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FOOD TECHNOLOGY

Replacement of chicken skin with canola oil in chicken nuggets

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ABSTRACT: This study developed chicken nuggets with emulsions with different percentages of canola oil (CO) to replace the chicken skin and evaluating the quality of the final product and the effect of frying. The experiment was set up in a 5x5 factorial scheme with the replacement of skin with different percentages of CO (0, 25, 50, 75, and 100%) and storage periods (0, 45, 90, 135,and 180 days), and parameters such as pH and oxidation were evaluated. It was considered a 5x2 factorial scheme (replacement of skin with CO and different sample conditions (raw and fried in CO)) for the analyses of the centesimal composition, fatty acid profile, atherogenic (AI), and thrombogenic (IT) indices. The sensory analysis evaluated the chicken nuggets at time zero. The pH values ranged from 6.62 to 6.86, as an effect of frying, there was an increase in lipid content with increasing levels of substitution (T0-raw: 5.36% and fried: 12.11%; T100-raw: 7.93% and fried: 15.43%) and a decrease in moisture content in relation to the raw sample. There was a decrease in AI (T0%-raw vs. T100%-fried, 0.39 vs. 0.10) and IT (T0%-raw vs. T100%-fried, 0.61 vs. 0.18) with CO addition and frying, and lipid oxidation increased with storage. The ω6/ω3 ratio decreased and the PUFA/SFA increased with the replacement of chicken skin with CO, but there was no difference in the sensory analysis. Even though chicken nuggets are prone to lipid oxidation, it is possible to improve their nutritional value either by adding CO to the meat mixture or by frying them.
Key words: chicken nuggets, canola oil, lipid oxidation, lipid profile.

Substituição de pele de frango por óleo de canola em nuggets de frango

RESUMO: Objetivou-se neste estudo desenvolver nuggets de frango com emulsões contendo diferentes percentagens de óleo de canola em substituição a pele de frango e avaliar a qualidade do produto final bem como os efeitos da fritura. O experimento foi desenvolvido em esquema fatorial 5x5 (substituição da pele por óleo de canola (0, 25, 50, 75, 100%) e tempo de armazenamento (0, 45, 90, 135, 180 dias)) avaliando parâmetros como pH e oxidação. Considerou-se fatorial 5x2 (substituição da pele por óleo de canola e condições da amostra (cru e frito em óleo de canola)) para as análises da composição centesimal, perfil de ácidos graxos e índices aterogênico (IA) e trombogênico (IT). Para a análise sensorial avaliou-se os nuggets de frango produzidos apenas no tempo zero. Houve efeito da fritura com aumento no teor de lipídios (T0%-cru:5,36% e frito:12,11%; T100%-cru:7,93% e frito: 15,43%), redução no teor de umidade em relação a amostra crua e aumento no teor de lipídios com aumento dos níveis de substituição. Com a adição de óleo de canola e fritura houve redução de IA (T0%, cru, IA:0,39; T100%, frito, IA:0,10) e IT (T0%, cru, IT:0,61; T100%, frito, IT:0,18) e com o tempo de armazenamento aumentou-se a oxidação lipídica. A proporção ω6/ ω3 diminuiu e a AGP/AGS aumentou com a substituição da pele de frango por óleo de canola e não houve diferença na análise sensorial. Apesar da propensão à oxidação lipídica, é possível melhorar nutricionalmente os nuggets de frango, tanto pela adição de óleo de canola à massa quanto pelo processo de fritura.
Palavras-chave: nuggets de frango, óleo de canola, oxidação lipídica, perfil lipídico.

INTRODUCTION

Chicken meat is well accepted by consumers as it is lean, rich in unsaturated fatty acids, has good digestibility, has a low economic value, and is widely accepted among several age groups. Therefore, the fatty acids present are prone to oxidation, which requires the food industry to use antioxidants in the preparation of processed products based on chicken meat, hindering the consumption of these products by people who have some kind of allergy to these preservatives (HENCHION et al., 2014; NADEEM et al., 2022; NOLLET et al., 2012).

According to MARTÍNEZ et al. (2020), low cost, long shelf life, and convenience in a ready-to-eat format make nuggets and other processed meat products well-accepted by consumers. Every day, consumers are following healthier food habits, and this has awakened industries to search for and produce foods containing reduced amounts of animal fat. Vegetable oils with lipids considered more favorable for cardiovascular health are promising alternatives (MONTEIRO et al., 2017; RITZOULIS et al., 2010).

Among vegetable oils, canola oil stands out because of its moderate level of linoleic acid

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(21%), a significant amount of α-linolenic acid (10%), and a high oleic acid content (18:1n-9; 60%), which contributes to the reduction of the risk of cardiovascular diseases (VISENTAINER et al., 2015).

Linoleic acids (C18:2, ω6) and α-linolenic acids (C18:3, ω3) are essential as, like all mammals, they cannot be produced by humans and must be acquired from the diet. The balance of these fatty acids in the human body is important in the prevention and treatment of coronary diseases, hypertension, diabetes, arthritis, osteoporosis, autoimmune disorders, cancer, and mental health (SIMOPOULOS et al., 2008). A ratio of linoleic to α-linolenic acids of 4:1 or less was previously shown to be favorable for good health (NOVELLO et al., 2008).

Various studies on meat products have been conducted to evaluate the increase in the nutritional value of the product by changing its fatty acid lipid profile with the replacement of animal fat with canola oil. MONTEIRO et al. (2017) and LACERDA et al. (2022) partially replaced animal fat with canola oil in Tuscany sausage and fresh chicken sausage, respectively. YUNES et al. (2013) evaluated the effects of the addition of vegetable oils, including canola, as substitutes for animal fat in *mortadella*. All of these studies concluded that the nutritional value of products changed with the canola oil addition instead of animal fat and that the results were better. But only a few studies have reported the replacement of animal fat with canola oil and monitored the quality of the meat product after it has been fried in canola oil.

The major technological challenges in the replacement of animal fat with canola oil are the destabilization of the emulsion due to the difference in melting points, the change in texture and succulence of the product, and the increase in lipid oxidation caused by the presence of unsaturated fatty acids. Replacing animal fat with canola oil in chicken nuggets and deep-frying in this same type of vegetable matrix is likely to preserve the quality of the fatty acids present. This study developed chicken nuggets with emulsions with different percentages of canola oil (CO) to replace the chicken skin and evaluating the quality of the final product and the effect of frying.

