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STABILITY OF EXTRUDED DIETS FOR DOGS

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ABSTRACT - Controlling the factors that influence the conservation of extruded dog foods can increase shelf life and/or guarantee the quality of shelf life of diets for these animals. Therefore, the objective was to evaluate the relationship among water activity (Aw), moisture (M), acidity, lipid peroxidation, crude protein (CP), ether extract (EE), and kibble size of extruded dog foods stored in sealed and open packages for 60 days (Experiment I). We also evaluated the stability of the Aw for up to 6.5 hours after coating with palatant (Experiment II). We manufactured four extruded dry dog foods: high CP and EE (HPE); low CP and EE (LPE); small kibble (SK); and large kibble (LK). In experiment I, the foods were stored in sealed 1 kg packages and 10 kg open packages, for a period of 60 days. We measured Aw, M, acidity, peroxide, CP, and EE of foods and the relative humidity (RH). Data were subjected to Pearson correlation analysis. For experiment II, samples were collected immediately after coating with palatant. Sub samples were collected every half hour to measure Aw. In experiment I, positive correlations were observed ($P<0.05$) among kibble size, M, and Aw; acidity, CP, EE, and M; and between Aw and RH, for open and sealed packages. There was also a positive correlation ($P<0.05$) for open packages among time, Aw, and peroxide. In experiment II, LK and LPE food presented Aw stabilized in less time. Diets with higher kibble size and high CP and EE levels are more unstable if kept in open packages. Extruded dry food with higher protein and lipid and smaller kibble size needs more time to stabilize its Aw.
Keywords: acidity, water activity, palatant, peroxide.

ESTABILIDADE DE ALIMENTOS EXTRUSADOS PARA CÃES

RESUMO - O controle dos fatores que influenciam a conservação de alimentos extrusados para cães pode aumentar o tempo de prateleira e/ou garantir a qualidade no tempo de validade dos alimentos destinados a esses animais. Diante disso, objetivou-se avaliar a relação entre atividade de água (Aa), umidade (UM), acidez, peroxidação lipídica, teores de proteína bruta (PB), extrato etéreo em hidrólise ácida (EE) e tamanho de extrusado em alimentos para cães armazenados em embalagens fechadas e abertas durante 60 dias (Experimento I). Ainda, avaliou-se a estabilidade da Aa até 6,5 h após recobrimento com palatabilizante (Experimento II). Foram fabricados quatro alimentos secos extrusados: alta PB e EE (APE), baixa PB e EE (BPE), extrusado pequeno (PQ) e extrusado grande (GR). No experimento 1 os alimentos foram armazenados em embalagens de 1 kg fechadas e de 10 kg abertas. Foram mensuradas a Aa, UM, acidez, peróxido, PB e EE dos alimentos e a umidade relativa do ar (URA). Os dados foram submetidos à análise de correlação de Pearson. No experimento II, foram coletadas amostras de 1 kg após o recobrimento. Subamostras foram coletadas a cada 30 minutos para mensuração da Aa. No experimento I foram observadas correlações positivas ($P<0.05$) entre tamanho do extrusado, UM e Aa; acidez, PB, EE e UM; e entre a Aa e URA, para embalagens abertas e fechadas. Houve correlações positivas ($P<0.05$) para as embalagens abertas entre o tempo, Aa e peróxido. No experimento 2, extrusados GR e alimentos BPE tiveram a Aa estabilizada em menor tempo. Dietas com extrusados maiores e elevados teores de PB e EE são mais instáveis, se mantidas em embalagens abertas. Alimentos secos extrusados com maiores teores de proteína e lipídios e extrusados menores apresentam maior tempo para estabilizar sua Aa.
Palavras-chave: acidez, atividade de água, palatabilizante, peróxido.

