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Pineapple by-product and canola oil as partial fat replacers in low-fat beef burger: Effects on oxidative stability, cholesterol content and fatty acid profile

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ABSTRACT

The effect of freeze-dried pineapple by-product and canola oil as fat replacers on the oxidative stability, cholesterol content and fatty acid profile of low-fat beef burgers was evaluated. Five treatments were performed: conventional (CN, 20% fat) and four low-fat formulations (10% fat): control (CT), pineapple by-product (PA), canola oil (CO), and pineapple by-product and canola oil (PC). Low-fat cooked burgers showed a mean cholesterol content reduction of 9.15% compared to the CN. Canola oil addition improved the fatty acid profile of the burgers, with increase in the polyunsaturated/saturated fatty acids ratio and decrease in the n-6/n-3 ratio, in the atherogenic and thrombogenic indexes. The oxidative stability of the burgers was affected by the vegetable oil addition. However, at the end of the storage time (120 days), malonaldehyde values of CO and PC were lower than the threshold for the consumer's acceptance. Canola oil, in combination with pineapple by-product, can be considered promising fat replacers in the development of healthier burgers.

1. Introduction

Burger is one of the most popular processed meat products in the world. It is highly accepted and consumed, mainly due to the current increase in the number of fast foods worldwide and its convenience and low price (Hoogenkamp, 1997). However, burgers are also known by some negative aspects, such as the quantity (20–30%) (Jiménez-Colmenero, 2000) and quality of its fat (mostly saturated fatty acids), as well as the cholesterol content, which are associated with the occurrence of some chronic and cardiovascular diseases (Fernández-Ginés, Fernández-López, Sayas-Barberá, & Pérez-Alvarez, 2005).

With the increased concerns about the relationship between fat intake and health, consumers have become more conscious regarding a healthy diet, demanding products with reduced fat, cholesterol content, and altered fatty acid profile (Ospina, Sierra, Ochoa, Pérez-Álvarez, & Fernández-López, 2012). Thus, due to the high fat content and popularity of the burgers, they are considered an attractive choice for fat reduction and fatty acid profile improvement.

However, the fat reduction of meat products, with its direct substitution by water, can bring a series of deleterious effects in both sensory quality (reducing flavor and juiciness, and modifying texture) (Jiménez-Colmenero, 2000) and technological characteristics (increasing cooking loss, reducing yield, affecting emulsion stability) (Hughes, Cofrades, & Troy, 1997). In order to minimize these issues and to improve the quality of reduced-fat products, some ingredients have been studied to act as animal fat replacers, such as dietary fibers (DF) and vegetable oils.

The use of dietary fiber as a functional ingredient is related to their interesting properties that can positively affect some technological characteristics of the meat products. Fibers have been successfully applied to improve water holding capacity (WHC), oil holding capacity (OHC) and swelling capacity, which are useful in products that require hydration, to improve yield, stabilize emulsions, and modify texture and viscosity (Elleuch et al., 2011). Furthermore, it is well-known that DF plays an important role in human health, acting as a bulking agent, normalizing intestinal motility and then preventing constipation (insoluble fibers) and decreasing the intestinal absorption of cholesterol and glucose (soluble fibers) (Silveira Rodríguez, Monereo Megías, & Molina Baena, 2003).

Dietary fibers are obtained mainly from cereals. However, fruits and vegetable by-products still have high DF content, with the advantage of presenting considerable amounts of antioxidants (Deng, Penner, & Zhao, 2011; Martínez et al., 2012). Pineapple is a widely consumed tropical fruit and part of its production is intended to the manufacture of juices, fruit salads, canned fruits and jams. The residues generated by this industrial activity are composed mainly by peel and core and

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represent about 25–35% of the fruit (Larrauri, Rupérez, & Calixto, 1997). According to a previous study, pineapple by-product (peel and heart) presents DF as its major component (75.8%), in addition to have high water holding capacity, swelling capacity and an interesting antioxidant activity (Martínez et al., 2012).

Besides the dietary fiber, the replacement of animal fat with vegetable oil has been used as an alternative to improve technological and sensory aspects of low-fat meat products, in addition to enhance its nutritional value, by reducing saturated fatty acids (SFAs), cholesterol content and increasing monounsaturated (MUFAs), and polyunsaturated fatty acids (PUFAs). Canola oil has an interesting fatty acid profile, showing the lowest level of SFAs (7.36%) among the most common vegetable oils, such as sunflower (10.30%), corn (12.94%), olive (13.80%), soybean (15.65%), and cottonseed (25.9%) oils, high levels of monounsaturated fatty acids (MUFAs) (63.27%), and intermediate levels of polyunsaturated fatty acids (PUFAs) (28.14%) (USDA, 2015). Its lipid composition has motivated some studies based on canola oil application in meat products, resulting in positive effects regarding technological (Youssef & Barbut, 2011) and nutritional characteristics (Pelser, Linssen, Legger, & Houben, 2007) of the products.