MATERIALS AND METHODS

Design and statistical analysis

To study the effect of frying, we chose chicken nuggets, which are meat products without wrapping, which can facilitate exchanges between the lipids of the frying oil and the product. The experiment was organized in a completely randomized design with a 5x5 factorial scheme for the physical-chemical and lipid oxidation analyses, with the same five replacements of chicken skin by canola oil (0, 25, 50, 75, and 100%) and five storage periods (0, 45, 90, 135, and 180 days). It was considered a 5x2 factorial scheme to analyze the centesimal and fatty acid lipid profiles. The five conditions were the percentages of replacement of chicken skin by canola oil (0, 25, 50, 75, and 100%), and the two conditions were raw and fried chicken nuggets. Samples were collected for analysis only at time zero (t0). Sensory analysis was performed considering the percentage of chicken skin replaced by canola oil at t0. All laboratory analyses were made in triplicate, except fatty acid profiles, which were made in duplicate.

The data were statistically evaluated using the MINITAB Program, version 17. The Kolmogorov–Smirnov normality test was used, and the data presented normality and were submitted to variance analysis (ANOVA) with one factor for centesimal composition and sensory characteristics and a factorial ANOVA for the other research results. In all cases, the Tukey test was used at the level of 5% significance ($P < 0.05$).

For lipid oxidation, the data did not present normality and were transformed into a logarithmic scale ($y = \log(x)$). After transformation, the Kolmogorov–Smirnov test was applied, and the values presented normality and were submitted to a factorial ANOVA and Tukey's test at the level of 5% significance ($P < 0.05$).

Chicken nuggets preparation

The raw materials and ingredients were purchased from commercial establishments in the city of Cuiabá-MT, Brazil. At first, the emulsion was made with the following fixed ingredients: chicken breast (15.6%), swine skin collagen (22.0%), cold water (30.0%), soy protein (6.0%), salt (1.0%), antioxidants: sodium erythorbate (0.1%), sodium tripolyphosphate (0.3%), and nonfixed ingredients: chicken skin and canola oil, whose percentages changed (0, 25, 50, 75, and 100%) as chicken skin was replaced with canola oil (Table 1).

For the emulsion preparation, the fat-free swine skin was cooked to obtain collagen. After cooking, it was transferred to a bench cutter (Model C5 L, G. Paniz) together with the other ingredients and beaten until a homogeneous dough was obtained, which was then frozen in a freezer and ground in a grinder (Model Picador 22, Beccaro) with an 8 mm disc.

Table 1 - Components of emulsion formulations with 0, 25, 50, 75 and 100% replacement of chicken skin with different percentages of canola oil.

Components of chicken nuggets (%)	-----------------------Levels of replacement of chicken skin with Canola oil----------------------				
	0%	25%	50%	75%	100%
Chicken breast	15.60	15.60	15.60	15.60	15.60
Swine skin collagen	22.00	22.00	22.00	22.00	22.00
Cold water	30.00	30.00	30.00	30.00	30.00
Soy protein isolate	6.00	6.00	6.00	6.00	6.00
Salt	1.00	1.00	1.00	1.00	1.00
Ssodium erythrobate	0.10	0.10	0.10	0.10	0.10
Sodium tripolyphosphate	0.30	0.30	0.30	0.30	0.30
Components of emulsion (%)					
Chicken skin	25.00	18.75	12.50	6.25	0.00
Canola oil	0.00	6.25	12.50	18.75	25.00
Total	100.00	100.00	100.00	100.00	100.00

To replace the chicken skin from 0 to 100% (mass/mass), which is equivalent to 8.5% in the product, 34% of canola oil of each replacement ratio of this emulsion was added to a mixer (Model MMS50I, Skymsen) along with the following other ingredients: chicken breast (46.5%), textured soy protein (1%), soy protein (1.5%), ice water (14.25%), chicken condiment (1.5%), salt (1%), white pepper (0.05%), sodium erythorbate (0.05%), and sodium tripolyphosphate (0.15%).

The dough was formatted, frozen, and manually breaded with wheat flour, eggs, and corn flour. After this process, the chicken nuggets were frozen in a freezer at -27 °C and then fried in canola oil (Vitaliv©) at 165 ± 5 °C for two minutes and 30 seconds.

According to the nutritional information contained in the canola oil package, it contains 9.2% saturated fats, 3.4% *trans*-fat, 61.3% monounsaturated fats, mainly oleic acid, and 26.0% polyunsaturated fat, with 18.3% ω6 and 7.5% ω3 resulting in an ω6/ω3 ratio of 2.4:1.

The samples were coded and stored in a freezer at a temperature of -12 °C for up to 180 days.

Centesimal composition analysis

The centesimal composition analysis was performed based on the analytical standards of the Association of Official Analytical Chemists (AOAC, 2012). The moisture content was determined by measuring the water loss by drying to a constant weight, the ash content was determined by incineration of organic compounds in a muffle furnace at 550 °C, and the protein content was determined by measuring total nitrogen using the Kjeldahl digestion process using a factor of 6.25 for the transformation of nitrogen to protein. The lipid content was obtained using a Soxhlet apparatus with a solvent extractor, and the percentage of total carbohydrates was estimated by a difference of 100% concerning the percentage amounts of moisture, protein, fat, and ash. The analyses were performed in triplicate on both raw and fried nuggets.

Physical and chemical analysis

For pH analysis, the samples were homogenized in a food processor. For each homogenized sample, 5 g of sample was weighed and added to 50 ml of distilled water, and then a pHmeter (model MB-10, Mars) was used to measure the pH of the diluted sample according to the analytical standards of the AOAC (2012).

The Minolta CM-700D colorimeter, calibrated to a white standard and programmed with the CIE System $L^*a^*b^*$ (luminosity (L^*), a^* (red–green), and b^* (yellow-blue) according to CIELAB (French: Commission International de L eclairage: International Commission of Lighting/Colour), was used for the objective determination of color. The illuminant used was D65; 10° was used for the standard observer angle and the excluded specular component, according to the methodology of RAMOS & GOMIDE (2017). Water activity (Aw) evaluation was performed using an AQUALAB 4TE Water Activity Metre, and the method used was that of AOAC (2012).

The chicken nugget samples that underwent the cooking process were cooled and cut into 4 cm samples with a 1 cm^2 cross-section, and their instrumental texture (shear force) was measured using the TA.XTPlus Texture Analyzer, aided by the XTRAD software, coupled to a Warner-Bratzler type probe, with the sample being sheared transversely.

The cooking loss (CL) of the nuggets was determined as described by the American Meat Science Association (AMSA, 1978). The samples were packed in aluminum foil and baked on a heating sheet at 150 °C until reaching an internal temperature of 72 ± 2 °C. The weight loss from cooking is equal to the difference between the initial and final weights of the samples.