INTRODUCTION

With the greater demand for extruded diets, some stores sell these products in bulk, to facilitate sales to customers. However, this type of commercialization can reduce the shelf life of extruded foods, since the food is exposed to light, oxygen, and moisture (M), which accelerate reactions of lipid oxidation and microbial development (HOLDA; GLOGOWSKI, 2016). For these feeds to reach pet animals with quality, some factors must be considered by the industry (CAPPELLI et al., 2016). Among them are the levels of M, water activity (Aw), acidity, and peroxide of products. The M is present in practically all foods through bound water to molecules and free water (MORGANO et al., 2008) and is a fundamental element for palatability (PIZZATO; DOMINGUES, 2008, ZANATTA et al., 2016). As free water is available for the formation of chemical, physical, and microorganism reactions, such as fungi and bacteria (BRITO et al., 2013), it can accelerate the deterioration of the product, and its

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control by the industry is very important (SOARES et al., 2012; COSTA et al., 2015).

Another important point for food quality is the process of lipid deterioration, due to peroxidation or bacterial hydrolysis (PIHLANTO, 2006). Peroxidation occurs when free radicals come into contact with an unsaturated fatty acid (LIMA, 2001), which can reduce the nutritional value of the food and lead to the formation of toxic compounds (FISCHER et al., 2005). The hydrolysis of triglycerides by bacteria can be measured by the acidity content (MELLO et al., 2015). Acidity determines the amount of free fatty acids in the fat present in the food (VIEIRA et al., 2018). Both lipid peroxidation and hydrolysis processes can be influenced by the chemical composition and mode of storage of the diet (MENDES et al., 2014). In addition, they depress the nutritional value of the diet and can cause the animal to refuse food (FÉLIX et al., 2010).

Considering the importance of these factors on the quality of pet food, the objective was to evaluate the relationship among Aw, M, acidity, lipid peroxidation, protein, and lipid contents, and kibble size of extruded dog food stored in sealed or open packages for 60 days. Besides, the objective was to evaluate the Aw stability of complete dog food within 6.5 h after coating with a liquid palatant.

MATERIAL AND METHODS

The experimental diets were manufactured and the experiments were conducted in a factory that produces extruded diets for pet animals, located in Três Barras (SC, Brazil). Four complete extruded dry dog foods were produced during seven days for the experiment. Two diets had different kibble sizes, being: large (LK), with 0.925 cm³ and small (SK), with 0.537 cm³, both with the same formulation. The other diets had different levels of crude protein (CP) and acid hydrolyzed ether extract (EE), being: high levels (HPE) or low (LPE). The diets were ground to 1 mm particle size and analyzed at the beginning of the experiment for M, CP, EE, ash, crude fiber, calcium, and phosphorus, according to AOAC (1995). The chemical composition of the experimental diets is found in Table 1.

TABLE 1 - Analyzed chemical composition (% as fed basis) of complete dog foods containing small (SK) and large (LK) kibble and with high (HPE) and low (LPE) protein and lipid contents.

Itens (%)	SK	LK	HPE	LPE
Moisture	8.15	11.53	6.00	11.53
Crude protein	19.54	19.79	26.79	19.86
Ether extract	11.07	10.11	16.46	12.57
Crude fiber	5.53	5.56	4.20	5.87
Ash	5.97	5.64	5.37	8.58
Calcium	0.91	1.08	0.91	1.67
Phosphorus	0.89	0.84	1.01	1.45

The SK and LK foods consisted of corn, broken rice, poultry offal meal, brown rice bran, chicken fat, chicken hydrolyzate, sodium chloride, dehydrated carrot, dehydrated spinach, white beet pulp, propionic acid, antioxidant (BHT), vitamin A, vitamin D3, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin K3, folic acid, pantothenic acid, niacin, biotin, magnesium, copper, iron, iodine, manganese, selenium, zinc, and choline.

