parallelism between the curves as presented in Table 3. Through the intercept equality test, a *p*-value less than 0.05 rejects the hypothesis that the intercepts are equal to the 5% significance level, i.e., the curves do not have the same intercept.

Table 2. Linearity and parameters of regression from the proposed method for determination of propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal

	Linear range / (μg g^{-1})	Linear regression ($y = ax + b$)	Correlation coefficient (R)
Propanal	0.49-19.57	$y = 2682x - 178.1$	0.999
Pentanal	0.49-19.57	$y = 375151.9x - 15036.5$	0.999
Hexanal	0.49-19.57	$y = 767.4x - 2.57$	0.999
Octanal	0.49-19.57	$y = 501.5x - 22.07$	0.999
trans-2-Nonenal	0.49-19.57	$y = 484.9x + 0.877$	0.999

Table 3. Statistical comparison tests between curves

	Test	LOD / (μg g^{-1})	Square sum	*F*-value	*p*-value
Propanal	intercept equality	1	87177400947.2153	126135.1328	0
	parallelism	1	24176310302.5149	34980.1907	0
	coincidence	2	92857419906.7263	67176.7159	0
Pentanal	intercept equality	1	199390504245679	18595.8186	0
	parallelism	1	6397360305403.83	596.639	0
	coincidence	2	310557992159161	14481.8333	0
Hexanal	intercept equality	1	675941508.5847	36750.5284	0
	parallelism	1	9775305.3598	531.4774	0
	coincidence	2	1141519997.9883	31031.8738	0
Octanal	intercept equality	1	983033594.4706	166614.3983	0
	parallelism	1	148870773.7869	25232.1127	0
	coincidence	2	1181792204.7842	100151.0011	0
trans-2-Nonenal	intercept equality	1	2199666806.2621	153519.9354	0
	parallelism	1	553314110.2871	38617.097	0
	coincidence	2	2385568676.0299	83247.2327	0

LOD: limit of detection.

The parallelism test assesses the impact of the matrix on the analytical methodology.[23] Curves parallels present an impact zero, thus the method can be developed in solvent. Therefore, a *p*-value less than 0.05 in the parallelism test rejects the hypothesis that the slope coefficients are equal to the 5% significance level, thus the curves are not parallel. Likewise, in the coincidence test, a *p*-value less than 0.05 rejects the hypothesis that the intercepts and slopes are equal to the 5% significance level. Thus, the curves are not coincident. Table 3 presents that all compounds obtained *p*-value less than 0.05, showing that the curves are neither parallel nor coincident. As a result, matrix interferences can cause a significant influence on the final response being necessary to take into account during the analysis. Figure 2 shows the analytical curves for each compound.

Limit of quantification (LOQ), precision, and accuracy

The limit of quantification (LOQ) is defined as the lowest concentration of the analyte which can be quantified in the sample, with acceptable accuracy and precision, under the experimental conditions adopted. In this sense, the lowest spiking level corresponded to LOQ was equal to 0.49 μg g^{-1}.[24]

The parameter that assesses the proximity between several measurements made on the same sample is the precision of the analytical method, i.e., refers to how close the measurements are to each other. Thereby, method precision must be evaluated through repeatability and reproducibility. The intra-day precision (repeatability) was verified through nine determinations covering the low, medium and high levels (0.5, 5.0, and 20.0 μg g^{-1}). The inter-day precision (reproducibility) was evaluated by means of a second repeatability carried out in the same laboratory, on a second day and prepared by different analysts. As demonstrated in Table 4, intra-day and inter-days assays ranged from 0.03 to 3.47% and from 1.22 to 3.93%, respectively, showing acceptable trueness and precision of the proposed method.

Accuracy is defined as the agreement between the real value of the analyte in the sample and that estimated by the analytical method, i.e., it is the degree of agreement between the individual results obtained by the method, regarding a reference value accepted as true. Therefore, the recovery experiment was carried out to evaluate the accuracy of the method, wherein the response of the three levels (0.5, 5.0, and 20.0 μg g^{-1}) of spiked working standard mixture solution. As presented in Table 5, the method presented satisfactory recoveries for all compounds studied, which ranged from 88-109% with relative standard deviation (RSD) (n = 3) from 0.87 to 6.95% for all levels evaluated. According to AOAC guide of validation, recoveries between 80-100% are acceptable. Thus, the results were suitable for the determination of propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal.