Lipid oxidation was evaluated using the thiobarbituric acid reactive substances (TBARS) method. TBARS was tested according to JORGE et al. (2015). The TBARS values were expressed in milligrams of malonaldehyde (MDA) per kilogram of the sample (mg MDA/kg sample). Lipid extraction was performed according to the method of FOLCH et al. (1957), and esterification was performed according to HARTMAN & LAGO (1973).

The fatty acid profile analyses were performed in a gas chromatograph (SHIMADZU brand, CG Solution) equipped with a flame ionization detector coupled to a microcomputer. Nitrogen gas was used as a carrier with a linear velocity programmed to 43.2 cm/s, and hydrogen gases and synthetic air formed the flame in the detector. The injector and detector temperatures were controlled isothermally at 220 °C and 240 °C, respectively. The initial column temperature was 150 °C (maintained for five minutes), increasing by 4 °C per minute until reaching 240 °C (maintained for 30 minutes). The drag gas flow in the column was 0.8 mL/minute. To produce and analyze the chromatograms, 1 µl of the sample was injected with the aid of a 10 µl syringe (Hamilton®) in a Split = 10 system. The compounds were separated into a capillary column (Carbowax; 30 m x 0.25 mm, internal diameter; 1.0 µm). The CG Solution software was used to integrate the peak areas and transform them into the amounts of each fatty acid derivative.

Atherogenic (AI), and thrombogenic (IT) indices were calculated according to the following formulas by ULBRICHT & SOUTHGATE (1991):

$AI = [(C12:0) + 4\,(C14:0) + (C16:0)]/[(\omega6) + (\omega3) + (\Sigma MUFA)]$

$TI = [(C14:0) + (C16:0) + (C18:0)]/[0.5(\Sigma MUFA) + 0.5(\omega6) + 3(\omega3) + (\omega3/\omega6)]$.

where MUFA denotes monounsaturated fatty acids.

Sensory analysis

This study was approved by the Research Ethics Committee of the Federal Institute of Mato Grosso (CAAE: 69103517.8.0000.8055). After this stage, sensory analysis of the chicken nuggets was performed in the laboratory. The analysis included 60 untrained judges between 18 and 44 years old, including students, teachers, technical consultants, and employees of the institution.

After preparing the chicken nuggets according to each treatment, they were fried by immersion in canola oil and given to the tasters according to codification. The judges were given a form for the evaluation of the attributes of color, flavor, texture, odor, and overall appearance using the 9-point hedonic scale test (1 = very much disliked up to 9 = very much liked). Additionally, each one received a dish with five samples (0, 25, 50, 75, and 100% of canola oil substitution) coded with three numbers and a glass of water to cleanse the palate between the tasting of each sample.

The acceptability index was reported by dividing the actual note of the tasters by the highest grade that could be given and then multiplying that number by 100 (DUTCOSKY, 2007).

RESULTS AND DISCUSSION

The mean values of the centesimal composition of raw and fried chicken nuggets are shown in table 2. There were no significant differences ($P > 0.05$) in protein percentages in either raw or fried chicken nuggets between treatments. There were also no differences in carbohydrate values for fried samples.

The percentages of proteins and carbohydrates in raw and fried nuggets are in accordance with the legislation for the current physicochemical standards, Normative Instruction No. 6 February 2001 (BRAZIL, 2001a).

The moisture, lipid, and ash levels were significantly different ($P > 0.05$), where it was observed that in the raw chicken nugget, the treatment containing 75% canola oil presented the lowest percentages of moisture and ash, and that with the canola oil addition in the product, both in its raw and fried forms, the lipid contents increased, as expected, being significantly different from the treatment with 50% substitution. In terms of protein levels, the nuggets became more concentrated after being fried, which indicates that protein loss by solubilization during frying is less than the loss of mass of the product. This beneficial effect of replacing animal fat with canola oil was more evident in the treatments with a 50% and 75% substitution. Similar results were reported by LEE et al. (2015) when replacing animal fat with a mixture of vegetable

Table 2 - Mean values of centesimal composition of raw and fried chicken nuggets with the replacement of chicken skin with different percentages of canola oil.

Parameters		Substitution levels (%)					P value*
		0	25	50	75	100	
Moisture (%)	Raw	61.17^{abA}	62.66^{aA}	59.37^{bcA}	59.00^{cA}	60.09^{bA}	0.0124
	Fried	52.78^{aB}	50.68^{bB}	49.34^{cB}	50.68^{bB}	50.45^{bB}	0.0143
	P value*	0.0013	0.0012	0.0021	0.0008	0.0016	
Ethereal Extract (%)	Raw	5.36^{cB}	5.40^{cB}	6.29^{bB}	6.37^{bB}	7.93^{aB}	0.0013
	Fried	12.11^{bA}	13.03^{bA}	14.33^{aA}	14.94^{aA}	15.43^{aA}	0.0007
	P value*	0.0006	0.0008	0.0005	0.0004	0.0010	
Crude Protein (%)	Raw	15.42^{aA}	14.95^{aB}	14.54^{aB}	14.52^{aB}	14.26^{aB}	0.1387
	Fried	15.91^{aA}	15.87^{aA}	16.07^{aA}	16.05^{aA}	15.47^{aB}	0.9335
	P value*	0.0897	0.0432	0.0342	0.032	0.0247	
Carbohydrates (%)	Raw	15.68^{bcB}	14.73^{cB}	17.44^{aA}	17.89^{aB}	15.49^{cB}	0.0064
	Fried	16.83^{aA}	18.15^{aA}	17.8^{aA}	15.93^{aA}	16.58^{aA}	0.5032
	P value*	0.0218	0.0024	0.2326	0.0043	0.0425	
Ash (%)	Raw	2.37^{aA}	2.26^{abA}	2.36^{abB}	2.22^{bB}	2.23^{abA}	0.0238
	Fried	2.37^{aA}	2.27^{abA}	2.46^{aA}	2.40^{aA}	2.07^{bB}	0.0193
	P value*	0.0734	0.0738	0.0064	0.0073	0.0087	

Averages that do not share the same lowercase letter in the rows and uppercase in the columns are significantly differents. *Tukey's test was applied. Significant at the level of 5% probability (P > 0.05).

oils in sausage. ALINA et al. (2009) also observed an increase in lipid content by replacing animal fat with palm oil in chicken nuggets. NGADI et al. (2007) reported that the increase in the percentage of lipids in fried chicken was caused by the unstable chemical structure of the nonhydrogenated oil used in the frying process, which degrades more easily and causes excessive absorption of the oil in the product.

The mean moisture and lipid contents of raw chicken nuggets were 60.46% and 6.19%, respectively, and 50.86% and 13.97% for fried nuggets. This change can be explained by the replacement of the product's water with lipids during the frying process. According to NGADI et al. (2007), during frying, the oil penetrates the food through pores formed by the moisture coming from it, and there is an exchange between water and oil.

