CAPÍTULO 2

PHYSICAL, CHEMICAL AND SHELF-LIFE CHARACTERISTICS OF CANINE BISCUITS CONTAINING NATURAL ANTIOXIDANTS

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Jessyca Caroline Rocha Ribas
Programa de Pós-Graduação em Zootecnia, Universidade Estadual de Maringá, Av. Colombo, 5790, J35, 87020-900, Maringá, PR, Brasil.
0000-0002-6046-2827

Livian Yumi Iwaoka
Graduação em Agronomia, Universidade Estadual de Maringá, Av. Colombo, 5790, J35, 87020-900, Maringá, PR, Brasil.
0000-0001-7923-2522

Paula Toshimi Matumoto-Pintro
Programa de Pós-Graduação em Engenharia de Alimentos, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, PR, Brasil.
0000-0002-9182-/5758

ABSTRACT: Snacks are foods that are increasingly being provided to dogs. The addition of natural antioxidants in this type of food improves its nutritional composition and brings benefits to animal health. The objective was to evaluate the effects of natural antioxidants (turmeric and marigold) on the physicochemical and technological characteristics and on the shelf life of canine biscuits made with three sources of fat (canola, linseed and olive oil). The parameters analyzed were: antioxidant activity, lipid oxidation, color and texture in biscuits with up to 60 days of storage. The addition of turmeric to the biscuits increased the antioxidant activity and intensified the color of the biscuits, although it did not influence the oxidative stability. The addition of linseed oil contributed to a greater formation of maloaldehyde. Storage reduced the hardness of the biscuits and increased lipid oxidation. It is concluded that the addition of turmeric increases the antioxidant activity, influences the color but does not influence the oxidative stability and texture of dog biscuits.
KEYWORDS: ABTS, DPPH, linseed oil, marigold, turmeric.

CARACTERÍSTICAS FÍSICO-QUÍMICAS E VIDA DE PRATELEIRA DE BISCOITOS CANINOS COM ANTIOXIDANTES NATURAIS

RESUMO: Petiscos são alimentos cada vez mais oferecidos aos cães. A adição de antioxidantes naturais neste tipo de alimento melhora sua composição nutricional e traz benefícios à saúde animal. O objetivo do trabalho foi avaliar os efeitos de

antioxidantes naturais (cúrcuma e calêndula) nas características físico-químicas, tecnológicas e na vida de prateleira de biscoitos caninos elaborados com três fontes de gordura (canola, linhaça e azeite de oliva). Os parâmetros analisados foram: atividade antioxidante, oxidação lipídica, cor e textura em biscoitos com até 60 dias de armazenamento. A adição de cúrcuma aos biscoitos aumentou a atividade antioxidante e intensificou a cor dos biscoitos, porém não influenciou a estabilidade oxidativa. A adição de óleo de linhaça contribuiu para uma maior formação de malonaldeído. O armazenamento reduziu a dureza dos biscoitos e aumentou a oxidação lipídica. Conclui-se que a adição de cúrcuma aumenta a atividade antioxidante, influencia na cor, mas não influencia na estabilidade oxidativa e na textura de biscoitos para cães.
PALAVRAS-CHAVE: ABTS, calêndula, cúrcuma, DPPH, óleo de linhaça.

1 I INTRODUCTION

Snacks for companion animals are becoming popular at the same rate that pets are increasingly being considered family members (White et al., 2016; Morelli et al., 2020). These snacks are provided for the purpose of pleasing or rewarding, being classified specific food or chewable product (BRASIL, 2009). The variety of snacks available on the market is notorious, which can be presented in extruded, roasted, semi-moist or wet forms, with a wide range of textures, formats, and flavors. The most popular snack offered to pets are biscuits and chewable products (White et al., 2016). Biscuits for dogs are dry and hard, low-calorie foods, composed of cereals, salt, binders and flavor enhancers (Zahra et al., 2021), and ingredients with therapeutic purposes can be added like mental health promoters (Ricca, 2020; Margutttti et al., 2022), oral health (Santos et al., 2021) and intestinal health (Baroncello et al., 2020) among other purposes.

In bakery products, when fats are mixed with flour that has not yet been hydrated, fat particles surround the flour particles, reducing the elasticity of the dough, which prevents the dough from shrinking after laminating the cookies. In addition, the fat has a lubricating action, preventing the products from sticking to the baking sheets and causing a sensation of melting in the mouth at the time of consumption (Giuffrè et al., 2022).

