Research Article

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The combined use of GC, PDSC and FT-IR techniques to characterize fat extracted from commercial complete dry pet food for adult cats

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Abstract: This study aims to compare the quality of fat extracted from different priced dry pet food for adult cats through classical and instrumental methods: pressure differential scanning calorimetry (PDSC), Fourier transform infrared spectroscopy (FT-IR) or gas chromatography (GC). Fat extracted from pet food was examined for induction time (IT), fatty acid composition, free fatty acid (FFA) content and peroxide value with the use of PDSC, GC, acid–base and iodometric titration, respectively. FT-IR data from the selected spectral regions correlate with the value of oxidation IT or the content of FFA. This resulted in construction of a reference model for IT with the following statistical features: \( R_{\text{calibration}} = 0.917 \) (RMSEC = 28.0) and \( R_{\text{validation}} = 0.841 \) (RMSEP = 34.6). For fatty acid content, model statistics were as follows: \( R_{\text{calibration}} = 0.912 \) (RMSEC = 0.61) and \( R_{\text{validation}} = 0.856 \) (RMSEP = 0.75). Discriminant model that uses spectral data alone, calculated with performance index 83.7 allowed distinguishing the studied pet food samples due to the price. Studies conducted proved PDSC and IR as reliable analytical techniques to control and monitor the quality of dry pet food for cats. Considering quality of the studied samples, it was proved that low-priced pet foods can be stored longer than premium-priced ones, while former is nutritionally more beneficial for adult cats.

Keywords: pet food, fat quality, cat complete food, commercial feeds, oxidative stability

1 Introduction

According to GfK Institute, a global increase in the amount of pet food sold in 2016 was 5% greater on average compared to that of 2015. However, in developing countries of eastern Europe, this increase was more than 7%, while western countries reported only a 2% increase. A significant increase in eastern European countries is accompanied by a rapidly increasing number of producers and a variety of products offered. Euromonitor International evaluates the value of the pet food market in Poland as 1.8 billion PLN and forecasts a rapid increase in this sector in the coming years [1]. Increased demand for pet food results in increased production that in turn requires intensified quality control. Quality control refers to controlling sources of starting materials, technological processes applied in the production and chemical composition of the final product. Pertaining to source fat used should be of good quality as it is the component most sensitive to environmental factors, hence mostly influencing the deterioration process. The fat contained in the final product is mainly animal fat, provided via the rendering process. Additional fat comes from the poultry by-product meal and/or corn, while smaller amounts come from meat and bone meal, wheat and soybean meal. Such mixture contains a range of fatty acids including the essential unsaturated fatty acid (UFA), e.g., linoleic acid. Some other commonly used fat sources in pet dry food production are vegetable, fish and flaxseed oils rich in linoleic acid and omega-3 fatty acids [2]. Appropriate
fat of good quality ensures proper growth, development and healthiness of animal fed. Cats require an especially well-balanced diet in terms of fatty acid composition. Their specific metabolism of n-3 and n-6 fatty acids requires feed containing those compounds in stable, degradation-resistant form. Fat components are air and water sensitive. They undergo chemical reactions especially if reactive double bonds are present. Stored fatty acids, especially UFAs, are prone to oxygen action. When oxygen reacts with fatty acids, intermediating including free radicals are formed and the main product is said to be oxidized or rancid. The function of antioxidants in pet foods is to prevent or stop the oxidation process. All dry products require an antioxidant (preservation) system to stop oxygen from destroying UFAs, which are first aim of environmental oxygen action. A rancid product is deficient in important nutrients, contains free radicals and probably has a bad taste/aroma that causes the pet to reject the food [2].

The Official Publication of the Association of the American Feed Control Officials (AAFCO), the National Research Council (NRC) recommendations or The European Pet Food Industry Federation – FEDIAF associated with Scientific Advisory Board have been uniform in terms of the development of standards for commercial cat food, and their recommendations are based on peer-reviewed scientific data and information from experts in the field. The recommendations published by NRC present the required amounts of each nutrient to be included in a diet. FEDIAF and AAFCO recommended minimum fat, linoleic acid, arachidonic acid, alpha-linolenic acid and eicosapentaenoic acid + docosahexaenoic acid level in pet food [3]. According to the study by Ahlstrom et al. (2004), it is known that the fatty acid profile consumed by pet cats has a ratio of n-6:n-3 fatty acids between 5:1 and 17:1, which differs from the ratio appropriate for feral cats (recommended ratio is 2:1) [4]. Unfortunately, there are no recommendations on the correct amount and ratio of saturated fatty acids (SFAs) to UFAs, especially mono-unsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) in cat diets. Therefore, the quality of pet food fat mainly refers to the total content of UFAs that are of particular importance in feeding cats.