Robustness

The robustness of the analytical method is assessed through its ability to remain unchanged under slight variations in its parameters. In the robustness tests, statistical experiments are applied to simultaneously examine the effects of changes in different method variables, indicating

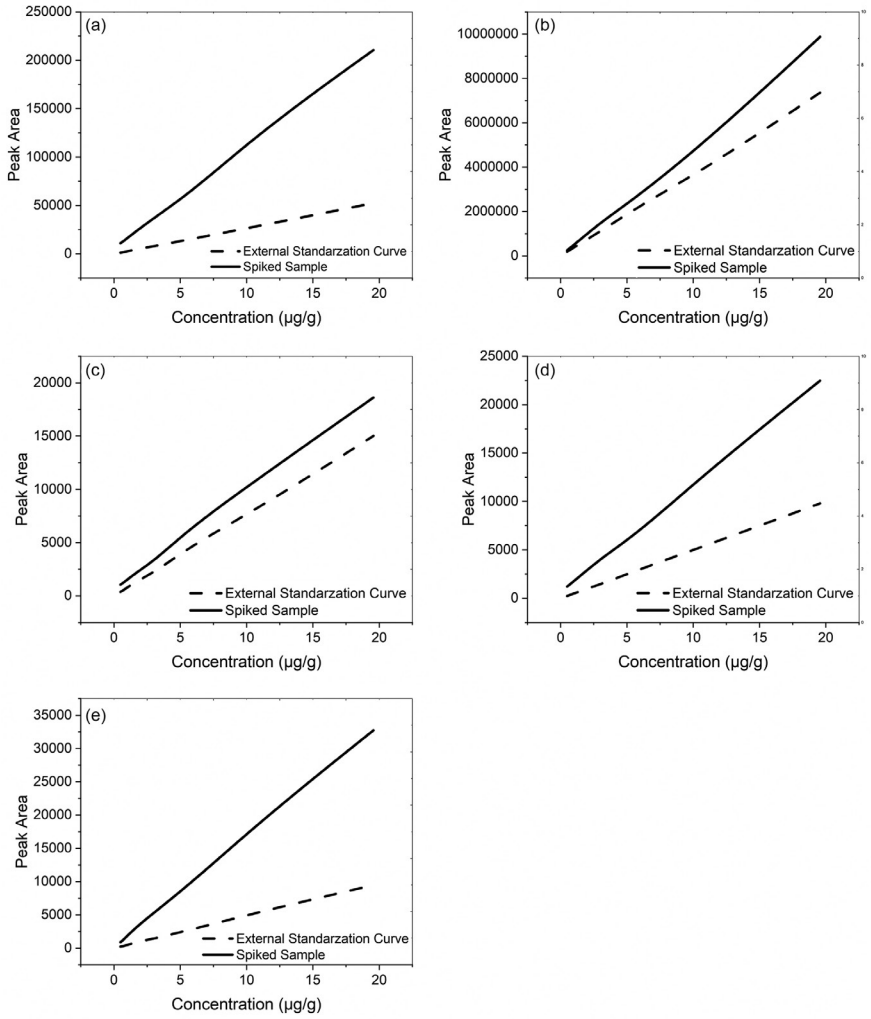

Figure 2. Analytical curves of the standard addition method and the external calibration of (a) propanal, (b) pentanal, (c) hexanal, (d) octanal, (e) *trans*-2-nonenal.

the factors that can significantly influence the response. In order to check the robustness of the present analytical method, it was performed deliberate changes, in the time, in flow rate, and in the initial oven temperature of the column. The effects of these changes were tested using the results obtained from the unchanged method as a reference in calculating the recovery of the residual solvent under the other conditions. The values of the parameters selected were oven temperature 38 and 42 °C, and flow rate of 1.4 and 1.8 mL min^{-1}. The results summarized in Table 5 show recovery values between 80-100% agreeing with the AOAC guide of validation. This studies indicated that there was no effect on the determination of propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal. Thus, the proposed method has proved to be robust for all the variations evaluated.

Solution stability

The stability of the solution aims to assess how long it remains stable the sample solution after its preparation. Solution stability studies were carried out using the working standard mixture solution in different days. The first analysis was performed on the day of preparation. Sequencing, the solution was stored at ambient temperature (25 °C) and after 48 h the analysis was carried out again. Table 6 shows the results concerning solution stability study. The recovery results are in accordance with the AOAC guide of validation, ranging from 0.54 to 0.81.