For the manufacture of biscuits, different sources of lipids can be used in the dough, such as animal fat or vegetable oils, which vary in relation to physical, chemical and nutritional characteristics. Vegetable oils when used as an alternative to solid fats produce biscuits with a healthier fatty acid profile (Tarancón et al., 2014). Canola oil, for example, is mainly composed of oleic acid (65%), linoleic acid (15%) and linolenic acid (4.5%). In linseed oil there is a predominance of linolenic acid (46.5%), oleic acid (25%) and linoleic acid (14%), while olive oil stands out for its oleic acid content (75%) (Alves et al., 2019).

However, it is important to consider the inclusion of fats in bakery products from a nutritional point of view, due to their tendency to oxidation and the consequent formation of free radicals (Giuffrè et al., 2022). Generally, polyunsaturated fatty acids present in vegetable oils are more prone to lipid oxidation than animal fat. Thus, one way to increase

the shelf life of biscuits and other high-fat pet foods is to use antioxidants such as synthetic butylhydroxytoluene (BHT), butylhydroxyanisole (BHA) and ethoxyquin (Campigotto et al., 2020). However, the use of synthetic antioxidants in foods has been related to deleterious effects to the human and animal organism. In this way, the search for the replacement of synthetic antioxidants for natural ones, such as turmeric and marigold, is increasing.

Turmeric (*Curcuma longa L.*) is an important spice with an intense yellow color and a strong flavor used in the cuisine of several countries. It has bioactive compounds known as curcuminoids, represented by curcumin (70%), demethoxy curcumin (20%) and bisdemethoxy curcumin (10%) (Ma, Du & Wang, 2017). Curcumin is a potent antioxidant and anti-inflammatory agent with hepatoprotective, anticarcinogenic and antimicrobial properties (Munekata et al., 2021).

Marigold (*Calendula officinalis*) is a plant belonging to the Asteraceae family. It has yellow or orange flowers that are used as food, dye, spice, tea, ointment, or cream in cosmetics. The species contains a variety of active biological constituents, such as carotenoids, flavonoids, saponins, sterols, phenolic acids, which have a wide range of pharmacological effects (AshwlayanVD & Verma, 2018). In this way, the inclusion of natural antioxidants in dog food aims to protect lipid oxidation during storage and their biological properties act to promote animal health (Campigotto, et al., 2020). The present work aims to evaluate the effects of different sources of fat and natural antioxidants on the physicochemical and technological characteristics and on the shelf life of canine biscuits.

2 I MATERIALS AND METHODS

2.1 Materials

The ingredients for the manufacture of biscuits were purchased from the local market (Maringá, Paraná, Brazil). Folin-Ciocalteau, sodium carbonate, gallic acid, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS), potassium persulfate and 2,2-Diphenyl-1-Picrylhydrazyl free radical (DPPH) are from Sigma Aldrich (Spruce Street, St Louis, USA). The other reagents used are analytical grade.

2.2 Characterization of turmeric and marigold

Turmeric and marigold extracts were prepared with 100% methyl alcohol in proportions of 1:10 (w/v). The supernatant was collected and used for analysis.

The antioxidant activity of turmeric and marigold were determined using the 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging methods described by Re et al., (1999) and the scavenging of the free radical 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) (Li, et al., 2009). Results were expressed as a percentage.

The color was determined in turmeric and marigold using a colorimeter (Konica Minolta, CR400, China) using a CIELAB scale, measuring L* (100 ¼ white; 0 ¼ black), a*

(+red; -green) and b* (+yellow; -blue) at three distinct points.

2.3 Preparation and characterization of biscuits

The biscuits were prepared with formulations based on rice flour and chickpea flour. Based on the IC50 value of DPPH (concentration necessary of the antioxidant to reduce the DPPH radical by 50%), the addition of 0.5% of turmeric or marigold was defined in treatments T2 and T3 respectively. The treatments were tested:

1. T1CA: biscuit made with canola oil without antioxidants;
2. T1LI: biscuit made with extra virgin linseed oil without antioxidants;
3. T1AZ: biscuit made with extra virgin olive oil without antioxidants;
4. T2CA: biscuit made with canola oil and turmeric;
5. T2LI: biscuit made with extra virgin linseed oil and turmeric;
6. T2AZ: biscuit made with extra virgin olive oil and turmeric;
7. T3CA: biscuit made with canola oil and marigold;
8. T3LI: biscuit made with extra virgin linseed oil and marigold;
9. T3AZ: biscuit made with extra virgin olive oil and marigold.