There are few studies evaluating the association of fiber and vegetable oils as fat replacers. Choi et al. (2010) studied the replacement of pork back fat by different vegetable oils (olive, grape seed, corn, canola and soybean oil) and rice bran fiber in frankfurters and reported that the products had a decrease in cholesterol and trans-fat levels, an increase in cooking yield and TBARS values, in addition to have showed sensory properties similar to control frankfurters containing pork fat. Another study found that the incorporation of sunflower seed oil and dietary fiber from *makgeolli* lees in reduced-fat frankfurters minimized the texture alterations associated with fat reduction, reduced cooking loss and improved emulsion stability of the product (Choi et al., 2013). The replacement of pork back fat by olive oil emulsion and wakame (brown seaweed high in fiber) fortified patties with dietary fiber and minerals, improved the texture and the fatty acid profile and resulted in a healthier meat product (López-López, Cofrades, Yakan, Solas, & Jiménez-Colmenero, 2010).

In this context, the present work aimed to study the impact of animal fat reduction and its partial substitution by pineapple by-product (peel and pomace) and canola oil on the oxidative stability (during 120 days of frozen storage), cholesterol content and fatty acid profile of beef burgers.

2. Material and methods

2.1. By-product preparation

Pineapple by-product (peel and pomace) was obtained from a fruit and vegetable processing industry (Jundiaí, SP, Brazil). At the industry, the fruits were sanitized with 200 ppm of sodium hypochlorite, rinsed with water and then passed through the pulp extractor, where the by-product was collected. The material was kept frozen until its transportation to the Laboratory of Food and Nutrition of the Universidade de São Paulo (ESALQ/USP, Piracicaba, SP, Brazil). Samples were freeze dried (EC Modulyo, EC Apparatus Inc., New York, USA), ground using a knife mill (Marconi, Piracicaba, SP, Brazil), passed through a 40-mesh sieve (420 μm) and stored at −18 °C. Before the burger processing, pineapple by-products underwent a thermal treatment (100 °C, 2 h) in order to inactive the bromelain.

2.2. Burger manufacture

Fresh beef (moisture 77.24%, fat 1.29%) and pork back fat (moisture 12.06%, fat 84.09%) were purchased from a local slaughterhouse (Piracicaba, SP, Brazil). Beef and fat were separately ground (Hobart 4B22-2, Troy, OH, USA) using a 0.8 cm plate and then beef was divided into 5 treatments. The first treatment was used as a conventional formulation (CN) and the fat content was adjusted to 20% by the addition of back fat. The second treatment was used as a low-fat control (CT) and the fat content was adjusted to 10%. For the other treatments, pineapple by-product (1.5%) and/or canola oil emulsion (5%) were used and the fat content was also adjusted to 10% (Table 1). The concentration of pineapple by-product was selected based on a previous experiment that evaluated different concentrations of pineapple by-product (1.0, 1.5, 2.0, 2.5%) and canola oil (5%) as fat substitutes in low-fat beef burger (Selani, Margiotta, Piedade, Contreras-Castillo, & Canniatti-Brazaca, 2015). Canola oil emulsion was prepared by mixing eight parts of mineral hot water (50–55 °C) with one part of soy protein isolate by using a high speed mixer (Ultra Turrax Ika T18 basic, Wilmington, NC, USA) at 10,000 rpm for 2 min, and then 10 parts of canola oil was gradually added to this mix and homogenized for 3 min at 10,000 rpm (Muguerza, Gimeno, Ansorena, Bloukas, & Astiasarán, 2001).

After the addition of the respective amount of beef, fat, pineapple by-product and canola oil emulsion, the treatments were mixed with salt (1.5%), a commercial mix for burger (salt, maltodextrin, sodium polyphosphate, sodium erythorbate, natural spices, monosodium glutamate) (IBRAC, Rio Claro, SP, Brazil), and cold water. The formulations were kneaded by hand for 5 min and from the homogenized meat mixture, 100 g portions were manually shaped using a burger-maker, to give the dimensions of 10 cm diameter and 1 cm thickness. The beef burgers were then placed in polyethylene packages. The processing occurred in triplicate (all the formulations were applied to three independent batches of meat and fat).

2.3. Storage of the samples

After packaging, raw burgers were stored under −18 °C, for further analyses. The cholesterol content and the fatty acid profile of the burgers were determined during the first 15 days of storage. For the oxidative stability, beef burgers were stored up to 120 days and the samples were analyzed at 30 days intervals (1, 30, 60, 90, and 120 days).