The HPE food contained: poultry offal meal, broken rice, whole corn, chicken fat, refined fish oil, corn gluten, wheat bran, powdered egg, fish meal, meat hydrolyzate, beet pulp, brewer's yeast, mannanoligosaccharides, yucca extract, sodium chloride, chelated minerals, propionic acid, antioxidant (BHT), vitamin A, vitamin D3, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin K3, folic acid, pantothenic acid, niacin, biotin, magnesium, copper, iron, iodine, manganese, selenium, zinc, and choline.

The LPE food contained the following ingredients: corn, wheat bran, meat and bone meal, brown rice bran, beef tallow, chicken liver hydrolyzate, sodium chloride, calcitic limestone, propionic acid, antioxidant (BHT), vitamin A, vitamin D3, vitamin B1, vitamin B2, vitamin B6, vitamin B12, Vitamin K3, folic acid, pantothenic acid, niacin, biotin, magnesium, copper, iron, iodine, manganese, selenium, zinc, and choline.

Experiment I: Stability of extruded dog foods stored in sealed or open packages.

Laboratory analysis – sealed packages:

To measure the stability of the diets, analyzes were carried out on sealed packages, which remained in the factory's stock on pallets, away from excessive moisture and sunlight. The treatments were separated into 42 sealed packages of 1 kg for each treatment. The analyzes started on the day the diets were produced. Fourth two samples were analyzed for 60 days. The analyzes were carried out for five consecutive days, with a two-day break (weekends). Room temperature and air relative humidity (RH) data were also recorded using an internal digital hygrometer (Incoterm, model: 7663.02.0.00, Brazil).

Daily Aw and M analyzes were performed. The Aw was measured with an Aw analyzer (BrasEq - Brasileira de Equipamentos Ltda, Jarinu, Brazil). This analysis was performed from samples collected from the feed packages. The sample was placed in a capsule and the Aw of the food was given according to the dew point determination technique in an encapsulated mirror.

The M was obtained from a halogen humidity meter (GEHAKA, São Paulo, Brazil). The measurement of M was performed with the same samples ground to measure Aw. The samples were weighed in the device that measured M in an amount of 1,000 g ± 0.100 g. The Aw and M analyzes were performed every day in duplicate. Analysis of acidity and cold peroxide index was also carried out according to the Brazilian Compendium of Animal Nutrition (1998). The latter is carried out once a week, totaling nine weeks.

The packages, which remained sealed until the time of the measurements, were taken to the analysis room every day and opened only when they were taken. Each day, four sealed packages were taken, one for each treatment.

Laboratory analysis – open packages:

The same four treatments were separated in a 10 kg package for each food, which remained open for the entire period on the floor to simulate the sale of bulk products. The analyzes of the diets stored in open packages began on the day the packages were opened, approximately one week after manufacture. Within the 10 kg packages, sub-samples were taken. The same analyzes were carried out as for the sealed packages.

Pearson correlation analyzes were performed on the SAS package using the CORR procedure (P<0.05) among SK, LK, HPE, and LPE and Aw, M, CP, EE, acidity, peroxide, size, room temperature, time, and RH for open and sealed packages. Except for the acidity and peroxide data, which totaled nine repetitions, the other data totaled 42 repetitions per treatment.

Experiment II: Water activity stability

Laboratory analysis:

To measure the Aw stabilization time of the diets, 1.00 kg samples of the four extruded dog foods were collected after coating with a liquid palatant (poultry liver hydrolyzate). Sub-samples were collected every half hour to perform Aw analysis for a period of 6.5 h. Room temperature and RH were measured on the days that these analyzes were performed. The samples were submitted to regression analysis by excel (OFFICE, 2010). The derivatives of the equations were made, equaling 'y' to zero, to estimate the Aw stabilization time of the different diets after application of the palatant.

RESULTS AND DISCUSSION

Experiment I: Stability of extruded dog food stored in open and sealed packaging

The initial (week 0) and final (week 9) values for each analyzed variable are shown in Table 2. For foods stored in sealed packages (Table 3), it can be seen that Aw and M showed a positive correlation (P<0.01). The same characteristic is observed in the correlations between Aw and the variables acidity, kibble size (P<0.01), and RH (P<0.05). Food acidity also showed a positive correlation with CP and EE (P<0.01).