Ingredients	**Grams**
Rice flour	400
Chickpea flour	220
Distilled water	200
Fat	100
Dehydrated whole egg	50
Sugar	27
Salt	2.5
Antioxidant (except T1)	0.5
Nutritional composition	
Moisture (%)	05.82 ± 0.05
Mineral matter (g $100g^{-1}$ DM)	02.81 ± 0.14
Carbohydrates (g $100g^{-1}$ DM)	64.57 ± 0.37
Crude protein (g $100g^{-1}$ DM)	11.47 ± 0.15
ethereal extract (g $100g^{-1}$ DM)	16.11 ± 0.17
Crude fiber (g $100g^{-1}$ DM)	05.05 ± 0.36

Table 01: Basic recipe to produce dog biscuits (1000 grams) and nutritional composition:

The process of preparing biscuits was carried out in the following steps: weighing the ingredients, mixing, molding, cutting, and baking in a conventional oven (Suggar FE1001BR, Brazil) at 200 °C for 12 minutes. After roasting, the biscuits were cooled to room temperature and packed in polypropylene plastic bags closed by pressing. The biscuits had a diameter of 4x4cm and a weight of around five grams. The samples were evaluated

in triplicate at intervals of 0, 15, 30 and 60 days of storage at room temperature. Table 01 shows the formulations of the biscuits and the nutritional composition.

The biscuits of each treatment were ground, and the extracts were prepared with 100% methyl alcohol in the proportions of 1:10 (m/v). To determine the antioxidant activity of biscuits, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging method as previously described.

Thiobarbituric acid reactive substances (TBA) resulting from lipid oxidation were determined according to the method of Juncher et al. (2001). TBA values were expressed in mg of malonaldehyde per kilogram of sample (mg malonaldehyde / kg of sample).

Biscuits from each treatment were ground and homogenized, and the color of the snacks was performed in each treatment at three different points with the aid of a portable colorimeter (Minolta CR400) and the color evaluation was based on the CIELAB system. Texture analysis was performed on the biscuits using a Brookfield-CT III texturometer. The equipment was calibrated in the following settings: Compression; test speed: 5 mm/s; trigger load: 30 g; probe: TA7; repair: TA-JTPB. The hardness was measured in grams.

2.4 Statistical analysis

The results obtained were submitted to analysis of variance using the statistical program SISVAR 5.6 (UFLA, MG, Brasil). Means and standard deviation were calculated for each variable. Antioxidant concentrations (T1: 0%; T2: 0.5% turmeric; T3: 0.5% marigold) and storage time (0, 15, 30 and 60 days) were considered fixed factors in the factor model for the variables: antioxidant activity, lipid oxidation, color, and texture. When differences were statistically significant, Tukey's test was used with a 5% significance level. The experiment was repeated three times and analyzes were performed in triplicate.

3 I RESULTS AND DISCUSSION

3.1 Characterization of turmeric and marigold

The results regarding the antioxidant activity and color of turmeric and marigold used as additives in canine biscuits are described in table 02. Turmeric showed higher antioxidant activity in relation to marigold both by scavenging the DPPH radical and ABTS.

	Tumeric	Marigold
Antioxidant activity		
DPPH (%)	94.03 ± 0.65^{A}	67.90 ± 2.60^{B}
ABTS (%)	66.39 ± 0.84^{A}	43.74 ± 0.64^{B}
Color		
L*	64.15 ± 0.26^{B}	77.13 ± 0.26^{A}
a*	13.26 ± 0.15^{A}	7.32 ± 0.16^{B}
b*	48.92 ± 0.16^{B}	52.99 ± 0.16^{A}

*Means with different uppercase letters in the same column have a significant difference (P<0.05).
**Results are expressed as mean ± standard deviation, in triplicate.

Table 02: Antioxidant activity and color of turmeric and marigold used in the preparation of canine biscuits.

