

Selected quality properties of lipid fraction and oxidative stability of dry dog foods under typical storage conditions

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Abstract Selected quality and oxidative stability parameters of the lipid fraction were analyzed in four complete dry dog foods with different main animal-derived ingredients. The measurements were taken at the time of bag opening and repeated after 7 months of continuous storage in normal room conditions. Fatty acid (FA) content and acid value (AV) were determined, followed by subsequent pressure differential scanning calorimetry (PDSC) measurements. From the resulting PDSC exotherms, maximum induction time (τ_{\max}) was determined and used for assessing the oxidative stability. The study revealed changes in lipid quality and oxidative stability of dry dog foods that appeared during storage. Results of FA and AV assays showed specificity and marked quality differences of lipid ingredients declared as used in the production process. Product with the lowest content of polyunsaturated FA had the highest oxidative stability. PDSC appeared to be an effective method for the analysis of lipid oxidation in pet foods.

Keywords Pet food · Storage · Fatty acid · Oxidation · PDSC

Introduction

As reported by the European Pet Food Industry Federation (FEDIAF), 8.5 million tons of pet food products was sold in EU in 2012 with a turnover of 13.8 billion euro [1].

Continuing growth in pet-related spending is currently explained with the increased devotion to pets [2]. Before making purchase decisions on the market, dog owners seek the best feeding option for their pets, often consulting the packaging information [3]. Pet food labels are widely promoted as the primary source of information for customers [4].

According to EU regulations that concern products for animal feeding all nutrient sources used in the production process have to be listed on the label either by specific names or categories [5].

However, regulatory guidelines forbid the inclusion of ingredient quality indicators on pet food labels. In fact, actual properties of finished product depend on selection of commodities providing the nutritional features [6].

Lipid ingredients are specifically prone to oxidation damage and rancidification causing major sensory alterations that occur during storage. Thus, the monitoring of oxidative stability status is crucial for pet foods quality control [7]. It was previously shown that oxidized dietary lipids negatively affect the growth, antioxidant status and some immune functions of growing dogs [8]. Various combinations of antioxidant compounds were tested for their efficacy in preserving the nutrient quality and protecting freshness of pet foods during storage [9]. It is essential to add that labeling the information on antioxidants added to the product is voluntary. In consequence, such additives may be indicated only as a functional group [10].

The technological parameters of production also affect the oxidative stability of the kibble. Dry foods manufacturing process (extrusion, baking or other) is expected to offer products with extended shelf life, when stored in factory sealed bags. After opening for everyday use, various environmental factors affect the chemical and physical

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properties of kibbles, especially when improperly handled or stored in risky conditions [11].

Differential scanning calorimetry (DSC) has a wide range of applications including classification of various categories of lipids in terms of their oxidative stability [12–15]. Recently, DSC was used to characterize the degree of starch gelatinization and amylose–lipid complexation of baked and extruded pet foods [16].

Here we report the preliminary results of lipid fraction quality measurements (i.e. acid value—AV, fatty acid composition) and oxidative stability assessment of complete dry products for dogs, before and after 7 months of storage in typical house environment. The aim of this study was to assume the relative oxidative safety of canine complete diets from the perspective of long-term use. To our knowledge, the applicability of PDSC method to evaluate oxidative stability in dry dog foods is hereby presented for the first time.

Experimental

Materials

Small size bags (1–1.5 kg) of four complete dry foods for growing dogs were purchased in local specialized pet stores, with the special attention put on the far best before date declared on the label. The formula of each product based on different type of main animal-derived ingredient. The labels were carefully checked for information on fat sources and antioxidant content (Table 1).

First set of analyses (0) was performed just after opening the bag and the second (7) after 7 months of storage, simulating typical, normal environmental conditions in the household (room temperature, constant humidity and no contact with the sun light). However, the regular (daily) opening for animal feeding was not simulated. The foods were kept in original bags, closed tightly but without using any additional devices or objects.

Methods

Total lipid extraction

The lipid fraction was extracted using the procedure described by Boselli et al. [17].

Approximately 10 g of the finely grained sample was mixed with 100 mL of a chloroform/methanol solution (1/1 v/v) in a Shott's bottle with a screw-cap. The bottle was kept at 60 °C for 20 min before adding an additional 100 mL of chloroform. After 3 min of a vigorous stirring the content was filtered. The filtrate was mixed thoroughly with 70 mL of 1 M KCl solution and left overnight at 4 °C for phase separation.

The organic phase was collected and dried with a rotary evaporator at 40 °C, dissolved in 5 mL *n*-hexane/isopropanol solution (4/1, v/v) and stored at –18 °C until analysis.