Infrared spectroscopy (IR) and differential scanning calorimetry (DSC) are modern methods that provide reliable information on the quality of fats. Gas chromatography (GC) provides about the fatty acid composition of studied samples. IR is a rapid and sensitive technique that provides information on the energy of vibrating groups of atoms in a molecule. For 20 years, it has been used for food analysis widely, including fats and oils analysis [5–7]. It was proven to be a proper instrument for the evaluation of milk, vegetables, various beverages and meets quality and chemical composition. The application of IR in quality analysis of fats and oils is one of the most common and documented trends in the area [8–11]. The major advantages of Fourier transform infrared spectroscopy (FT-IR) over other techniques include rapid and easy equipment operations along with the possibility to conduct nondestructive measurement of samples at any state, i.e., solid, liquid, paste, gas with convenient and environmentally friendly sample preparation with no chemical waste production.

Pressure differential scanning calorimetry (PDSC) is a rapid technique that requires quite small amount of sample. Although it has many advantages, it is undiscovered fully so far. Only limited literature data are available on the application of PDSC in food and feed analysis. DSC has also been used to monitor the oxidative stability of fats and oils contained in feed samples, as well as studied alone [12,13]. GC used for fatty acid composition is currently considered a standard method and therefore is widely applied in many laboratories worldwide [14,15].

The current investigation provided feedback on some of the social requirements and doubts related to feed quality and its controlling measures, e.g., allowed time of storage, materials used to produce feed or methods used to control the quality of fats and oils contained in the feed, the nutritional value of feed. Data obtained by PDSC, GC and IR methods were used to evaluate cat feeds of different prices. It was expected that, if present, variation in the quality of different price feeds can be due to the use of different starting materials in various ratios in the production process. Initial materials used have a significant impact on the oxidative stability of fat in the final product. The lower oxidative stability results in the faster oxidation and the lower quality. Higher oxidative stabilities were expected to be greater for more premium-priced products that can be checked with the use of various analytical methods. Therefore, the scientific aim of this study is to check the oxidative stability of oils extracted from the feed, indicated by induction time (IT), free fatty acids (FFAs) and peroxide value (PV). Oxidative stability results from the chemical composition of the oil, which in turn exhibits in wavelength and intensity of bands occurring in IR spectra. Reference models were constructed to correlate oxidative stability with spectral information from selected specific regions. As chemical difference between price-differing feeds was expected, the next scientific aim was to construct a statistically significant discriminant model, with the use of spectral data exclusively, to rapidly differentiate feeds due to their price or quality. Both discriminant and reference
models equipped with statistically significant power of prediction can be further used for rapid and versatile evaluation of feed quality of any unknown cat feed sample.

2 Materials and methods

2.1 Pet food for cat

Commercial complete pet foods for adult cats were purchased at different prices from the local market in Warsaw, Poland. Each group of studied products, i.e., low-priced (L), fairly average priced (A) and premium-priced (P), consisted of samples from three different companies (L1, L2 and L3; A1, A2 and A3; P1, P2 and P3, respectively). To ensure random diversity, each one of three different low-priced, fairly average priced and premium-priced dry pet food composed of ten samples purchased from various retailers. For example, P1 composed of ten samples of the same feed, meaning feed with the identical label, purchased from different retailers. Boxes were opened and subjected to further analysis starting with fat extraction.

2.2 Extraction of fat

The Folch method [16,17] with further improvement by Boselli was applied for studied feed samples to chemically extract a fat from them with the use of hexane.

2.3 PV and FFA content

The PV of the mixture was determined by the iodometric technique with visual endpoint detection. The FFA content was calculated based on acid values and the value of the molar mass of oleic acid. The acid value was determined by titration of fat samples with 0.1 M ethanolic potassium hydroxide solution.