The curcumin present in turmeric has an antioxidant function related to the number of hydroxyl groups in the structure of its aromatic ring. The number of these functional groups is directly proportional to the free radical scavenging activity of this polyphenolic compound (Ma, Du & Wang, 2017). Marigold has flavonoids and carotenoids which inhibit the production of various reactive oxygen species and free radicals due to its ability to inhibit oxidases, activate antioxidant enzymes, act as a chelator and by transferring electrons to free radicals (Jan, Andrabi & John, 2017).

From the color analysis, it is observed that turmeric has a lower luminosity value, greater intensity of red and lesser intensity of yellow in relation to marigold. The marigold is characterized by having a lesser shade of red and a greater shade of yellow. The characteristic coloring of these products mainly refers to the presence of carotenoids such as curcumin present in turmeric and lutein esters predominant in marigold (Rodrigues, Mercadante & Mariutti, 2018).

3.2 Characterization of biscuits

The antioxidant activity of dog biscuits made with different sources of fat and with the addition of turmeric or marigold over 60 days of storage is presented in Table 03.

The antioxidant effects of turmeric when used as a food additive are reported by several authors (Carvalho et al., 2020; El-Sayed et al., 2021; Roy et al., 2021; Yildiz et al., 2019). Biscuits made with the addition of turmeric (T2) showed scavenging activity of DPPH and ABTS radicals superior to the standard biscuits (T1) and with the addition of marigold (T3). Turmeric also has beneficial effects on the health of dogs due to its bioactive compound, the curcumin. Curcumin has anti-inflammatory effects and stimulates the activity of several antioxidant enzymes in the blood serum of dogs fed rations containing this compound (Campigotto et al., 2020). The incorporation of marigold as an antioxidant did not result in scavenging of DPPH and ABTS radicals at levels higher than biscuits without the addition of natural antioxidants. Marigold has bioactive compounds with antioxidant activity

(Table 01), although the content added to the biscuits may have been insufficient.

The antiradical activity of DPPH for biscuits made with turmeric reduced during the 60 days of storage, however, it remained constant for biscuits made with marigold except for the T3LI treatment. The antiradical activity of ABTS shows that the storage time did not influence the values obtained. The fat source used did not influence the antioxidant activity of the treatments. It is observed that the addition of turmeric influences the antioxidant activity by the DPPH and ABTS methods in biscuits. The lipid sources tested had no effect on this variable. Storage time can reduce antiradical activity for DPPH.

	DPPH (%)			
	DAY 0	DAY 15	DAY 30	DAY 60
T1CA	10.46 ± 0.90^{BCa}	09.25 ± 2.71^{Ba}	09.63 ± 2.57^{Ba}	08.70 ± 1.22^{Ba}
T1LI	08.87 ± 2.35^{Ca}	08.84 ± 1.77^{Ba}	08.56 ± 1.37^{Ba}	08.80 ± 1.12^{Ba}
T1AZ	09.93 ± 1.00^{BCa}	08.87 ± 1.89^{Ba}	08.71 ± 1.72^{Ba}	09.17 ± 1.15^{Ba}
T2CA	18.18 ± 0.81^{Aa}	16.44 ± 1.49^{Aab}	15.01 ± 2.67^{Ab}	15.51 ± 1.43^{Aab}
T2LI	19.10 ± 1.56^{Aa}	16.57 ± 1.53^{Aab}	15.16 ± 1.62^{Ab}	14.52 ± 0.99^{Ab}
T2AZ	18.93 ± 0.82^{Aa}	15.08 ± 2.57^{Ab}	15.77 ± 1.85^{Aab}	16.36 ± 0.90^{Aab}
T3CA	09.86 ± 0.95^{BCa}	10.24 ± 1.73^{Ba}	09.89 ± 1.39^{Ba}	09.75 ± 1.06^{Ba}
T3LI	11.92 ± 1.77^{Ba}	10.57 ± 2.32^{Ba}	11.03 ± 1.30^{Ba}	08.02 ± 0.66^{Bb}
T3AZ	11.27 ± 2.09^{Ca}	10.68 ± 1.34^{Ba}	09.88 ± 1.75^{Ba}	08.81 ± 1.15^{Ba}
	ABTS (%)			
	DAY 0	DAY 15	DAY 30	DAY 60
T1CA	04.11 ± 0.90^{BCa}	03.82 ± 0.28^{Ba}	04.07 ± 0.84^{Ba}	04.21 ± 0.49^{Ba}
T1LI	04.04 ± 0.67^{BCa}	03.92 ± 0.32^{Ba}	04.87 ± 0.77^{Ba}	04.03 ± 0.51^{Ba}
T1AZ	03.40 ± 0.42^{Ca}	04.44 ± 2.60^{Ba}	04.16 ± 0.81^{Ba}	03.78 ± 0.77^{Ba}
T2CA	09.47 ± 1.79^{Aa}	09.66 ± 1.93^{Aa}	11.43 ± 0.48^{Aa}	09.71 ± 1.44^{Aa}
T2LI	10.15 ± 2.69^{Aa}	09.11 ± 1.03^{Aa}	10.93 ± 1.04^{Aa}	10.35 ± 2.01^{Aa}
T2AZ	10.80 ± 1.16^{Aa}	09.68 ± 0.94^{Aa}	10.13 ± 0.76^{Aa}	10.54 ± 1.63^{Aa}
T3CA	05.45 ± 1.51^{Ba}	04.17 ± 1.13^{Ba}	05.28 ± 0.46^{Ba}	04.65 ± 0.53^{Ba}
T3LI	05.21 ± 0.76^{BCa}	04.58 ± 0.75^{Ba}	05.52 ± 0.41^{Ba}	04.78 ± 0.37^{Ba}
T3AZ	04.98 ± 0.95^{BCa}	04.00 ± 1.02^{Ba}	04.93 ± 1.19^{Ba}	04.66 ± 0.42^{Ba}