Fatty acid analysis

Methyl esters of FA (FAME) were prepared in accordance with the procedure given in PN-EN ISO 12966-2:2011 [18]. Esterification was conducted following the general methylation method. According to this standard, both bound FA and free fatty acids (FFA) were converted into FAMES.

Reference kit of FAMES was used for the identification of particular isomers. FA content was determined and results were calculated according to the PN-EN ISO 12966-4:2015 [19]. The chromatographic conditions were similar to those reported by Verardo et al. [20]. The composition of FA was expressed as g 100 g⁻¹ of FAMES.

Physicochemical characterization of lipids

Acid value (AV) determinations were carried out in triplicate according to the Polish Standard PN-EN ISO 660:2010 [21].

Table 1 Characteristics of the analyzed dry dog foods as declared on the label

Product	A	B	C	D
Main animal-derived ingredient	Salmon	Lamb	Beef	Chicken
Crude fat/%	21	18	14.5	22
Main fat sources	Animal fat, fish oil	Chicken fat (preserved with tocopherols), salmon oil	Mixed vegetable oils (rapeseed oil, linseed oil), salmon oil	Animal fats, salmon oil
Antioxidant declarations	Tocopherol rich extracts of natural origin 65 mg kg ⁻¹	Contains antioxidants	No added colorants or preservatives	Antioxidants: tocopherols

Pressurized differential scanning calorimetry measurements

The thermooxidative measurements were taken with DSC Q20 calorimeter coupled with a high pressure cell (Q20P) (TA Instruments, New Castle, DE, USA). The apparatus was calibrated with high-purity indium standards. Samples of approximately 4 mg were analyzed under oxygen atmosphere, pressurized in an isobaric module (1400 kPa). The open pans were heated from ambient temperature at a heating rate $10\text{ }^{\circ}\text{C min}^{-1}$ until isothermal temperature $100\text{ }^{\circ}\text{C}$. Each analysis was carried out in triplicate.

The time of reaching the maximum heat flow (τ_{max}) was determined from the resulting PDSC exotherms (Fig. 1) [22]. The assumptions given previously by Kowalski et al. [23] were applied for the assessments of the oxidative stability.

Data analysis

For the statistical analysis, paired t-tests were performed, for each evaluated product the means from both sets of analyses were compared, as described above. Results are presented in Table 2 as means plus SD for each pair of measurements with *P* values. IBM SPSS Statistics software, version 21 (IBM Warsaw, Poland) was used for calculations.

Results and discussion

Fatty acid content

Amylose–lipid complexations, occurring during extrusion, are expected to decrease free fat in the pet food matrix. Such ‘entrapment’ in protein helixes reduces amount of fat

available for oxidation, apparently extending the shelf life [16].

Therefore, it can be expected that changes observed in lipid properties after storage mainly resulted from alterations in coatings and palatants sprayed over dried kibbles.

Results presented in Table 2 show that products evaluated in the current study had different FA content, subsequently modified during storage. Sum of saturated fatty acids (SFA) generally decreased, oppositely to monounsaturated fatty acids (MUFA) mainly due to a significant increase in oleic acid (C18:1; OA). One plausible explanation can be the reactivation of plant-derived lipases, linked with unavoidable increase in humidity after opening the packages and during storage in room temperature [24, 25]. In fact, all analyzed products declared rice at the top of the ingredients list in quantities that can be estimated in the range of 10–20 % of DM (not showed). However, the form (kernels, bran or both) was not revealed by manufacturers. Previously, it was shown that in rice bran stored in open bags in ambient temperature for 5 months, palmitic acid (C16:0) was strongly reduced of about 80 % of its initial content. An apparent reason given for this phenomenon was the lipase preference to cleave the specific positions of triacylglycerols but not higher affinity to this FA [26].

At the beginning of the study products, A, C and D showed similar sum of polyunsaturated fatty acids (PUFA) (18.42; 24.38 and 20.96 %, respectively), while product B had drastically lower amount (7.5 %). Opposite directions of FA changes, revealed in all pet foods after 7 months of storage, likely reflect combined effect of fat type and antioxidant applied in the formula [27].

All products studied (except C) had tocopherols declared as an antioxidant on the label (Table 1), whereas on the packaging of product B an additional claim: ‘contains antioxidants’ was placed, allowing for the use of other additives within permitted maximum level [5].

Fish (or namely salmon) oil was declared as a minor fat source in all studied formulas (Table 1). From the group of long-chain PUFAs typically abundant in this feedstock, only docosahexaenoic acid (C22:6; DHA) was detected in the lipid fraction (not shown). Product A had highest DHA content in freshly opened kibbles that markedly decreased during storage. Interestingly, product B showed no detectable amounts of any FA typical for fish oil addition. Ahlstrøm et al. [28] reported substantial differences in FA content in commercial dry foods for dogs (mainly puppy foods), suggesting that no DHA or eicosapentaenoic acid (C20:5; EPA) practically reflects the absence of marine oils or products in the kibble.