2.4 GC measurements

The determination of fatty acid composition was carried out by GC analysis of fatty acid methyl esters. Methyl esters of fatty acids were prepared through the saponification of triacylglycerols and esterification with methanol according to ISO 5509:2001. An YL6100 GC chromatograph equipped with a flame ionization detector and a BPX-70 capillary column of 0.20 mm i.d. × 60 m length and 0.25 µm film thickness was used. The oven temperature was set at 60°C for 5 min, and then it was increased from 10°C min⁻¹ to 180°C; from 180°C to 230°C, temperature was increased by 3°C min⁻¹ and then maintained at 230°C for 15 min. The temperature of the injector was 225°C, with a split ratio of 1:100, and the detector temperature was 250°C. Nitrogen flowing with the rate of 1 mL min⁻¹ was used as the carrier gas. The identification of fatty acids was carried out using the lipid standard.

2.5 DSC measurements

A differential scanning calorimeter (DSC Q20 TA) coupled with a high-pressure cell (PDSC) was used. Fat samples of 3–4 mg were weighed in an aluminum open pan and placed in the sample chamber under an oxygen atmosphere with an initial pressure of 1,400 kPa and the oxygen flow rate of 100 mL min⁻¹. The isothermal temperature for each sample was 120°C. Obtained diagrams were analyzed using TA Universal Analysis 2000 software. For each sample, the output was automatically recalculated and presented as amount of energy per one gram. The maximum PDSC oxidation time (IT) was determined based on the maximum rate of oxidation (maximum rate of heat flow).

2.6 FT-IR measurements

The background spectrum was registered with an empty measuring chamber for each sample separately to eliminate the influence of carbon dioxide and water vapor present in the air on the spectra of samples. A drop of the sample was placed on a round ZnSe (zinc selenide) plate and covered with another identical plate to form a film of a sample between them. Therefore, the path length of IR light passing through the sample was considered constant. Both plates were then placed in a holder that was mounted in the measuring chamber of Perkin Elmer System 2000 instrument using dedicated slides. After closing measuring chamber doors, recording of spectrum has been started. Region selected was 4,000–400 cm⁻¹.
with the resolution of 2 cm\(^{-1}\). Twenty-five scans were taken for background spectrum followed by 25 scans for spectrum of each sample. After recording of spectrum, sample holder plates were washed with hexane and then shortly with water and dried with paper towel to remove traces of given sample before further recording. The computer runs on Windows 95 with GRAMS AI software that operated with System 2000 spectrometer.

### 2.7 Modeling

Two types of models, i.e., discriminant and reference were constructed. Discriminant models were obtained with spectral data alone. Wavenumbers and intensities of selected bands occurring in different spectral regions were used to form homologous groups of samples, i.e., groups of samples with identical or very similar properties. Specific regions expected to contain different information on every homologous group of samples were tested to obtain the best separation with the appropriate assignment of samples. Some of the studied samples were ignored within model construction as considered outliers. For discriminant models, Mahalanobis distances within the own group and distances from the next groups were calculated. Mahalanobis distance is the distance of a given sample from the mean of a set of samples, was square error of prediction mean square error of calibration for both calibration and prediction meaning smallest residual software expected to contain different information on every homologous group of samples served to evaluate discrepancies between groups. Distances from other than the own group served to evaluate discrepancies between groups. For reference models, some outlier spectra were ignored as well. The trials with different spectral regions were done to obtain models with the highest correlation coefficient values for both calibration and prediction meaning smallest residual mean square error of calibration (RMSEC) and residual mean square error of prediction (RMSEP), respectively.

Mahalanobis distance, that is, the distance of a given sample from the mean of a set of samples, was calculated as follows:

\[
D^2 = (X - X_{\text{ave}})^T S^{-1} (X - X_{\text{ave}}),
\]

where \(D\) is the distance (as a scalar); \(X\) is the data vector \((n \times 1)\); \(X_{\text{ave}}\) is the mean data vector \((n \times 1)\); \(S\) is the covariance matrix \((n \times n)\); \((X - X_{\text{ave}})^T\) denotes the transpose of \((X - X_{\text{ave}})\); \(n\) is the number of data points in \(X\).