TABLE 2 - Averages of the initial (day 0) and final (day 60) values of the variables evaluated in diets kept in open or sealed packages.

Items	Moisture (%)		Water activity		Acidity (mg NaOH g^{-1})		Peroxide (meq kg^{-1})	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Open packaging								
SK	8.49	8.85	0.529	0.546	1.21	1.28	0	0.99
LK	10.86	11.21	0.623	0.677	1.36	1.5	0	0.98
HPE	7.72	8.41	0.516	0.567	1.68	1.91	0	2.22
LPE	8.85	10.18	0.531	0.566	1.34	1.44	0	1.96
Sealed packaging								
SK	8.49	8.62	0.529	0.533	1.21	1.28	0	0
LK	10.86	10.36	0.623	0.637	1.36	1.47	0	0
HPE	7.72	7.93	0.516	0.502	1.68	1.9	0	0
LPE	8.85	8.86	0.531	0.517	1.34	1.36	0	0
SEM	0.281		0.015		0.072		0.286	

SK: small kibble, LK: large kibble, HPE: high protein and ether extract, LPE: low protein and ether extract, SEM: standard error of the mean.

There was no correlation between peroxide and Aw (P>0.05), since the diets were properly packed and well stored. There was also no correlation between RH and M of the diet and the time that the food remained stored with Aw and M for the sealed packages (P>0.05). During the period of the experiments, the maximum and minimum temperatures and RH were 25.1°C and 16.4°C and 74% and 29%, respectively.

TABLE 3 - Correlation among water activity (Aw), moisture (M), acidity, peroxide, crude protein (CP), ether extract (EE), kibble size, time (weeks), and air relative humidity (RH) of dog foods in sealed packages.

	M	Aw	Acidity	Peroxide	CP	EE	Size	Time	RH
M	NE	-	-	-	-	-	-	-	-
Aw	0.931**	NE	-	-	-	-	-	-	-
Acidity	0.776**	0.722**	NE	-	-	-	-	-	-
Peroxide	0.000	0.000	0.000	NE	-	-	-	-	-
CP	-0.903**	-0.682**	0.592**	0.000	NE	-	-	-	-
EE	-0.903**	-0.682**	0.592**	0.000	NE	NE	-	-	-
Size	0.951**	0.844**	0.834**	0.000	NE	NE	NE	-	-
Time	0.030	-0.149	0.276	0.000	NE	NE	NE	NE	-
RH	0.225	0.509*	-0.009	0.000	NE	NE	NE	-0.134	NE

NA = not evaluated. *P<0.05. **P<0.01.

In open packages (Table 4) Aw showed a positive correlation with M, acidity, kibble size, and storage time (P<0.01). Likewise, acidity showed a positive correlation with the variables mentioned above (P<0.01), except for storage time (P>0.05). Acidity also showed a positive correlation with CP and EE (P<0.01). There was a positive correlation between the peroxide index and storage time (P<0.01). There was no correlation between peroxide and Aw, time, and M and between RH and M (P>0.05).

TABLE 4 - Correlation among water activity (Aw), moisture (M), acidity, peroxide, crude protein (CP), ether extract (EE), kibble size, time (weeks), and air relative humidity (RH) of dog foods in open packaging.

	M	Aw	Acidity	Peroxide	CP	EE	Size	Time	RH
M	NE	-	-	-	-	-	-	-	-
Aw	0.873**	NE	-	-	-	-	-	-	-
Acidity	0.850**	0.755**	NE	-	-	-	-	-	-
Peroxide	-0.193	0.428	0.354	NE	-	-	-	-	-
CP	-0.808**	-0.09	0.862**	0.347	NE	-	-	-	-
EE	-0.808**	-0.09	0.862**	0.347	NE	NE	-	-	-
Size	0.953**	0.773**	0.851**	-0.242	NE	NE	NE	-	-
Time	0.134	0.680**	0.09	0.826**	NE	NE	NE	NE	-
RH	0.216	0.688**	0.09	0.334	NE	NE	NE	0.208	NE

NE = not evaluated. *P<0.05. **P<0.01.