Table 3 and figure 1 show the average pH values of fried chicken nuggets prepared with different percentages of canola oil and their behavior over the storage period. It was observed that the average pH values ranged from 6.62 to 6.86 and that there was an increase in pH followed by a reduction and maintenance over the storage period. In relation to reaching the maximum pH, figure 1 shows that for treatments with a lower percentage of canola oil addition (0 and 25%), the maximum occurred at 90 days, and for the other samples, this peak was not observed until the end of shelf life. For microbiological reasons, the closer to pH 7.00, the greater the possibility of microbial growth. It is suggested that treatments with 50, 75, and 100% canola oil presented more stable results when compared to other treatments. In their studies with chicken breading that had clove powder added, KUMAR & TANWAR (2011) reported that the meat protein broke down significantly with the increase in pH value during product storage.

There was no significant difference (P > 0.05) in the water activity values between treatments; however, regarding the time of storage of chicken nuggets, a significant difference (P < 0.05) was observed at 90 and 180 days compared to t0 (Table 3). The mean water activity (Aw) of the chicken nuggets treatments during storage ranged from 0.954 to 0.978. Figure 1 shows that the lowest Aw values were found in treatments 0 and 25% at 90 days. The same behavior occurred in the pH values, where maximum values were obtained. It is possible that the higher water activity contributed to higher enzymatic proteolysis in this storage period (WONG & KITTS, 2002).

In the texture analysis, increased shear strength was observed for all treatments, with significant differences between treatments at 135 and 180 days for samples with 25% and 100% canola oil, which had the highest shear force values, while samples

Table 3 - Average pH, water activity (Aw), shear force (SF) and cooking loss (CL) values of fried chicken nuggets with the replacement of chicken skin with different percentages of canola oil and storage periods.

Parameters	Treatments (T)	---Storage (S) (days)---					---P value*---		
		0	45	90	135	180	T	S	TxS
pH	0%	6.65^{cA}	6.70^{bcA}	6.86^{aA}	6.76^{bA}	6.74^{bA}	0.0042	0.0063	0.0256
	25%	6.63^{cAB}	6.70^{bA}	6.83^{aA}	6.72^{bB}	6.73^{bAB}			
	50%	6.62^{cB}	6.70^{bA}	6.77^{aAB}	6.72^{abB}	6.77^{aA}			
	75%	6.63^{cAB}	6.67^{bcAB}	6.71^{bB}	6.77^{aA}	6.75^{aA}			
	100%	6.65^{bA}	6.64^{bB}	6.71^{abB}	6.76^{aA}	6.69^{abB}			
Aw	0%	0.969^{bA}	0.968^{bA}	0.954^{cB}	0.965^{b}	0.979^{aA}	0.3485	0.0063	0.0332
	25%	0.970^{bcA}	0.965^{cA}	0.956^{dB}	0.971^{abA}	0.975^{aA}			
	50%	0.968^{bA}	0.965^{bcA}	0.963^{cA}	0.968^{bA}	0.979^{aA}			
	75%	0.968^{bA}	0.965^{bA}	0.964^{bA}	0.970^{abA}	0.976^{aA}			
	100%	0.968^{bA}	0.964^{bcA}	0.961^{cAB}	0.973^{aA}	0.964^{bcB}			
SF (Kgf)	0%	0.53^{cB}	0.73^{bA}	0.76^{bA}	0.86^{aAB}	0.90^{aB}	0.0015	0.0036	0.0243
	25%	0.59^{cAB}	0.73^{bA}	0.72^{bB}	1.01^{aA}	1.01^{aA}			
	50%	0.45^{cC}	0.68^{bAB}	0.71^{bB}	0.67^{bC}	0.87^{aBC}			
	75%	0.46^{dC}	0.60^{cB}	0.72^{bB}	0.81^{aB}	0.84^{aC}			
	100%	0.61^{dA}	0.62^{dB}	0.75^{cA}	1.11^{aA}	0.91^{bB}			
CL (%)	0%	4.24^{dA}	5.44^{cA}	6.03^{bA}	6.07^{bA}	6.77^{aA}	0.0605	0.0041	0.9354
	25%	4.37^{dA}	5.45^{cA}	6.12^{bA}	6.15^{bA}	6.73^{aA}			
	50%	4.90^{dA}	5.41^{cA}	6.09^{bA}	6.29^{abA}	6.63^{aA}			
	75%	5.05^{bA}	5.39^{bA}	6.18^{aA}	6.40^{aA}	6.39^{aA}			
	100%	5.27^{eA}	5.84^{dA}	6.43^{cA}	6.64^{bA}	6.83^{aA}			

Averages that do not share the same lowercase letter in the rows and uppercase in the columns are significantly different. The *Tukey test was applied. Significant at the level of 5% probability (P < 0.05).

with 50% presented the lowest values when compared to the standard 0% treatment (Table 3 and Figure 1). YOUSSEF & BARBUT (2009), when replacing 25% of animal fat with canola oil in ground beef, observed an increase in the product's hardness, showing that the fat or oil ratio influences the size of blood cells and has a significant effect on texture parameters. JIMÉNEZ-COLMENERO et al. (2010) reported that when the product formulations are similar, the difference in texture is mostly caused by the characteristics of the emulsion and the type of oil used.

We observed an increase in CL values over the storage period for all treatments. The increase in CL over the storage period can indicate destabilization of the emulsion during the storage period.

Even though there was a significant difference in the CL of the treatments of chicken nuggets (Table 3), this does not cause harm to the product. Similar results were found by ÁLVAREZ et al. (2011) reported that the weight loss of the sausage was insignificant when comparing the control sample to those with the replacement of animal fat by canola and canola-olive oil (20%) and that the oils studied have a high capacity to hold the water and fat of the sausage, forming an emulsion more stable than commercial sausage. JIMÉNEZ-COLMENERO et al. (2010) replaced animal fat with an oil-water emulsion in sausage and observed no significant differences in CL, attributing this result to emulsion stability.

When comparing treatments and during the storage period, there was a significant difference ($P < 0.05$) for the parameters L^*, a^*, and b^* (Table 4). With canola oil added to the emulsion, there was a significant difference between the 25% (75.24) and 100% (72.07) treatments for the L^* parameter, and there was a significant increase in the values of a^* and b^* for the 100% treatment compared to the 0% standard. DOMÍNGUEZ et al. (2016) also observed an increase in the red (a^*) and yellow (b^*) color parameters in liver pates that had animal fat replaced by fish oil and attributed this to the color of the oil itself. YOUSSEF & BARBUT (2009) reported in their study that a higher L^* value may occur because canola oil globules are smaller and therefore reflect more light than animal fat globules. However, YUNES et al. (2013) reported that decreasing the fat content can lead to darker products with a lower L^* content.