### Ethical approval

The conducted research is not related to either human or animal use.

### 3 Results

#### 3.1 Fat characterization by PDSC and GC

SFAs, MUFAs, PUFAs and TRANS (fatty acids with trans configuration at double bond) were analyzed. Figure 1 shows the percentage composition of studied fats.

On the left \(y\)-axis, one can observe a similar percentage level of SFA and MUFA and a significantly lower percentage of PUFA.

The ratio of PUFA/SFA calculated from the data presented in Figure 1 is 0.32, 0.50, 0.95, 0.67, 0.37, 0.43, 0.78, 0.31 and 0.79 for L1, L2, L3, A1, A2, A3, P1, P2 and P3, respectively. This ratio, although no recommendation of daily intake, is provided in the literature so far, as mentioned in Section 1, is considered an important parameter from the nutritional point of view and hence is a clear novelty of current studies [16,17]. IT in which value is denoted on the right auxiliary \(y\)-axis is plotted in Figure 1, with white squares connected with solid line. Experimental data of PV, FFA and IT, each followed by appropriate standard deviation, are presented in Table 1. The value for L3 is unexpectedly lower compared to L1 and L2.

In Table 1, IT is different for different groups; however, expected high average values for premium-priced feeds group are not confirmed.

Low-priced feeds have quite high average IT value (173.9), significantly greater than average values for fairly average priced and premium-priced groups, i.e., 88.5 and 73.6, respectively. Small IT values for L3 make average value even smaller than could be. Other values measured were FFA and PV (Table 1). PVs were higher for low-priced feeds on average and lower for fairly average priced and premium-priced samples. FFAs presented in Table 1 are in the following order: expensive < fairly average priced < low priced.

#### 3.2 Fat characterization by FT-IR

IR spectra of fat of studied three groups of samples were registered and averaged. Averaged spectra of low-priced, fairly average priced and premium-priced feeds are presented in Figure 2.
Each spectrum was averaged from 18 spectra (2 replications each) registered for 9 samples from every group. Spectra presented in Figure 2 are quite similar to each other. Characteristic strong bands at 2,800–3,000 cm\(^{-1}\) are present in every spectrum. Bands in this region are generated by C–H vibrations, with C–H occurring over 3,020 cm\(^{-1}\) and C–H groups forming carbon chain under 3,020 cm\(^{-1}\)\cite{18}. If bands over 3,020 cm\(^{-1}\) are not present, samples were considered to not contain chemicals with benzene ring.

Samples that do not contain water or contain very small amount of water are indicated by weak but relatively broad bands at around 3,321 cm\(^{-1}\) and 3,429 cm\(^{-1}\) generated by vibrations of O–H water group\cite{13,20}. Next region with very strong bands is at 1,800–1,580 cm\(^{-1}\). Bands in this region are generated by C=O vibrations, i.e., symmetric and asymmetric stretches\cite{19}.

Moving right on the spectrum toward lower wavenumbers, dactyloscopy region starts\cite{20}. Small differences, e.g., different intensity ratios, or slightly different shapes of selected bands occur; however, region 800–650 requires more detailed analysis. Figure 3 presents the extension for the region.

In the region presented on Figure 3, there are two distinct bands located at 759 and 723 cm\(^{-1}\), respectively. For each studied cat feed spectrum those bands are pointed with arrows. Their mutual ratios, however, differ significantly depending on the type of the sample. In the case of low-priced (solid line) and premium-priced (dotted line) feeds, the ratio of intensity is 759–723 cm\(^{-1}\) and bands are significantly bigger. In the case of medium-priced feed (dashed line), this ratio is much smaller. This shows one of the differences in the chemical composition of three studied samples. In addition, in the low-priced feed (solid line), there is a band at 668 cm\(^{-1}\) that is significantly more intense than bands at the same wavenumber in two remaining feeds. Only few examples of differences performed by visual inspection are presented here, but some other less clear differences certainly occur, which can be detected only with appropriate software, which is explained in the following section.