Application

Following the validation of the chromatographic method, the compounds hexanal, octanal, and propanal were identified and quantified in six different cat food samples by comparison of their peak retention times with those of the standards. As shown in Table 6, the three compounds were determined in all cat food samples evaluated, ranging from 00 to 0.75 µg g^{-1}. Furthermore, the compounds were found in quite similar amounts. The

Table 4. Intra-day and inter-days precision for propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal

	Intra-day[a]				Inter-days[b]	
	Day 1		Day 2			
Propanal	Average concentration / (μg g^{-1})	RSD / %	Average concentration / (μg g^{-1})	RSD / %	Average concentration / (μg g^{-1})	RSD / %
	0.47 ± 0.01[c]	1.49	0.51 ± 0.01[c]	1.18	0.49 ± 0.02[c]	3.93
	2.94 ± 0.07[d]	2.43	2.94 ± 0.01[d]	0.37	2.94 ± 0.06[d]	1.94
	20.05 ± 0.1[e]	0.03	19.60 ± 0.41[e]	0.41	19.82 ± 0.25[e]	1.25
	Intra-day				Inter-days	
	Day 1		Day 2			
Pentanal	Average concentration / (μg g^{-1})	RSD / %	Average concentration / (μg g^{-1})	RSD / %	Average concentration / (μg g^{-1})	RSD / %
	0.51 ± 0.00[c]	0.80	0.53 ± 0.01[c]	1.10	0.52 ± 0.01[c]	2.33
	2.94 ± 0.08[d]	2.76	2.94 ± 0.01[d]	0.37	2.94 ± 0.05[d]	1.76
	20.16 ± 0.01[e]	0.03	20.88 ± 0.13[e]	0.64	20.52 ± 0.41[e]	1.99
	Intra-day				Inter-days	
	Day 1		Day 2			
Hexanal	Average concentration / (μg g^{-1})	RSD / %	Average concentration / (μg g^{-1})	RSD / %	Average concentration / (μg g^{-1})	RSD / %
	0.49 ± 0.00[c]	0.57	0.47 ± 0.01[c]	3.01	0.48 ± 0.01[c]	3.00
	2.94 ± 0.02[d]	0.59	2.94 ± 0.07[d]	2.49	2.94 ± 0.05[d]	1.62
	19.73 ± 0.16[e]	0.81	17.38 ± 0.08[e]	0.46	18.55 ± 1.297[e]	6.95
	Intra-day				Inter-days	
	Day 1		Day 2			
Octanal	Average concentration / (μg g^{-1})	RSD / %	Average concentration / (μg g^{-1})	RSD / %	Average concentration / (μg g^{-1})	RSD / %
	0.47 ± 0.01[c]	1.62	0.44 ± 0.01[c]	1.45	0.45 ± 0.02[c]	3.82
	2.94 ± 0.04[d]	1.28	2.94 ± 0.01[d]	0.50	2.94 ± 0.03[d]	0.87
	19.71 ± 0.13[e]	0.67	18.87 ± 0.29[e]	1.55	19.29 ± 0.51[e]	2.63
	Intra-day				Inter-days	
	Day 1		Day 2			
trans-2-Nonenal	Average concentration / (μg g^{-1})	RSD / %	Average concentration / (μg g^{-1})	RSD / %	Average concentration / (μg g^{-1})	RSD / %
	0.49 ± 0.00[c]	0.41	0.49 ± 0.02[c]	3.47	0.49 ± 0.01[c]	2.25
	2.94 ± 0.03[d]	1.11	2.94 ± 0.05[d]	1.82	2.94 ± 0.04[d]	1.35
	18.69 ± 0.14[e]	0.77	18.84 ± 0.30[e]	1.61	18.77 ± 0.23[e]	1.22

[a]n = 3; [b]n = 6; [c] low level (0.5 μg g^{-1}); [d]medium level (5.0 μg g^{-1}); [e]high level (20.0 μg g^{-1}); RSD: relative standard deviation.

results proved that the developed method was accurate and effective to assess the presence of these degradation products in this kind of sample.