* T1 (biscuits made without the addition of natural antioxidants. T2 (biscuits made with the addition of 0.5% turmeric) and T3 (biscuits made with the addition of 0.5% of marigold) CA: canola oil; LI: linseed oil; AZ: olive oil. **Means with different uppercase letters in the same column have a significant difference (P<0.05). Means with different lowercase letters in the same row have a significant difference (P<0.05). ***Results are expressed as mean ± standard deviation in three true samples per treatment.

Table 03: Antioxidant activity of canine biscuits by the DPPH and ABTS methods with up to 60 days of storage

Lipid oxidation is one of the most common processes that occurs in food and is influenced by many factors such as exposure to air, light and temperature, unsaturated fatty acid content and manufacturing processes (Kozłowska et al., 2014). The oxidation

process results in the modification of the food's taste and odor, at the same time as the nutritional value, shelf life and food safety decrease due to the formation of primary and secondary compounds (Bialek et al., 2016). To verify and monitor the occurrence of this process, the method adopted in this work considered the analysis of thiobarbituric acid reactive substances (TBARS) based on the concentration of malonaldehyde (MDA). Figure 01 shows the behavior of lipid oxidation of canine biscuits over 60 days of storage.

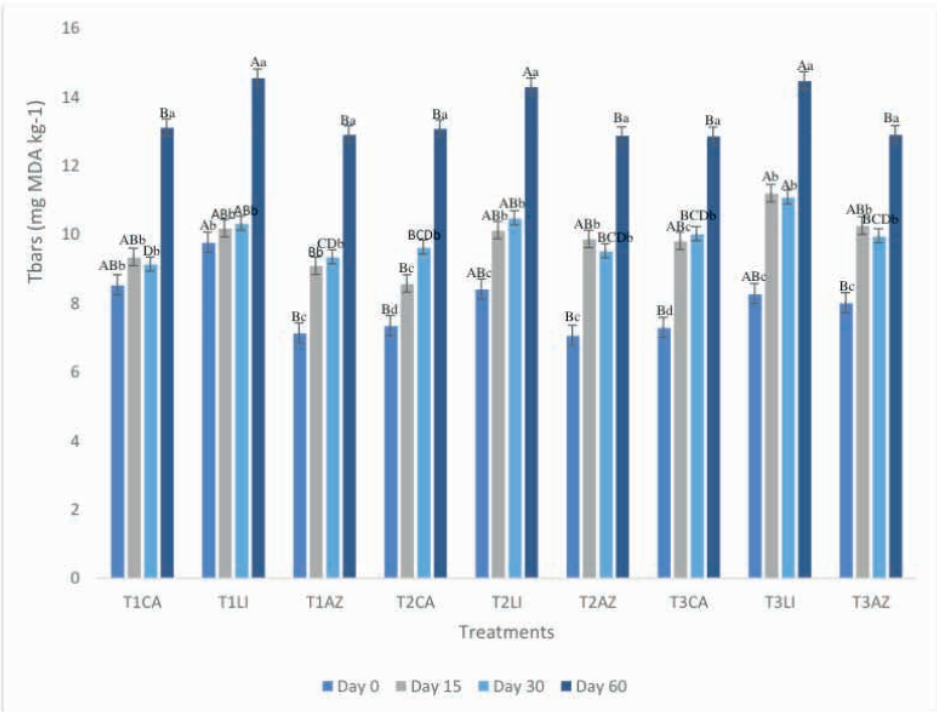

Figure 01: Comparative analysis of MDA levels (mg/kg) in canine biscuits samples prepared with up to 60 days of storage.