Experiment II: Stability of water activity

With the derivatives of the equations generated by regressing diets with different kibble sizes (Figure 1), it was possible to observe that the diet with smaller kibble obtained Aw stability in approximately 350 min. or 5.8 h. During the 6.5 h period, the lower volume kibble had an Aw variation between 0.652 (initial Aw) and 0.515 (final Aw) with stabilization with 0.599 Aw. The larger kibble diet stabilized in 250 min. or 4.2 h. During the Aw observation period, the highest volume kibble varied between 0.744 (initial Aw) and 0.666 (final Aw) and stabilized with 0.695 Aw.

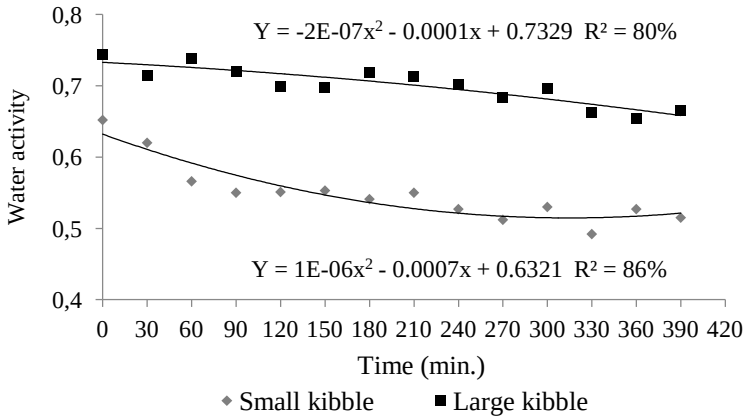

FIGURE 1 - Relationship between water activity and time after coating with palatant in complete foods for dogs with different kibble sizes.

In Figure 2, it is possible to verify that the Aw stabilization time for the LPE diet was 133 min. or 2.2 h. During the 6.5 h period, the LPE diet had an Aw variation between 0.663 (initial Aw) and 0.552 (final Aw), stabilizing with 0.629 Aw. While the Aw stabilization time for the HPE diet was 400 minutes or 6.7 h. During the Aw observation period, the LPE diet varied between 0.613 (initial Aw) and 0.507 (final Aw), and Aw stabilization occurred with 0.514.

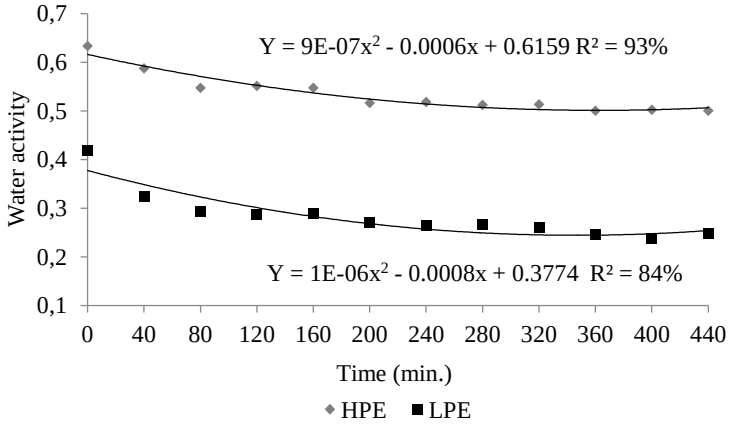

FIGURE 2 - Relationship between water activity and time after coating with palatant in complete foods for dogs with high (HPE) and low protein and ether extract (LPE).