On the other hand, product B with lamb declared as its main animal-derived component, had very low content of linoleic acid (C18:2; LA), typical for FA profile of this meat [29]. It seems plausible that the declared inclusion of

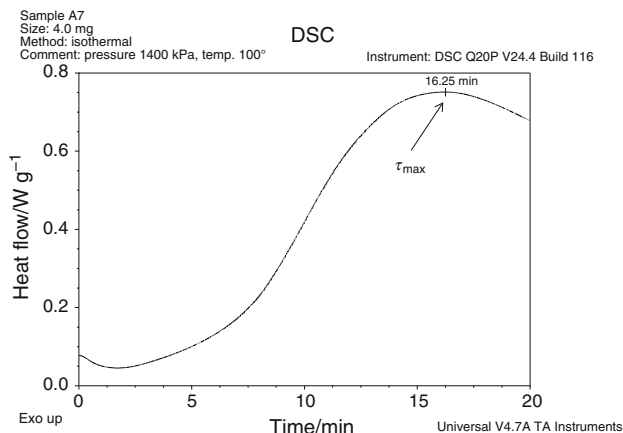


Fig. 1 PDSC scan of dry dog food lipid fraction obtained at $100\text{ }^{\circ}\text{C}$ and 1400 kPa of oxygen pressure. Time of τ_{max} is shown

Table 2 The parameters of lipid fraction

Product	A				B				C				D			
	0	7	SD	P	0	7	SD	P	0	7	SD	P	0	7	SD	P
Fat content/ g 100 g ⁻¹	11.71	15.48	0.24	0.074	11.14	20.18	0.67	0.053	11.79	12.15	0.55	0.067	16.90	20.99	0.49	0.061
Fatty acids/g 100 g ⁻¹ of FAMES																
C12:0	0.18	0.10	0.03	0.038	0.43	0.38	0.04	0.377	0.55	0.46	0.04	0.058	0.44	0.42	0.06	0.502
C14:0	2.16	1.59	0.06	0.004	3.25	3.10	0.15	0.408	2.57	1.69	0.13	0.007	1.66	1.52	0.04	0.026
C16:0	22.53	21.37	0.33	0.026	24.67	24.94	0.25	0.392	30.38	28.44	0.59	0.030	22.35	22.40	0.19	0.681
C16:1	2.70	2.44	0.14	0.085	6.15	6.13	0.08	0.815	2.20	1.96	0.07	0.030	2.10	2.17	0.07	0.208
C18:0	12.63	11.78	0.33	0.047	10.30	9.03	0.53	0.139	5.79	5.02	0.82	0.242	13.58	13.15	0.04	0.002
C18:1	37.70	41.08	0.10	0.000	43.48	46.34	0.26	0.008	31.84	35.23	0.45	0.006	35.83	37.06	0.13	0.004
C18:2	16.80	14.70	0.13	0.002	6.45	5.38	0.35	0.093	21.12	19.29	0.68	0.031	15.21	15.17	0.08	0.127
C18:3	1.59	1.46	0.05	0.049	1.05	1.02	0.04	0.582	3.26	3.10	0.10	0.121	5.78	5.52	0.04	0.006
C20:0	0.30	0.16	0.03	0.011	0.48	0.36	0.07	0.204	0.35	0.22	0.07	0.085	0.19	0.19	0.02	0.529
C20:1	1.71	1.28	0.16	0.042	0.39	0.33	0.25	0.833	1.29	1.12	0.22	0.312	0.79	0.72	0.03	0.053
ΣSFA/%	37.81	35.00	0.36	0.005	39.14	37.82	0.35	0.023	39.65	35.84	0.30	0.002	38.23	37.69	0.24	0.057
ΣMUFA/%	41.11	44.80	0.33	0.003	50.02	52.80	0.49	0.010	35.33	38.31	0.36	0.005	38.73	39.96	0.16	0.006
ΣPUFA/%	18.42	16.21	0.16	0.003	7.50	6.41	0.61	0.090	24.38	22.40	0.60	0.028	20.96	20.80	0.07	0.069
ΣUFA/%	57.54	63.02	0.37	0.001	57.52	59.21	0.33	0.012	57.72	62.71	0.78	0.008	59.69	60.76	0.22	0.013
AV/mg KOH g ⁻¹	8.35	12.14	0.02	0.000	13.04	11.48	0.12	0.002	32.77	37.11	0.22	0.001	9.58	12.25	0.37	0.006
τ _{max} /min	13.58	16.39	0.10	0.000	17.64	20.06	0.74	0.030	9.85	8.35	0.46	0.029	9.20	6.32	0.11	0.000

Data expressed as means ($n = 3$)

SD standard deviation, FAME fatty acid methyl ester, SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids, AV acid value, τ_{max} maximum induction time

chicken fat and salmon oil had negligible effects on the lipid properties of this dog food.