* T1 (biscuits made without the addition of natural antioxidants. T2 (biscuits made with the addition of 0.5% turmeric) and T3 (biscuits made with the addition of 0.5% of marigold) CA: canola oil; LI: linseed oil; AZ: olive oil. **Results are expressed as mean ± standard error in three true samples per treatment. ***Means with different uppercase letters have a significant difference (P<0.05) between treatments. Means with different lowercase letters have a significant difference (P<0.05) between days of storage.

Biscuits made with linseed oil (T1LI, T2LI and T3LI) showed higher production of secondary products of lipid oxidation (malonaldehyde) when compared with biscuits made with canola oil and olive oil. This is due to the chemical composition of linseed oil, which has more than 50% of omega 3 (alpha-linolenic acid) in its composition. Omega 3 oxidizes 20 to 40 times faster than oleic acid (found in olive oil) and 2 to 4 times faster than linoleic acid (Odeh et al., 2021). Despite the consumption of omega 3 brings benefits to human and animal health. Flaxseed oil has a limited shelf life and low oxidative stability. Storage time negatively affected the oxidative stability of the biscuits. This difference was even greater

between 30 and 60 days of storage where there was an increase of more than 35% in the formation of secondary oxidation products. Other authors also report an increase in lipid oxidation of biscuits over storage time (Bialek et al., 2016).

Biscuits and crackers have low humidity, high lipid content and a large surface area, which facilitates the action of pro-oxidizing agents such as light and oxygen, leading to the degradation of fatty acids contained in the food over the shelf life (Gebreselassie & Clifford, 2016). The addition of turmeric or marigold as sources of natural antioxidants was not effective in reducing the formation of lipid oxidation products in dog biscuits. During the cooking, several physical and biochemical reactions take place, like water evaporation, protein denaturation, partial gelatinization of starch, Maillard reactions, and dough deformation (Chevallier et al., 2000). The high temperature to which the biscuits were subjected during baking can reduce the antioxidant capacity of turmeric and marigold and increase the oxidation processes of vegetable oils added to the mass. This way, the oxidative stability of the biscuits was not influenced by the added antioxidant, only the fat source and storage period.

Dogs use their sense of smell and taste to select foods to eat. Although color is for dogs a factor of little significance in food preference, it is one of the most attractive sensory factors for tutors at the time of purchase (Gomez Baquero et al., 2018). The biscuits showed differences in color, as shown in table 04. The luminosity of the biscuits was higher for those prepared without the addition of antioxidants, followed by biscuits with the addition of marigold and turmeric. With storage time, there was a tendency to reduce luminosity. The a* scale indicates that the biscuits presented a green color while the b* scale indicates that all of them presented a yellow color. The presence of canola oils, linseed and olive oil did not influence the color of the biscuits, although the addition of the antioxidant compound provided a difference between the treatments. The addition of turmeric provided stronger coloration in the a* and b* scales, followed by the addition of marigold.