Complete foods stored open and those kept in sealed packages showed a positive correlation between Aw and M. These data do not corroborate the data of Murakami et al. (2018), in which the authors report that Aw does not necessarily correlate with the M of the finished kibble. In the case of the present study, this may have happened because the stability of Aw is influenced by the ingredients of the diet. Aw is one of the parameters capable of measuring osmotic properties, which makes it a good indicator of the availability of water capable of participating in chemical reactions (TROLLER et al., 2012). Thus, the final osmolality of the food is directly correlated with Aw. If the diet contains less hygroscopic ingredients, the water is less bound and consequently, Aw remains more correlated to M.

The positive correlation between RH and Aw for open packaging can be explained since open packaging undergoes a process called "hygroscopic balance" (CAETANO et al., 2012). As a result, they suffer rapid hydration when the RH is greater than M of the food (HOLTZ; REIS, 2013). This hydration can cause an increase in Aw and, consequently, microbial development (FERREIRA NETO et al., 2005). The reduction in Aw in the external medium to the microorganism increases the osmotic stress of the cell since they seek to maintain osmolarity lower than that of the medium. If Aw is lower in the food, there will be more solute in the external environment, resulting in dehydration and inhibition of the development of microorganisms (LABUZA; RAHMAN, 2007). Thus, by increasing Aw, the safety of the product is at risk.

Sealed packaging also suffered interaction with the environment, this should not happen with packaged food. Therefore, it is possible that the packaging material used in this study has not effective in preventing gas exchange between the environment and food entirely. Kibble with greater volume have more difficulty in losing M during the drying process, due to the relatively smaller surface contact with the air. This could explain the increase of the Aw and M over kibble sizes for open and sealed packages. Another factor that led to an increase in Aw for open packaging was time. The longer the packages remained open, the greater the Aw value. This may have occurred possibly due to the longer time available for hygroscopic equilibrium to occur, as previously discussed.

There was also an increase in acidity in diets that had higher levels of CP and EE. This factor is mainly related to the increase in EE in the diet, as acidity is formed mainly due to the presence of free fatty acids in the fat present in the food (ORTOLAN et al., 2010; SANTOS et al., 2017). Generally, formulations with higher levels of EE also have higher levels of CP, being the case of diets in the premium and super premium category (CARCIOFI et al., 2009). Therefore, the correlation is also positive about the CP of the complete food. In addition, diets with high CP levels generally use more animal-based flours in their formulation, which can contribute to the acidity of the diet. The acidity index can also be accelerated with the presence of factors such as M, temperature, and oxygen. Besides, food storage is the main responsible for acidity (GREGGIO; BONINI, 2014). This may be related to the larger kibble size, since the greater kibble volume is related to the increase in M, depending on where the food is stored open, there may be the formation of peroxides. This is what may have happened since the opened packages had direct contact with high temperatures, light, and oxygen (BELLAVER; ZANOTTO, 2004).

Regarding the second experiment, it can be seen that although the larger kibbles of complete dog food may have higher Aw and M, they stabilized the Aw values after

the palatant immersion bath 100 minutes before the lower volume kibbles. The LPE diet showed Aw stability faster, compared to the HPE diet. This may have happened because of the higher levels of CP and EE in the HPE diet, which can make the diet less stable. Despite this, the ingredients and formulas were different between diets, other factors such as hygroscopicity of the raw materials used, density and expansion of the diets may also have influenced Aw stabilization time after application of the liquid palatant. Thus, considering that extruded foods have different Aw stabilization times, the industry must consider this factor before packaging the diets. Commercial diets, when exposed to the environment, exhibit loss of quality and food security over time, which may cause risks to the health of pet animals. Quality control must be strict in both drying and storing food. Studies - mainly, on the monitoring of parameters such as water activity, peroxide, acidity, and humidity must be conducted to guarantee the quality of finished extruded foods and shelf life.

CONCLUSION

Diets with larger kibble and high levels of proteins and lipids require more care in drying and storage, as they are unstable if kept in open packaging.

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