### 3.3 Discriminant model

Table 2 provides statistical data obtained when the discriminant model was calculated.
Mahalanobis distances, i.e., “distance to actual,” “distance to low-priced” and “distance to fairly average,” are presented for each investigated sample. Three classes, i.e., homologous groups, were obtained with 12 spectra for low-priced feed, 17 spectra for fairly average priced feeds and 14 spectra for premium-priced feeds. Remaining spectra, i.e., five for low-priced and three for fairly average priced feed samples, were rejected from the model as outstanding. Samples were best assigned to individual groups with the use of spectral data from 3,057 to 574 cm\(^{-1}\) region, which covers almost entire spectrum while not overfitted. The performance index of the model constructed with the use of ten principal components equals 83.7, which indicates statistically high match and calibration.

The “next class” term in Table 2 refers to the Mahalanobis distance between the given sample and the second close class (homologous group). Data presented in Table 2 allow to state that low-priced samples are significantly different from premium-priced samples (biggest Mahalanobis distances), and for both classes, fairly average priced group is the next class. On the other hand, fairly average priced group samples have random similarity to premium-priced and low-priced feed samples as appropriate Mahalanobis distances differ. Figure 4 shows graphically how the samples are divided into separate homologous groups.

Figure 2: IR spectra registered for low-priced, fairly average priced and premium-priced cat feeds as a radiation transmittance against wavenumber in the range 4,000–400 cm\(^{-1}\).

Figure 3: IR spectral region 900–500 cm\(^{-1}\) extension registered for low-priced, fairly average priced and premium-priced cat feeds as a radiation transmittance against wavenumber.
Table 2: Class assignment and Mahalanobis distances calculated for studied samples with calibrating and validating discriminant model for studied low-priced, fairly average priced and premium-priced cat feeds