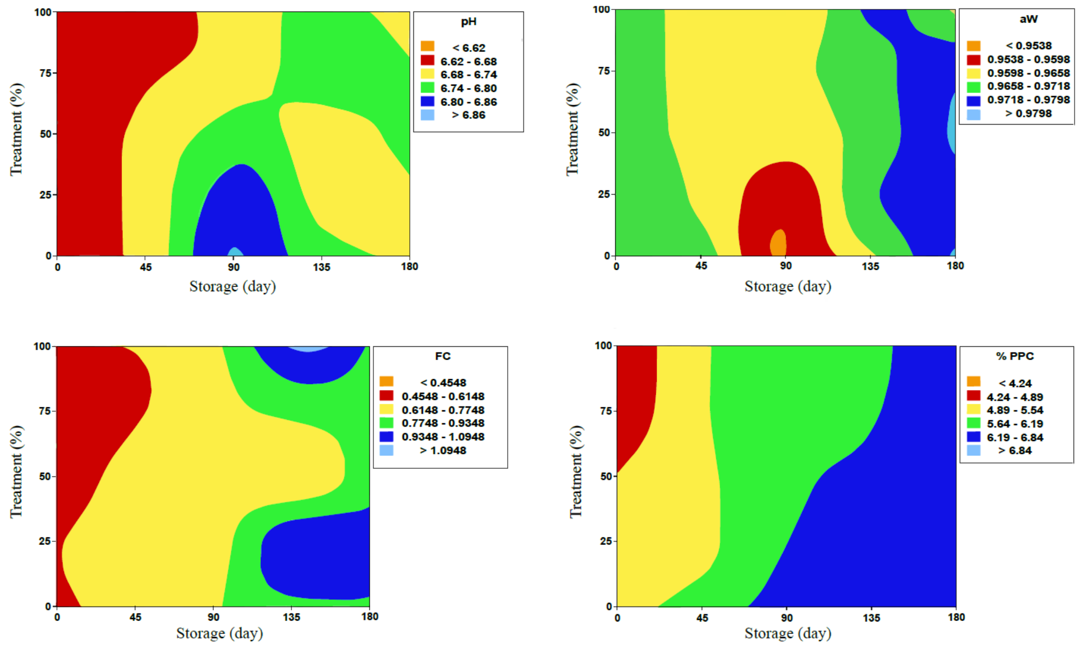

Figure 1 - Contour graph of the interaction of the parameters pH, Aw, FC and CL of fried chicken nuggets with the replacement of chicken skin with different percentages of canola oil and storage periods.

During the storage period, it was observed that for the L*, the treatments with 0, 25, 50, and 100% canola oil replacement showed significant differences, with lower values at 180 days and 135 days for the 75% sample compared to the t0. In the present study, the decrease in L* can be related to lipid oxidation, as an increase in TBARS values was observed with the increase in storage period and with the increase in the

Table 4 - Mean values of objective colour parameters of chicken nuggets with the replacement of chicken skin with different percentages of canola oil and storage periods.

Parameters	Treatments (T)	------------------------------Storage (S) (days)------------------------------					-----------P value*----------		
		0	45	90	135	180	T	S	T x S
L*	0%	73.60aB	71.55abB	73.86aAB	72.14abAB	70.64bB	0.0035	0.0282	0.0347
	25%	75.24aA	73.43abB	73.32abAB	73.38abAB	70.40bB			
	50%	74.86aA	75.28aA	74.20aA	74.75aA	71.15bAB			
	75%	74.12aAB	74.12aAB	71.61bB	71.21bB	72.09bA			
	100%	72.07bC	69.63bcC	73.81aAB	71.27bB	68.74cC			
a*	0%	4.22bAB	4.32bAB	3.03cB	3.83abA	4.49aA	0.0138	0.0224	0.0452
	25%	3.89bB	4.04abB	3.68bAB	3.78bA	4.61aA			
	50%	5.22aA	4.56bAB	4.01bcA	3.68cA	4.37bA			
	75%	4.35aAB	4.09abB	3.28bAB	3.50abA	4.33aA			
	100%	5.67aA	5.38aA	3.98bcA	3.45cA	4.29bA			
b*	0%	19.38abC	21.29aB	17.54cB	18.7bB	17.73cAB	0.0136	0.0025	0.0476
	25%	21.40aB	20.92bcB	18.62cAB	20.25bA	18.63cA			
	50%	22.92aAB	20.85bB	18.74cAB	18.09cC	16.34dC			
	75%	21.20aB	20.80aB	17.95cB	19.67bAB	17.25cB			
	100%	23.89aA	23.47aA	19.46bA	18.66bB	16.87cC			

Averages that do not share the same lowercase letter in the rows and uppercase in the columns are significantly different. *Tukey's test was applied. L*: luminosity; a*: color parameter green-red; b*: color parameter blue-yellow. Significant at the level of 5% probability (P < 0.05).

replacement of animal fat with vegetable oil. BACKES et al. (2013) also observed a decline in L* during the storage period.

At 90 days, both a* and b* showed T100% higher than T0% (P < 0.05). It was observed to have an a* value of 3.98 at T100% and 3.03 at T0%, and a b* value of 19.46 at T100% and 17.5 at T0%. GANHÃO et al. (2010) observed in their study a negative correlation between a* values and protein oxidation.

The TBARS values for the treatments increased as the chicken skin was replaced by canola oil (Table 5). The control sample (0%) presented a significant increase (P < 0.05) from the 50% treatment. During storage, there was a significant increase in lipid oxidation up to 90 days, with no significant differences until 180 days in relation to t0. According to ALMEIDA-COUTO & CESTARI. (2017), the reduction or stabilization of TBARS values is indicative of the complexation of MDA with other compounds originating from lipid oxidation, and once oxidized, the product cannot reverse the oxidation.

The mean TBARS values found in the treatments ranged from 2.69 to 5.59 mg MDA/kg during the 180-day storage period at -12 °C (Table 5), a result very similar to what KOMIYAMA et al. (2009) found in their study with chicken nugget fillets stored for 180 days at -18 °C, with an average of 3.21 to 4.92 mg MDA/kg.

WANG et al. (1976) conducted a study with breaded, fried, and frozen chicken with TBARS values ranging from 2.1 to 9.2 mg MDA/kg, with an average of 4.7 mg MDA/kg, and although these values were considered high, in this research, no rancidity odor was detected in any of the samples. RAHIM et al. (2009), when studying breaded chicken stored at -18 °C for three months, did not observe a rancidity odor in the product.