2.4. Cooking procedure

The burgers were cooked before the analyses (without previous defrosting), in an electrical grill (Edanca, São Bernardo do Campo, SP, Brazil) pre-heated at 150 °C. The core temperature of the beef burgers was measured using a digital thermometer (Incoterm, Porto Alegre, RS, Brazil) to ensure an internal temperature of 75 °C was reached. Right after the samples have reached 75 °C, they were placed on trays and cooled at room temperature for about 30 min before the analysis.

Table 1
Formulation of beef burgers.

Ingredients	Treatments (%)				
	CN	CT	PA	CO	PC
Beef meat	70	70	70	70	70
Back fat	20	10	10	10	10
Cold water	7.5	17.5	16	12.5	11
Canola oil emulsion	0	0	0	5	5
Pineapple by-product	0	0	1.5	0	1.5
Salt	1.5	1.5	1.5	1.5	1.5
Mix for burger*	1	1	1	1	1

CN: conventional, with 20% fat; CT: control, with 10% fat; PA: with 10% fat and 1.5% of pineapple by-product; CO: with 10% fat and 5% of canola oil; PC: with 10% fat, 1.5% of pineapple by-product and 5% of canola oil.

* Commercial mix for burger: salt, maltodextrin, sodium polyphosphate, sodium erythorbate, natural spices and monosodium glutamate.

2.5. Cholesterol content

Cholesterol was extracted in triplicate, by direct saponification, according to the method described by Almeida, Perassolo, Camargo, Bragagnolo, and Gross (2006). Two grams of the sample was saponified with 4 mL of a 50% (*w*/*v*) aqueous solution of potassium hydroxide and 6 mL of solvent (ethanol:water – 95:5, *v*/*v*). The samples were incubated in a shaking water bath (Solab model SL157, Piracicaba, SP, Brazil) at 40 °C until complete solubilization and then they were heated for 10 min at 60 °C. After this, 5 mL of distilled water was added and the samples were cooled in cold water. The non-saponifiable matter was extracted three times with 10 mL of hexane. The hexane extract was dried using a vacuum rotary evaporator (Fisatom model 801, São Paulo, SP, Brazil) and nitrogen gas. After this, the sample was diluted with 1 mL of acetonitrile:isopropanol (85:15, *v*/*v*), filtered through a 0.45 μm PTFE membrane into autosampler vials and then injected (20 μL) in a high performance liquid chromatography (HPLC).

The HPLC system (Shimadzu LC-20AT, Kyoto, Japan) was equipped with a UV–visible detector (Shimadzu SPD-20A, Kyoto, Japan), an ODS column and its corresponding guard column (Zorbax Eclipse plus C18, 4.6 mm × 250 mm, 5 μm). The column was eluted isocratically at 40 °C with acetonitrile and isopropanol (85:15, *v*/*v*) at a flow rate of 2 mL/min and an analysis time of 15 min. The detector was set at 210 nm. Cholesterol quantification was done by external standardization, using a cholesterol authentic standard (Sigma Aldrich, St. Louis, MO, USA).

2.6. Fatty acid profile

Fat was extracted in triplicate according to the method of Bligh and Dyer (1959) and the fatty acid methyl esters (FAMEs) were obtained according to the methodology described by Hartman and Lago (1973), with modifications. FAMEs were analyzed using gas chromatography (GC) (Shimadzu, Model GC 2010, Kyoto, Japan) equipped with a fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (Restek Stabilwax, PA, USA) and a flame ionization detector. Gas chromatograph oven program temperature was as follows: initial temperature of 180 °C for 3 min, then it was raised to 210 °C at a rate of 5 °C/min and kept at this temperature for 13 min, and finally the temperature was raised to 220 °C at a rate of 10 °C/min and kept at 220 °C for 7 min. The injector and detector temperatures were 180 °C and 250 °C, respectively. Carrier gas was nitrogen at a flow rate of 4 mL/min. The split ratio was 1:10. Samples (1 μL) were injected using an automatic injector (Shimadzu AOC-20i). Individual FAME peaks were identified by comparison of their retention times with those of the standards (FAME Mix C8-C24, Supelco, USA). Quantification was performed using tridecanoic acid as an internal standard. The results were expressed in grams per 100 g of detected FAMEs and the analysis was carried out in triplicate.

Based on the FAME results, the atherogenic (AI) (Eq. (1)) and thrombogenic indexes (TI) (Eq. (2)) were calculated according to Ulbricht and Southgate (1991):

$$AI = \frac{C12:0 + 4 * C14:0 + C16:0}{MUFA + n{-}3\ \text{PUFA} + n{-}6\ \text{PUFA}} \quad (1)$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{0.5 * MUFA + 0.5 * n{-}3\ \text{PUFA} + 3 * n{-}6\ \text{PUFA} + \frac{n{-}3\ \text{PUFA}}{n{-}6\ \text{PUFA}}} \quad (2)$$

where C12:0, C14:0, C16:0, C18:0, n - 6 PUFA, and n - 3 PUFA are lauric, miristic, palmitic, stearic, linoleic and linolenic acids, respectively.