| Usage          | Actual class | Calculated class | Distance to actual | Next class               | Next distance | Distance to low-priced | Distance to fairly average priced |
|----------------|--------------|------------------|--------------------|--------------------------|---------------|------------------------|----------------------------------|
| Calibration    | Low priced   | Low priced       | 1.0991             | Fairly average priced    | 1.3896        | 1.0991                 | 1.3896                           |
| Calibration    | Low priced   | Low priced       | 0.7793             | Fairly average priced    | 0.8538        | 0.7793                 | 0.8538                           |
| Calibration    | Low priced   | Low priced       | 0.7002             | Fairly average priced    | 0.7437        | 0.7002                 | 0.7437                           |
| Calibration    | Low priced   | Low priced       | 1.1056             | Fairly average priced    | 1.5863        | 1.1056                 | 1.5863                           |
| Calibration    | Low priced   | Low priced       | 0.7703             | Fairly average priced    | 1.2852        | 0.7703                 | 1.2852                           |
| Calibration    | Low priced   | Low priced       | 0.9383             | Fairly average priced    | 1.0081        | 0.9383                 | 1.0081                           |
| Validation     | Low priced   | Low priced       | 1.0198             | Fairly average priced    | 1.2482        | 1.0198                 | 1.2482                           |
| Calibration    | Low priced   | Low priced       | 0.6176             | Fairly average priced    | 0.6864        | 0.6176                 | 0.6864                           |
| Validation     | Low priced   | Low priced       | 1.0864             | Fairly average priced    | 1.559         | 1.0864                 | 1.559                            |
| Validation     | Low priced   | Low priced       | 0.8901             | Fairly average priced    | 1.4061        | 0.8901                 | 1.4061                           |
| Calibration    | Low priced   | Low priced       | 0.9954             | Fairly average priced    | 1.365         | 0.9954                 | 1.365                            |
| Calibration    | Low priced   | Low priced       | 1.4429             | Fairly average priced    | 1.8456        | 1.4429                 | 1.8456                           |
| Validation     | Fairly average priced | Fairly average priced | 0.8521             | Premium priced            | 0.8959       | 0.9839                 | 0.8521                           |
| Calibration    | Fairly average priced | Fairly average priced | 0.8090             | Low priced                | 0.9066       | 0.9066                 | 0.809                           |
| Calibration    | Fairly average priced | Fairly average priced | 0.7545             | Low priced                | 0.8154       | 0.8154                 | 0.7545                           |
| Calibration    | Fairly average priced | Fairly average priced | 0.6891             | Premium priced            | 0.7502       | 1.0469                 | 0.6891                           |
| Validation     | Fairly average priced | Fairly average priced | 0.6928             | Low priced                | 0.743        | 0.743                  | 0.6928                           |
| Calibration    | Fairly average priced | Fairly average priced | 0.8216             | Premium priced            | 0.8493       | 0.9026                 | 0.8216                           |
| Calibration    | Fairly average priced | Fairly average priced | 0.7497             | Low priced                | 0.8239       | 0.8239                 | 0.7497                           |
| Calibration    | Fairly average priced | Fairly average priced | 0.9488             | Premium priced            | 1.0015       | 1.497                  | 0.9488                           |
| Calibration    | Fairly average priced | Fairly average priced | 1.1767             | Premium priced            | 1.3102       | 1.5595                 | 1.1767                           |
| Calibration    | Fairly average priced | Fairly average priced | 1.0752             | Low priced                | 1.2757       | 1.2757                 | 1.0752                           |
| Calibration    | Fairly average priced | Fairly average priced | 0.8541             | Premium priced            | 1.3841       | 1.4236                 | 0.8541                           |
| Calibration    | Fairly average priced | Fairly average priced | 0.6980             | Low priced                | 1.0464       | 1.0464                 | 0.698                           |
| Calibration    | Fairly average priced | Fairly average priced | 0.6937             | Low priced                | 0.8451       | 0.8451                 | 0.6937                           |
| Calibration    | Fairly average priced | Fairly average priced | 0.7736             | Low priced                | 0.806        | 0.806                  | 0.7736                           |
| Validation     | Fairly average priced | Fairly average priced | 1.2276             | Premium priced            | 1.4403       | 1.5959                 | 1.2276                           |
| Calibration    | Fairly average priced | Fairly average priced | 1.0072             | Low priced                | 1.4866       | 1.4866                 | 1.0072                           |
| Calibration    | Fairly average priced | Fairly average priced | 0.7879             | Premium priced            | 1.3005       | 1.4405                 | 0.7879                           |
| Calibration    | Premium priced | Premium priced    | 1.0379             | Fairly average priced     | 1.061        | 1.5109                 | 1.061                            |
| Calibration    | Premium priced | Premium priced    | 0.9969             | Fairly average priced     | 1.2611       | 1.6036                 | 1.2611                           |
| Validation     | Premium priced | Premium priced    | 1.0001             | Fairly average priced     | 1.2176       | 1.7707                 | 1.2176                           |
| Calibration    | Premium priced | Premium priced    | 0.8308             | Fairly average priced     | 1.0321       | 1.5837                 | 1.0321                           |
| Calibration    | Premium priced | Premium priced    | 1.3171             | Fairly average priced     | 1.7186       | 2.1402                 | 1.7186                           |
| Validation     | Premium priced | Premium priced    | 1.3371             | Fairly average priced     | 1.9181       | 2.0734                 | 1.9181                           |
| Calibration    | Premium priced | Premium priced    | 1.2761             | Fairly average priced     | 1.6487       | 2.0545                 | 1.6487                           |
| Calibration    | Premium priced | Premium priced    | 0.6687             | Fairly average priced     | 0.8973       | 1.2713                 | 0.8973                           |
| Calibration    | Premium priced | Premium priced    | 0.7424             | Fairly average priced     | 1.0962       | 1.3693                 | 1.0962                           |
| Calibration    | Premium priced | Premium priced    | 0.8023             | Fairly average priced     | 1.0937       | 1.3358                 | 1.0937                           |
3.4 Reference models

Reference models calculated for studied cat feeds refer to correlation of spectral data with IT, FFA content and PV. In terms of fat quality followed by feed quality and ability to store, IT is the most important parameter. Figure 5 presents as an example, calculated versus actual values of IT for low-priced, fairly average priced and premium-priced cat feeds.