Even though YILDIZ-TURP & SERDAROGLU (2008) reported that it is possible to detect rancidity odors with TBARS values in the range of 0.6 to 2.0 mg MDA/kg of the sample, in our research, the TBARS values did not interfere with the sensory analysis of chicken nuggets.

Twelve fatty acids were identified in the raw chicken nuggets and 13 in the fried ones (Tables 6 and 7), with oleic (C18:1, ω9), linoleic (C18:2, ω6), and palmitic (C16:0) fatty acids being the most representative among the fatty acids in both the raw and fried nuggets. MONTEIRO et al. (2017) also observed these three fatty acids at higher concentrations when evaluating the lipid profile of raw and cooked Tuscan sausage with partial canola oil replacement of animal fat. According to VISENTAINER et al. (2015), oleic acid (C18:1 ω9) reduces cholesterol and low-density lipoprotein levels in the blood, and the latter is directly related to increased risks of cardiovascular disease.

Except for the results of tridecanoic fatty acid (C13:0) in fried nuggets, all other raw and fried products showed significant differences (P < 0.05), suggesting that the canola oil addition as a substitute for animal fat directly alters their fatty acid profile. There was a significant increase in the percentages of oleic (C18:1, ω9), linolenic (C18:3, ω3), and γ-linolenic (C18:3, ω6) fatty acids in relation to the standard sample (0% canola oil replacement). For fried nuggets, there was a significant reduction in the percentages of palmitic (C16:0), stearic (C18:0), and palmitoleic (C16:1) fatty acids and a significant increase in the percentages of linolenic acid (C18:3, ω3) in relation to the standard (0% canola oil replacement).

The differences observed in the fatty acid profiles in this study are due to the differences between the lipid profiles of the raw material of the product and the lipid profiles of canola oil. MONTEIRO et al. (2017) also found similar changes with the partial replacement of animal fat with canola oil in Tuscan fresh sausage.

Table 5 - Mean thiobarbituric acid reactive substances (TBARS) method values of chicken nuggets with the replacement of chicken skin with different percentages of canola oil and storage periods.

Parameter	---Treatments (T)---					--P value*--
	0%	25%	50%	75%	100%	
TBARS (mgMDA/kg)	2.74[c]	2.69[c]	3.74[b]	5.11[a]	5.59[a]	0.0053
	---Storage (S) (days)---					
	0	45	90	135	180	
	2.14[c]	3.64[b]	4.86[a]	4.53[a]	4.80[a]	0.0028

The results are not in the logarithmic scale. Averages that do not share the same letter in the lines are significantly different. The *Tukey test was applied. * Significant at the level of 5% probability (P < 0.05).

Table 6 - Mean values in percentage of fatty acids and sum of saturates (SFA) of raw and fried chicken nuggets with the replacement of chicken skin with different percentages of canola oil.

Fatty acids	Type of nuggets	Treatments (T) (%)					P value*
		0%	25%	50%	75%	100%	
C 13:0	Raw	0.00^{b}	0.00^{b}	0.00^{b}	0.26^{aA}	0.23^{aA}	0.005
	Fried	0.13^{a}	0.16^{a}	0.18^{a}	0.18^{aB}	0.16^{aB}	0.331
P value*		-	-	-	<0.001	<0.001	
C 14:0	Raw	0.65^{aA}	0.52^{bA}	0.41^{cA}	0.35^{dA}	0.24^{eA}	0.006
	Fried	0.36^{aB}	0.28^{bB}	0.27^{bcB}	0.24^{cB}	0.19^{dB}	0.004
P value*		<0.001	<0.001	<0.001	<0.001	0.004	
C 16:0	Raw	25.60^{aA}	20.83^{bA}	17.32^{cA}	14.08^{dA}	11.16^{eA}	<0.001
	Fried	15.06^{aB}	12.38^{bB}	11.45^{cB}	10.43^{dB}	8.78^{eB}	0.002
P value*		<0.001	<0.001	<0.001	0.002	<0.001	
C 18:0	Raw	6.88^{aA}	6.07^{bA}	5.44^{cA}	4.89^{dA}	4.23^{eA}	0.001
	Fried	4.71^{aB}	4.22^{bB}	4.17^{cB}	3.81^{dB}	3.40^{eB}	0.002
P value*		<0.001	<0.001	<0.001	<0.001	0.001	
C 20:0	Raw	0.26^{cB}	0.44^{bcB}	0.47^{bB}	0.69^{bB}	0.79^{aB}	0.010
	Fried	0.67^{cA}	0.72^{bcA}	0.75^{bA}	0.82^{aA}	0.84^{aA}	0.023
P value*		<0.001	0.006	0.008	0.005	0.024	
Σ SFA	Raw	33.43^{aA}	27.88^{bA}	23.80^{cA}	20.28^{dA}	16.66^{eA}	<0.001
	Fried	20.86^{aB}	17.80^{bB}	16.72^{cB}	15.41^{dB}	13.37^{eB}	<0.001
P value*		<0.001	<0.001	<0.001	<0.001	<0.001	

Σ SFA = sum of C13:0, C14:0, C16:0, C18:0 and C20:0. Averages that do not share the same lowercase letter in the rows and uppercase in the columns are significantly different. The *Tukey test was applied. Significant at the level of 5% probability (P < 0.05).

Among these changes, we make particular note of the reduction of palmitic acid (C16:0), which went from 25.60% in the control treatment (0% replacement of animal fat) to 11.60% in the treatment with 100% replacement of animal fat by canola oil and 15.06% when fried in canola oil. Linolenic acid (C18:3, ω3) increased from 1.30% in the control treatment to 7.14% in the treatment with 100% replacement of animal fat by canola oil and to 5.47% when fried in canola oil. These changes nutritionally favor the quality of the product because the saturated fatty acids (SFA) that are reduced (myristic and palmitic) are associated with hypercholesterolemia (ULBRICHT & SOUTHGATE, 1991), while oleic and linolenic fatty acids contribute to the improvement of the ω6/ω3 ratio, which has importance in the prevention and treatment of coronary diseases, hypertension, diabetes, arthritis, osteoporosis, autoimmune disorders, cancer, and mental health (SIMOPOULOS et al., 2008).

Significant differences (P < 0.05) were observed in the sum of SFA, MUFA and polyunsaturated fatty acids (PUFA) of both raw and fried nuggets. The sums of SFA in raw and fried products decreased significantly with the canola oil addition, from 33.43 to 20.86% in the 0% treatment to 16.66 to 13.37% in the 100% treatment. In relation to MUFA and PUFA, in fried nuggets, there was a significant increase from the treatment with 25% and 50% canola oil addition, respectively. LEE et al. (2015) observed a reduction in SFA and an increase in MUFA and PUFA in all treatments after adding a mix of vegetable oils to sausage and concluded that the replacement resulted in a healthier product and was desired by consumers.