COLOR				
	L*			
	DAY 0	DAY 15	DAY 30	DAY 60
T1CA	84.07 ± 2.23^{Aa}	81.29 ± 0.95^{Aa}	81.75 ± 1.91^{ABa}	83.20 ± 3.06^{Aa}
T1LI	84.07 ± 2.20^{Aa}	79.67 ± 1.78^{ABCb}	82.68 ± 2.32^{Aab}	82.61 ± 3.03^{ABCab}
T1AZ	84.02 ± 1.57^{Aa}	80.46 ± 1.94^{ABa}	83.02 ± 2.25^{Aa}	82.73 ± 4.41^{ABa}
T2CA	81.24 ± 3.99^{Ca}	77.43 ± 2.09^{CDa}	79.81 ± 1.41^{Ca}	80.01 ± 3.19^{EFa}
T2LI	82.09 ± 2.09^{BCa}	75.97 ± 2.31^{Db}	79.87 ± 1.62^{Ca}	79.72 ± 3.61^{Fa}
T2AZ	81.28 ± 3.27^{Ca}	77.83 ± 2.39^{CDa}	80.77 ± 1.51^{BCa}	79.92 ± 3.01^{Fa}
T3CA	81.66 ± 2.83^{BCa}	79.30 ± 2.34^{ABCa}	81.72 ± 3.27^{ABa}	80.87 ± 1.86^{DEFa}
T3LI	82.71 ± 2.56^{ABCa}	77.42 ± 3.72^{CDb}	81.30 ±1.93^{ABCab}	81.29 ± 3.26 ^{CDEab}
T3AZ	83.23 ± 1.34^{ABa}	78.15 ± 0.60^{BCDb}	79.75 ± 0.85^{Cb}	81.82 ± 2.52^{BCDa}
	a*			
T1CA	-1.01 ± 0.26^{Bab}	-0.77 ± 0.30^{Cb}	-1.09 ± 0.17^{Dab}	-1.30 ± 0.44^{Ba}
T1LI	-1.40 ± 0.45^{Ba}	-0.18 ± 0.79^{Cb}	-1.27 ± 0.32^{CDa}	-1.39 ± 0.70^{Ba}
T1AZ	-1.08 ± 0.68^{Bbc}	-0.68 ± 0.26^{Cb}	-1.73 ± 0.16^{CDa}	-1.44 ± 0.41^{Bab}
T2CA	-6.74 ± 1.88^{Aa}	-4.58 ± 0.82^{BAb}	-6.07 ± 0.54^{ABab}	-6.12 ± 1.63^{Aab}
T2LI	-7.27 ± 0.91^{Aa}	-3.33 ± 1.34^{Bb}	-5.46 ± 0.57^{Ba}	-5.79 ± 2.66^{Aa}
T2AZ	-6.89 ± 1.55^{Aa}	-4.94 ± 1.49^{Ab}	-6.35 ± 0.16^{Aab}	-6.01 ± 1.79^{Ab}
T3CA	-1.21 ± 0.93^{Ba}	-1.28 ± 1.13^{Ca}	-1.89 ± 1.25^{Ca}	-1.56 ± 0.67^{Ba}
T3LI	-2.00 ± 1.11^{Ba}	-0.00 ± 1.69^{Cb}	-1.54 ± 0.32^{CDa}	-1.63 ± 0.72^{Ba}
T3AZ	-2.22 ± 0.23^{Ba}	-1.09 ± 0.40^{Cb}	-1.89 ± 0.41^{Ca}	-2.07 ± 0.55^{Ba}
	b*			
T1CA	24.80 ± 1.46^{Ca}	26.86 ± 0.92^{Da}	26.31 ± 1.13^{Ca}	24.48 ± 2.59^{CDa}
T1LI	26.53 ± 1.79^{Cab}	28.86 ± 0.71^{Ca}	26.11 ± 2.54^{CDb}	26.58 ± 2.02^{Cab}
T1AZ	24.86 ± 1.04^{Cab}	26.33 ± 0.89^{Da}	24.52 ± 1.67^{Dab}	23.21 ± 1.79^{Db}
T2CA	53.81 ± 2.39^{Aa}	52.73 ± 1.48^{Aa}	54.60 ± 0.60^{Aa}	53.68 ± 1.38^{Aa}
T2LI	55.15 ± 3.36^{Aa}	51.72 ± 2.03^{Ab}	54.59 ± 0.57^{Aa}	53.88 ± 1.11^{Aab}
T2AZ	54.14 ± 0.61^{Aa}	52.51 ± 1.96^{Aa}	53.63 ± 1.76^{Aa}	53.17 ± 1.15^{Aa}
T3CA	33.78 ± 2.14^{Ba}	36.34 ± 1.08^{Ba}	35.20 ± 1.51^{Ba}	35.01 ± 2.64^{Ba}
T3LI	34.88 ± 2.06^{Bb}	37.53 ± 1.10^{Ba}	36.16 ± 2.03^{Bab}	35.39 ± 1.82^{Bab}
T3AZ	34.27 ± 2.24^{Bb}	37.11 ± 0.63^{Ba}	35.51 ± 1.23^{Bab}	33.87 ± 2.43^{Bb}

* T1 (biscuits made without the addition of natural antioxidants. T2 (biscuits made with the addition of 0.5% turmeric) and T3 (biscuits made with the addition of 0.5% of marigold) CA: canola oil; LI: linseed oil; AZ: olive oil. **Means with different uppercase letters in the same column have a significant difference (P<0.05). Means with different lowercase letters in the same row have a significant difference (P<0.05). ***Results are expressed as mean ± standard deviation in three true samples per treatment.