Figure 5 presents as an example, calculated versus actual values of IT for low-priced, fairly average priced and premium-priced cat feeds. Model construction resulted in calculating the following model parameters: correlation coefficient $R = 0.917$ ($R^2 = 0.841$) for calibration and $R = 0.856$ ($R^2 = 0.733$) for prediction set. The corresponding errors were $RMSEC = 28.0$ and $RMSEP = 34.6$. Of 53 analyzed spectra, nine were randomly selected for the validation set and others were used for calibration. The number of factors used was 8 of 10 calculated, which produced the smallest value of RMSEC. The spectral range used in modeling was 3,130–570 cm$^{-1}$.

The FFA value is the parameter classically determined by acid–base titration. Data concerning this value are presented earlier. The trials to find a correlation between IR spectral data and FFA content were conducted to establish model for the rapid determination of FFA in the unknown fat sample extracted from cat feed, relying on the spectral data exclusively. Once model is constructed to determine the FFA content of an unknown sample, it is necessary to introduce spectral data (spectrum) of an unknown sample to TQ Analyst software and use “Quantify” function to let the software calculate the amount of FFA in this sample. High correlation coefficients, i.e., 0.912 and 0.840 for calibration and validation sets, respectively, were determined in the model calculated. Small values of $RMSEC = 0.61$ and $RMSEP = 0.75$ confirm statistical significance of model as well. Figure 6 presents the distribution of residuals, i.e., differences between calculated and actual values (y axis) and actual values (x axis).

As the distribution is uniform, the correlation calculated can be considered statistically valid and significant. Spectral regions used for FFA model construction were 3,500–3,420 and 1,485–1,331 cm$^{-1}$. Extension or narrowing of any of the two regions led to worse statistics of the model. Other regions did not improve the model as well. That means the content of FFAs affects the spectral data of fat in those two regions exclusively.

The aforementioned statistical relations correlate reference data and spectral data or spectral data alone (discriminant analysis). Results obtained show the potential of correlation of data from different methods; hence, certain values, e.g., FFA content, are determined with the use of spectral data registered for an unknown sample. The use of the calculated discriminant model
and IR spectrum of any unknown sample allows us to conduct “yes” or “not” analysis with respect to fat extracted from cat feeds to assign an unknown sample to a proper group of feeds with different prices. It is possible as different price samples have unique chemical composition. Indirect information on this composition is contained in the calculated model.

4 Discussion

The highest values of IT clearly show that the low-priced pet feed can be stored longest without significant deterioration. This is confirmed by data presented in Figure 1, which shows that saturated acid content is highest for low-priced samples. Chemically, SFAs undergo oxidation/deterioration much slower than unsaturated, and thus, IT is of the highest value [21]. Determined PV values seem to be in contrast to this statement, as higher values of PV, determined for low-priced samples, denote a worse quality of these samples and hence suggest a shorter time for the sample to deteriorate. However, PV is characteristic for the primary oxidation process and only refers to the formation of oxides and peroxides [22,23]. The secondary stage of oxidation that leads to final deterioration, i.e., formation of aldehydes, ketones and short chain fatty acids, has not occurred in low-priced samples yet. Hence, premium-priced
samples have shorter IT. In premium-priced samples, the primary oxidation is already over or at least on, and entire time required for complete deterioration is shorter. This discussion shows that premium-priced feed samples were best balanced in terms of their nutritional value proved by their fat composition. According to the literature data, the average SFA, MUFA and PUFA contents in commercial cat foods for the adult cat purchased from the Australian supermarkets or pet shops were 36.7%, 41.0% and 22.3%, respectively. The total PUFA content ranged from 5.1% to 47.1% for the wet foods [24]. In samples collected and studied in this study, the average amounts of these acids in fat extracted from appropriate samples are as follows: SFA (C): 38.4%; (M): 39.5%; (E): 34.7%; MUFA: (C): 39.8%; (M): 41.1%; (E): 43.7%; PUFA: (C): 21.2%; (M): 18.9% and (E): 20.4%.

Presented results and statistical analysis show that fat chemical composition and shelf life do not directly relate to the price of a given group. The chief point is that deterioration in low-priced samples is due to hydrolytic rancidity, whereas deterioration in premium-priced samples is due to oxidative rancidity. It is well known that oxidative rancidity is a faster process than hydrolytic rancidity [25].