Table 8 shows the amounts of ω6 and ω3 fatty acids, the relationship between them, the ratio between PUFA and SFA, and the AI and TI. The analysis of these items shows significant differences (P < 0.05), both for the issue of canola oil substitution and for frying, except for the variable ω6.

For the sum of ω6, a significant difference was observed between both the raw and fried treatments, but because the Σω6 in the product is high, the differences between treatments are of little nutritional importance. Contrastingly, in Σω3, it was observed that both canola oil addition and frying showed significant effects of nutritional importance. Frying promoted significant increases in Σω3 fatty acid levels in all treatments. The observed effect of frying and canola oil addition on the ω3 fatty acid

Table 7 - Mean values in percentage of unsaturated fatty acids and sum of monounsaturated (MUFA) and polyunsaturated (PUFA) of raw and fried chicken nuggets with the replacement of chicken skin with different percentages of canola oil.

Fatty acids	Type of nuggets	-------------------------------------Treatments (T) (%)-------------------------------------					---P value*---
		0%	25%	50%	75%	100%	
C 16:1	Raw	5.41^{aA}	4.01^{bA}	2.98^{cA}	2.11^{dA}	1.31^{eA}	<0.001
	Fried	2.72^{aB}	1.98^{bB}	1.66^{cB}	1.42^{dB}	0.89^{eB}	0.002
P value*		<0.001	<0.001	0.003	0.0002	0.004	
C 17:1	Raw	0.00	0.00	0.00	0.00	0.00	----
	Fried	0.22^{a}	0.00^{d}	0.00^{d}	0.09^{c}	0.19^{b}	0.004
P value*		-	-	-	-	-	
C 18:1ω9	Raw	36.97^{aB}	42.05^{bB}	45.44^{cB}	49.20^{dB}	51.91^{eA}	<0.001
	Fried	46.90^{cA}	50.45^{bA}	51.12^{bA}	51.51^{bA}	52.96^{aA}	<0.001
P value*		<0.001	<0.001	<0.001	0.021	0.0351	
Σ MUFA	Raw	42.64^{aB}	46.20^{bB}	48.65^{cB}	51.31^{dB}	53.22^{eB}	<0.001
	Fried	49.79^{cA}	52.43^{bA}	52.78^{abA}	52.99^{abA}	53.95^{aA}	<0.001
P value*		<0.001	<0.001	<0.001	<0.001	0.035	
C 18:2ω6	Raw	21.82^{abB}	22.05^{aA}	22.06^{aA}	21.68^{bB}	22.11^{aB}	<0.001
	Fried	22.65^{abA}	22.32^{bA}	22.51^{abA}	22.94^{abA}	23.03^{aA}	<0.001
P value*		0.016	0.064	0.073	0.042	0.031	
C 18:2ω6 *trans*	Raw	0.11^{aB}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.003
	Fried	0.50^{aA}	0.00^{c}	0.12^{b}	0.00^{c}	0.14^{b}	0.001
P-value*		<0.001	-	-	-	-	
C 18:3ω3	Raw	1.30^{aB}	2.99^{bB}	4.61^{cB}	5.87^{dB}	7.14^{eB}	<0.001
	Fried	5.47^{eA}	6.76^{dA}	7.29^{cA}	7.89^{bA}	8.78^{aA}	<0.001
P value*		<0.001	<0.001	<0.001	<0.001	0.004	
C 18:3ω6	Raw	0.00^{a}	$0.22B^{b}$	0.33^{cB}	0.42^{dB}	0.51^{eA}	0.031
	Fried	0.42^{c}	0.45^{bcA}	0.48^{bA}	0.53^{aA}	0.56^{aA}	0.004
P value*		-	<0.001	0.002	0.023	0.074	
C 20:4ω6	Raw	0.69^{aA}	0.63^{bA}	0.55^{cA}	0.43^{dA}	0.35^{eA}	0.009
	Fried	0.32^{aB}	0.29^{aB}	0.29^{aB}	0.24^{bB}	0.22^{bB}	0.031
P-value*		<0.001	<0.001	<0.001	<0.001	0.006	
Σ PUFA	Raw	23.93^{aB}	25.92^{bB}	27.54^{cB}	28.41^{dB}	30.12^{eB}	<0.001
	Fried	29.36^{dA}	29.77^{cdA}	30.50^{bcA}	31.60^{abA}	32.68^{aA}	<0.001
P value*		<0.001	<0.001	<0.001	0.004	0.014	

Averages that do not share the same lowercase letter in the rows and uppercase in the columns are significantly different. The *Tukey test was applied. Significant at the level of 5% probability (P < 0.05).

content can be explained by the difference between the content of this component in the raw material and its content in canola oil. CARVALHO et al. (2020) also reported a significant increase in ω3 in relation to the standard sample when replacing porcine skin with chia and flax oils in cooked lamb sausages.

The ω6/ω3 ratio showed a significant change between all treatments, both in raw and canola oil-fried chicken nuggets. Frying with canola oil also showed a significant effect on all treatments. Raw chicken nuggets had a reduced ω6/ω3 ratio of 17.38 in the standard sample (with no canola oil addition), 3.22 in the sample with 100% replacement of animal fat by canola oil, and 4.37 when fried in canola oil. The balance between ω6 and ω3 fatty acids is important for normal development and lifelong mental health. Recommendations vary by country, with Japan being the most stringent, setting a ratio of 2:1, and the Food and Agricultural Organization being the least demanding, recommending an intake of ω6/ω3 at a ratio of 5–10:1. However, several studies state that a ω6/ω3 ratio of 1:1 to 4:1 is adequate for good health (DUBOIS et al., 2007, VISENTAINER et al., 2015).

It was observed in this research that all fried chicken nuggets presented a ω6/ω3 ratio of up to 4:1 and that the process of frying with canola oil provided the treatments with a great improvement in the nutritional quality regarding the lipid fraction. Our findings are in contrast to the widespread concept that frying foods reduces their nutritional value. This

Table 8 - Percentage values of the ω6 and ω3 series, ω6/ω3 ratio, PUFA/SFA ratio, atherogenic and thrombogenic indices of raw and fried chicken nuggets with the replacement of chicken skin with different percentages of canola oil.