Table 04: Color stability of canine biscuits during storage.

The texture of canine biscuits was evaluated by the hardness parameter (g) and is shown in figure 02.

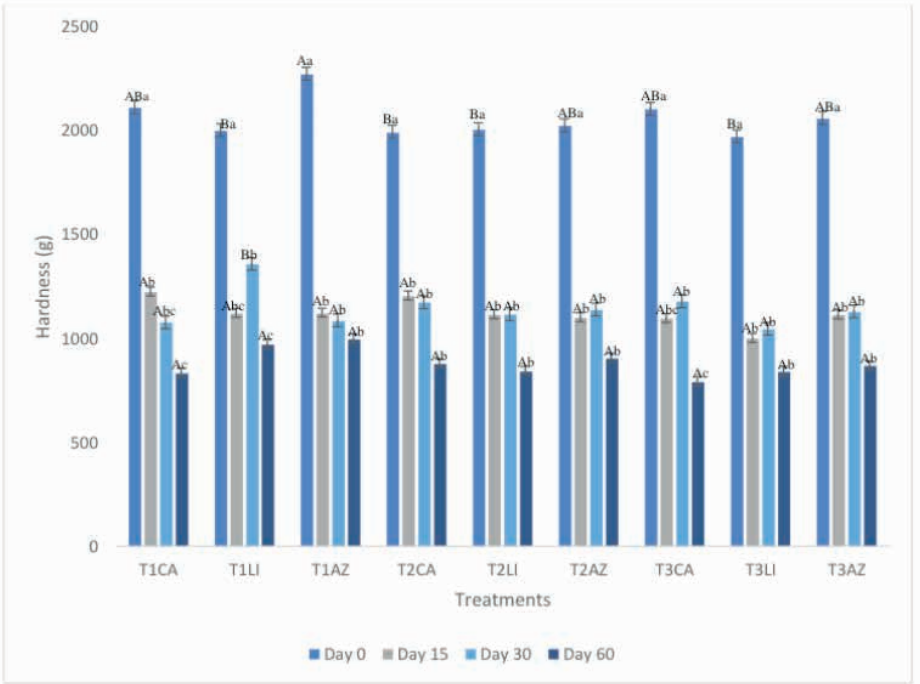

Figure 02: Comparative analysis of hardness levels (g) in canine biscuits samples made with 60 days of storage.

* T1 (biscuits made without the addition of natural antioxidants. T2 (biscuits made with the addition of 0.5% turmeric) and T3 (biscuits made with the addition of 0.5% of marigold) CA: canola oil; LI: linseed oil; AZ: olive oil. **Results are expressed as mean ± standard error in three true samples per treatment. ***Means with different uppercase letters have a significant difference (P<0.05) between treatments. Means with different lowercase letters have a significant difference (P<0.05) between days of storage.

Generally speaking the proportion of ingredients contained in the mass can determine the mechanical properties of the biscuits and thus affect the final texture of the product. In all treatments. the same proportion of vegetable oil was added to the dough, this way, there was no difference in the hardness of the biscuits when compared to the source of oil used (canola oil, linseed oil or olive oil). Regarding storage time, the biscuits showed a reduction in hardness after 15 days of storage, which remained at 30 days. Between 30 and 60 days of storage, there was a reduction of hardness above 20%. Other authors have also reported reduced hardness in biscuits with increasing shelf life (Shafi et al., 2022; Bhat et al., 2018). Exposure to oxygen and air humidity can adversely affect the texture of biscuits, decrease in hardness over time. This way, the packaging of this product must be carried out to reduce the exposure of the food to these conditions.

4 | CONCLUSIONS

The addition of natural antioxidants can increase the antioxidant activity of canine biscuits; however, it was not able to maintain oxidative stability. The addition of linseed oil to biscuits resulted in greater formation of secondary oxidation products. Storage time influenced the hardness and oxidative stability of canine biscuits for all treatments.

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