On the other hand, these compounds are directly responsible for the rancid smell present in the animal food. Given that palatability is an important factor in the acceptance of pets, since pet noses are more sensitive than humans, they can detect the presence of off-odors and flavors easily. The taste is detected by gustatory receptors present in the tongue, and their noses can detect some aroma compounds at levels lower than the limit of detection of many technologies.[25,26]

According to data of lethal dose (LD_{50}), the acute oral toxicity value in rats is 4.89 μg g^{-1} and acute dermal toxicity in rabbits is 5.5 μg g^{-1} for hexanal. The values of LD_{50} for octanal are 4.62 μg g^{-1} for acute oral toxicity in rats and

Table 5. Recovery test of the proposed method for determination of propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal

Compound	Level[a] / (μg g^{-1})	Recovery / %	RSD / %
Propanal	0.49	96-105	3.9
	4.89	98-103	1.94
	19.57	100-102	1.25
Pentanal	0.49	103-109	2.33
	4.89	97-102	1.76
	19.57	103-107	1.99
Hexanal	0.49	93-100	3.01
	4.89	98-103	1.61
	19.57	89-102	6.95
Octanal	0.49	88-97	3.82
	4.90	99-100	0.87
	19.60	95-101	2.63
trans-2-Nonenal	0.49	98-104	2.25
	4.89	98-101	1.35
	19.57	95-98	1.22

[a]n = 3; RSD: relative standard deviation.

Table 6. Content of propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal in cat food samples

Sample	Propanal / (μg g^{-1})	Pentanal / (μg g^{-1})	Hexanal / (μg g^{-1})	Octanal / (μg g^{-1})	*trans*-2-Nonenal / (μg g^{-1})
1	0.54	0.00	0.68	0.77	0.00
2	0.56	0.00	0.70	0.77	0.00
3	0.59	0.00	0.71	0.70	0.00
4	0.65	0.00	0.79	0.70	0.00
5	0.56	0.00	0.81	0.75	0.00
6	0.59	0.00	0.79	0.70	0.00
RSD / %	6.65	0.00	7.51	4.85	0.00

RSD: relative standard deviation.

5.20 μg g^{-1} for acute dermal toxicity in rabbits. Lastly, for the propanal, the value of LD_{50} is 1.41 μg g^{-1} for acute oral toxicity in rats, and 5.00 μg g^{-1} for acute dermal toxicity in rabbits. In this setting, the values found to hexanal in all samples ranged from 0.68-0.81 μg g^{-1}, which is below LD_{50}, offering no imminent risk to the pet. The same behavior was found to octanal, wherein the values ranged from 0.70-0.77 μg g^{-1}, which is lower than the LD_{50}. For the propanal, the values ranged from 0.54 to 0.59 μg g^{-1} also below the LD_{50} values.

Normally, dogs prefer their food moister and with the portion of fat originating from an animal source, while cats prefer dry food containing lipids of vegetable or animal origin.[13] Lin *et al.*[4] reported the effects of fat type, fat content, and processing conditions on lipid oxidation of extruded dry animal food and verified that the lipid oxidation rate was dependent on the fat type, added fat content, and feed moisture content.[4]

Koppel *et al.*[25] identified fifty-four different aromatic compounds in six samples of grain-free and eight samples of grain-added dry dog foods, including aldehydes, which were the most abundant group of volatiles found in dry dog foods. In the same context, Di Donfrancesco *et al.*[26] evaluated three extruded dry dog food diets manufactured with different fractions of red sorghum and a control diet containing corn, brewer's rice, and wheat as a grain source, and checked that the total concentration of volatile compounds was similar across the different diets, as well as the concentration of the different volatile compound groups. However, aldehydes represented the main compounds in the samples, corroborating with the results obtained in the present study, which proves that the presence of this kind of degradation compounds can influence the animal food quality.

In this sense, these evidence suggest that future research assessing samples of the same lot at different times will help to better understand the relationships between the quality of the animal food with the presence of volatile compounds. Besides, the influence of animal food storage also can provide information regarding the quality.[13]

Conclusions

Headspace coupled to GC-FID method for the identification and quantitation of propanal, pentanal, hexanal, octanal, and *trans*-2-nonenal in cat food samples was successfully validated. In addition, it was possible to verify the influence of possible interferences present in the sample, causing the ME, emphasizing the importance of taking it into account in routine analyzes. Furthermore, the method proved to be useful when applied to different commercial cat food samples, in which three analytes were determined. Thus, the proposed method showed good linearity, excellent accuracy, satisfactory precision, and robustness, indicating its usefulness in routine analyzes.

Supplementary Information

Supplementary data are available free of charge at https://jbcs.sbq.org.br as PDF file.

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Author Contributions

Bruno M. Carvalho was responsible for methodology, investigation, formal analysis, and writing original draft; Patrícia D. S. Santos for investigation, formal analysis, and validation; Geovane A. R. da Silva for investigation, formal analysis, and validation; Carlos E. R. Senes for writing review and editing; Jesuí V. Visentainer for funding acquisition and supervision; Oscar O. Santos for conceptualization, visualization and supervision.

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