Fatty acids	Type of nuggets	----------Treatments(T) (%)----------					--P value*--
		0%	25%	50%	75%	100%	
ω6 series	Raw	22.62^{bcB}	22.90^{abA}	22.94^{abA}	22.53^{cB}	22.98^{aB}	0.001
	Fried	23.88^{aA}	23.01^{bA}	23.21^{abA}	23.71^{abA}	23.91^{aA}	0.008
P value*		<0.001	0.432	0.335	0.001	<0.001	
ω3 series	Raw	1.30^{aB}	2.99^{bB}	4.61^{cB}	5.87^{dB}	7.14^{eB}	<0.001
	Fried	5.47^{eA}	6.76^{dA}	7.29^{cA}	7.86^{bA}	8.78^{aA}	<0.001
P value*		<0.001	<0.001	<0.001	<0.001	<0.001	
ω6/ω3 ratio	Raw	17.38^{aA}	7.66^{bA}	4.98^{cA}	3.84^{dA}	3.22^{eA}	<0.001
	Fried	4.37^{aB}	3.41^{bB}	3.19^{cB}	3.01^{dB}	2.73^{eB}	<0.001
		<0.001	<0.001	<0.001	<0.001	<0.001	
PUFA/SFA ratio	Raw	0.72^{aB}	0.93^{bB}	1.16^{cA}	1.40^{dB}	1.81^{eB}	<0.001
	Fried	1.41^{eA}	1.67^{dA}	1.83^{cA}	2.05^{bA}	2.45^{aA}	<0.001
P value*		<0.001	<0.001	<0.001	<0.001	<0.001	
Atherogenic index	Raw	0.39^{aA}	0.30^{bA}	0.24^{cA}	0.18^{dA}	0.14^{eA}	<0.001
	Fried	0.19^{aB}	0.15^{bB}	0.14^{cB}	0.13^{dB}	0.10^{eB}	<0.001
P value*		<0.001	<0.001	<0.001	<0.001	<0.001	
Thrombogenic index	Raw	0.61^{aA}	0.54^{bA}	0.42^{cA}	0.33^{dA}	0.25^{eA}	<0.001
	Fried	0.35^{aB}	0.27^{bB}	0.25^{cB}	0.22^{dB}	0.18^{eB}	<0.001
P value*		<0.001	<0.001	<0.001	<0.001	<0.001	

Series ω6 = sum of 18:2 ω6 18:2 ω6 trans, 18:3 ω6 and 20:4 ω6; Series ω3 = 18:3 ω3; ω6/ ω3 = Series ω6/Series ω3; PUFA/SFA = Σ PUFA/SFA. Averages that do not share the same lowercase letter in the rows and uppercase in the columns are significantly. The *Tukey test was applied. Significant at the level of 5% probability ($P < 0.05$).

study has shown that frying chicken nuggets with canola oil increases the essential fatty acid (ω9, ω6, and ω3) contents and reduces the SFA content. This is very important because the lipid sources that are part of our diet are poor in these fatty acids, making our so-called cholesterolemic, atherogenic, and thrombogenic diets. However, a small increase in calories due to frying can be balanced with reduced carbohydrate intake.

The PUFA/SFA ratio increased significantly from 0.72 in the standard sample (with no canola oil addition) to 1.81 in the sample with 100% replacement of animal fat by canola oil and to 1.41 when fried in canola oil. Treatment with 100% canola oil provided the greatest improvement. ALFAIA et al. (2009) reported that the PUFA/SFA ratio in human diets must be higher than 0.45. All raw and fried chicken nuggets in our study had PUFA/SFA ratios higher than 0.45.

Regarding nutritional indices (TI and AI), in both raw and fried chicken nuggets, there was a significant reduction between treatments with the canola oil addition. Raw products presented AI and TI with a reduction of 0.39–0.61 to 0.14–0.25, respectively, and in fried nuggets, they presented a reduction of 0.19–0.35 to 0.10–0.18, respectively. DOMINGUÉZ et al. (2017) observed a reduction in the AI (0.43 to 0.38) and TI (0.95 to 0.66) by adding fish and olive oils as a partial replacement for the animal fat of a sausage. MONTEIRO et al. (2017) reported in their study that canola oil addition lowers AI and TI. This is because canola oil has much higher percentages of unsaturated fatty acids, especially oleic and linolenic acids, than other oils.

According to ULBRICHT & SOUTHGATE (1991), only three fatty acids are hypercholesterolemic: lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0). So, the fewer of these SFAs there are, the lower the AI and TI are. As a result, the food will be nutritionally superior.

In the color, odor, and overall appearance attributes, there was no significant difference between treatments ($P > 0.05$) (Table 9). The notes of the analyzed parameters ranged from 6.77 to 7.98, which represent "liked regularly" to "liked much," demonstrating the acceptance of the product by the tasters. POLIZER et al. (2015) performed a sensory analysis of chicken nuggets with partial meat and fat substitution by pea fiber and obtained grades of 7.20 to 7.48.

Table 9 - Mean results of sensory analysis of fried chicken nuggets with the replacement of chicken skin with different percentages of canola oil.

Parameters	-----------------------------------Treatments-----------------------------------					P value*
	0%	25%	50%	75%	100%	
Overall appearance (%)	7.42[a]	6.95[a]	7.05[a]	7.22[a]	7.43[a]	0.0804
Smell (%)	7.23[a]	6.77[a]	6.80[a]	7.00[a]	7.17[a]	0.2416
Colour (%)	7.08[a]	6.97[a]	6.98[a]	6.92[a]	7.20[a]	0.6206
Flavour (%)	7.98[a]	7.08[b]	7.32[ab]	7.40[ab]	7.75[a]	0.0327
Texture (%)	7.43[ab]	6.92[b]	7.17[ab]	7.28[ab]	7.57[a]	0.0306
Acceptability index (%)	82.56[a]	77.07[b]	78.48[ab]	79.59[ab]	82.48[a]	0.0418

Means that do not share the same letter in the lines are significantly different. *Tukey's test was applied. Significant at the 5% probability level ($P < 0.05$).

All treatments had an acceptance rate above 70%, which, according to DUTCOSKY (2007), means good acceptance of the product in the market. The control treatment did not present a significant difference in relation to treatments with 50, 75, and 100% canola oil (Table 9). Chicken nuggets from all treatments met the microbiological requirements of Resolution n. 12 of 2001 (BRAZIL, 2001b) during the storage period.

CONCLUSION

In the chicken nugget, the process of frying with canola oil promoted an exchange of its fatty acids with the fatty acids of the frying oil, promoting a nutritional improvement in the chicken nuggets with regard to their fatty acid profile and AI and TI. Even though fats tend to go rancid, the nutritional value of chicken nuggets can be improved by adding canola oil to the meat mixture or by frying them.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

All authors contributed equally to the design and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

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