AV

AV is a classic measure of FFA content in food lipids, indicating insufficient processing, lipase activity or other hydrolytic actions [30]. Commonly accepted tendency is that the lower AV of the oil or fat, the better the quality and freshness it possesses [31].

According to Codex Alimentarius, AV of edible fats and oils should not exceed 0.6 for refined and 4.0 mg KOH g⁻¹ for virgin and cold pressed [32]. Animal fats used in pet food plants are produced in the process of rendering various animal-derived by-products [33]. In general, low qualified oils with high AV are used as raw materials in dog food formulas.

In the current study, all products showed high initial AV that noticeably changed during storage (Table 2). In the case of product C that declared a mixture of vegetable oils as a main fat source, the AV was particularly high (32.77 and 37.11 mg KOH g⁻¹ of fat, respectively in first and second phase of analyses). After FFA% calculation

described in PN-EN ISO 660:2010 [21], a similarity with the feed fat acidity (restaurant grease denoted as waste frying oil) could be observed [34]. Considering that this product had ‘no preservatives’ claim on the label and was the only one packaged in a paper bag, the discrepancy in AV can be at least partially explained.

It has to be underlined that in dry pet foods, lipid quality is a vector of the properties of various ingredients used in processing plants. For example, AV increase in fish oil during long-term storage was previously reported [35]. However, most likely the addition of fish oil to the formulas of all currently studied dog foods had little effect on quality and oxidative parameters on the contrary to main animal-derived product used. Commodities typically used in pet food production have to be listed on the label in decreasing order by mass [5]. First ingredient on the list (if not declared as dehydrated or dried) should also be considered not defatted [4], i.e. having prevalent effects on lipid fraction quality of the final product. Therefore, due to the specifics of labeling regulations, allowing for different ways of presenting the content, it may become complicated for the consumers to pick up exact product meeting their expectations.

Another interesting phenomenon detected in product B deserves further elucidation. For this product, a noticeable decrease in AV was observed between the first and second phase of analysis (13.04 and 11.48 mg KOH g⁻¹ of fat, respectively, Table 2). Toci et al. [36] suggested that a decrease in FFA during storage of roasted coffee was a consequence of their oxidative degradation regardless of the storage temperature and atmosphere. Compared to products A, C and D, it is possible that the rate of loss overcame the rate of FFA production through triacylglycerols (TAG) hydrolysis.

Oxidative stability

In a recently published study, the FA concentration of various commodity fats and oils was shown to be highly correlated with the results of numerous methods of oxidative stability measurements [37].

We attempted to use PDSC techniques to estimate the oxidation effects of long time storage with reference to the proportions of FA in dry dog foods. The highest oxidative stability was revealed for product B. During 7 months of storage its τ_{\max} significantly increased (17.64 vs 20.06 min; Table 2) probably due to changes in the ratio of particular isomers. This dog food had smallest amount of PUFA and the highest of MUFA (namely OA).

Similar trend was noted for the product A with more than double PUFA but with initially lower oxidative resistance. Freshly opened products C and D had similar proportions of FA and showed comparable stability that substantially decreased during storage. These distinctions need further research.

The data in Table 2 revealed that OA was the most abundant isomer in the lipid fraction of product B. Many authors have previously shown that vegetable oils with the highest OA content were the most resistant to autoxidation (with or without added oxidants) [38–40]. Kerrihard et al. [37] justified the magnitude of fat oxidation with the corresponding composition and proportions of monounsaturated, diunsaturated and triunsaturated FA (MUFA:DiUFA:TriUFA) in foods.

On the example of tendencies revealed for product B, we suggest that the high prevalence of OA can be most likely attributed to increased oxidative stability during storage of dry dog food. Additional studies are necessary to confirm this hypothesis.

Conclusions

In summary, it can be concluded that ingredients used in dry dog foods processing have a substantial impact on their quality and stability. Declarations present on the labels not

always accurately describe the properties of the product. Our preliminary results showed that main animal-derived ingredient characteristics may closer reflect the actual properties of the lipid fraction of the kibble than those of additional fat sources. It can be seen that the typical storage of dry dog kibbles has a moderate effect on the lipid fraction properties. Further studies are needed to determine the oxidative consequences of modified storage conditions and handling of the product and its resistance against oxidation. PDSC index τ_{\max} appears to be appropriate and effective in such measurements of oxidative stability in pet foods.

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