4.1 Fat characterization by FT-IR

Differences in spectra occur due to different chemical compositions of studied samples. They can serve to differentiate samples that rely on spectral information. This refers to the construction of the discriminant model [26,27]. As spectra differ, it is also possible to establish the relation between spectral data and certain properties such as IT, which results in the construction of the reference model [28].

4.2 Discriminant model

Data presented in Table 2 are used to plot the graph presented in Figure 4. Both Table 2 and graph 4 show that feed samples can be distinguished by spectral analysis of fat extracted from solid samples. The question arises whether solid-state samples could have been distinguished with the use of their IR spectra without the necessity of oil extraction. Unfortunately, this approach has not been done in this study. Figure 4 shows how the fat samples are divided into separate homologous groups, meaning that low-priced, fairly average priced and premium-priced samples can be distinguished with the use of only spectral data. This model can be used practically for rapid monitoring or controlling the quality or price group of unknown samples of cat feeds.

4.3 Reference models

In the model for IT, very wide spectral region, i.e., region 3,130–570 cm\(^{-1}\), has been used for calibration. Figure 2 presents averaged IR spectra of fat extracted from low-priced, fairly average priced and premium-priced feeds in whole registered range, i.e., 4,000–400 cm\(^{-1}\), and one can observe low-intensity bands at the region above 3,130 cm\(^{-1}\) not used for IT model construction. They are most probably generated by small amounts of water contained in fat as O–H vibrations generated bands are present in this spectral range [29,30]. Also, region below 570 cm\(^{-1}\) was not used for this calibration, meaning data are not informative for changes IT. During model construction, data were randomly divided into calibration and validation sets by software itself. The constructed model has high, statistically significant correlation coefficients of 0.917 and 0.841 between actual and calculated values for calibration and validation data sets, respectively. RMSEC is 28.0 and RMSEP is 34.6. As measured with the reference method, values of IT range from 44.11 to 249.37 and model characteristics are quite good and its statistical strength of prediction is high. That means unknown samples of fat extracted from cat feeds can be measured with the use of this model and single IR spectrum, and recording the spectrum takes not
more than a few minutes. The great advantage of current results is that the same spectrum of an unknown sample can be simultaneously entered for both models to rapidly determine IT or FFA values.

The model for FFA content correlates spectral and reference data at a statistically significant level for both calibration and validation sets. There are small values of RMSEC and RMSEP that prove the quality of the model and its ability to determine or predict the content of FFAs in an unknown sample being fat extracted from pet food. In the case of FFA, data from two separate spectral regions produced the best model. Interestingly, the use of each region separately did not result in good statistics. This means that FFAs present themselves in both spectral regions and bands generated by vibrations are mutually dependent.

5 Conclusions

The aim of this study is to verify spectral data—stability dependence for price-different cat feeds. This was completed by (1) constructing reference models correlating data from spectral and reference methods and (2) constructing the discriminant model with the use of spectral data exclusively, to rapidly differentiate feeds. The aim is achieved, which is presented in Sections 3.3 and 3.4. The following conclusions were drawn.

1. Fat from low-priced product samples exhibits longer IT, higher PV and greater content of FFA compared to fat from premium-priced samples. Premium-priced feeds can be stored for shorter period than low-priced feeds without a significant decrease in quality; however, premium-priced samples have better initial nutritional value.

2. The group of low-priced feeds differs from the fairly average price group and premium-priced group significantly by means of chemical composition. This was proved by the presence of distinct homologous groups occurring in the discriminant model constructed.

3. It is generally possible to differentiate the fairly average priced feeds from premium-priced feeds by chemical composition; however, some fairly average priced and premium-priced feeds seem to be chemically very similar as the statistical distances between samples in those two homologous groups indicated in the discriminant model are small.

4. Constructed models prove the ability to correlate analytical data from different methods, i.e., PDSC and IR. This evidences that results obtained by each method separately are robust and reliable and can be used exclusively for the determination of desired parameters, e.g., IT to evaluate product oxidative stability.

5. Models constructed might have practical application. They can be used for monitoring or controlling the quality of feeds; however, introduction to practice requires further study, e.g., use of a much greater number of samples for calibration and validation.

6. Spectra of feed alone without fat extraction should have been measured or reference materials should be included in the study to find out whether the correlation between spectral data of solid-state samples and fat quality parameters exist.

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