2.7. Oxidative stability

Malonaldehyde (MA) was extracted in triplicate, through the method described by Vyncke (1970, 1975) and Sørensen and Jørgensen (1996) with modifications, and quantified using an HPLC, according to Saldaña et al. (2015). For extraction, 5 g of meat was homogenized in Ultra Turrax (Ika T18 basic, NC, USA) at 10,000 rpm for 30 s with 15 mL of a 7.5% trichloroacetic acid, 0.1% propyl gallate, and 0.1% ethylene diamine tetraacetic acid aqueous solution. After filtration, 5 mL of the extract was mixed with 5 mL of a 0.02 M thiobarbituric acid aqueous solution in capped test tubes. The samples were incubated in a water bath (Solab SL157, Piracicaba, SP, Brazil) at 100 °C for 40 min and then cooled in cold water. After that, the samples were filtered through a 0.45 μm PTFE membrane into autosampler vials and injected (20 μL) into a HPLC.

The HPLC system (Shimadzu LC-20AT, Kyoto, Japan) was equipped with a fluorescence detector (Shimadzu RF-20A, Kyoto, Japan), an ODS column and its corresponding guard column (Zorbax Eclipse plus C18, 4.6 mm × 250 mm, 5 μm). The column was eluted isocratically at 40 °C, with sodium phosphate buffer (pH 7.0, 5 mM):isopropanol (85:15, *v*/*v*) at a flow rate of 1 mL/min and an analysis time of 10 min. The fluorescence detector was set at 515 nm (excitation) and 543 (emission). A standard curve was performed with 1,1,3,3-tetraethoxypropane and the results were expressed as mg of MA per kg of sample. The oxidative stability of the samples was evaluated after 1, 30, 60, 90, and 120 days of frozen storage (−18 °C).

2.8. Experimental design and statistical analysis

The study was a randomized block design, with three blocks (each block corresponding to an independent burger processing).

For malonaldehyde, a randomized block design with two blocks was considered, and for this analysis a 5 × 5 full factorial experiment was conducted, considering as factors the treatments (CN, CT, PA, CO, PC) and the storage times (1, 30, 60, 90, and 120 days).

Analysis of variance (ANOVA) was carried out to analyze the results and the comparisons of treatments and storage times were performed by the Tukey's test ($P < 0.05$), using the software SAS.

3. Results and discussion

3.1. Cholesterol content

The results of the raw samples showed that CN had a cholesterol content of 82.42 mg/100 g while low-fat burgers had values ranging from 73.33 to 76.75 mg/100 g (Table 2). Despite this decrease, the reduction in the cholesterol content was not significant. Similar to these findings, no differences in the cholesterol content of full-fat and low-fat treatments were observed in the study of Bond, Marchello,

Table 2
Cholesterol content[1] (mg/100 g sample) of raw and cooked burgers (mean ± standard deviation).

Treatments	Cholesterol	
	Raw burger	Cooked burger
CN	82.42 ± 13.20[a]	117.42 ± 12.64[a]
CT	76.75 ± 20.87[a]	107.06 ± 14.43[b]
PA	74.70 ± 16.89[a]	105.31 ± 15.41[b]
CO	73.33 ± 17.40[a]	107.63 ± 15.76[b]
PC	74.44 ± 16.26[a]	106.66 ± 12.30[b]

Different letters in the same column differ significantly ($P < 0.05$) by the Tukey's test.
CN: conventional, with 20% fat; CT: control, with 10% fat; PA: with 10% fat and 1.5% of pineapple by-product; CO: with 10% fat and 5% of canola oil; PC: with 10% fat, 1.5% of pineapple by-product and 5% of canola oil.
[1] The cholesterol content of the beef burgers was determined during the first 15 days of frozen storage.

and Slanger (2001), who evaluated conventional (20% fat) and reduced-fat burgers (10% fat), and in the study of Rodríguez-Carpena, Morcuende, and Estévez (2012), comparing control (10% fat) and low-fat burgers (5%) with vegetable oils. On the other hand, Choi et al. (2010) reported significantly lower cholesterol content in reduced-fat frankfurters with vegetable oil and rice bran fiber (10% fat) than the control (30% fat). The differences among studies are certainly related to the amount of fat removed from the formulation. Higher levels of fat reduction, as those observed in the study of Choi et al. (2010), probably led to a higher impact on the cholesterol amount, and could have more reasonably caused its significant decrease.

According to the Brazilian Table of Food Composition (TACO), pork back fat (raw) has 73 mg/100 g (Unicamp, 2011) and according to the USDA Nutrient Database for Standard Reference, the cholesterol content of lard (raw) is 95 mg/100 g (USDA, 2015). Taking into account these two databases and considering 100 g of burger, the reduction of fat from 20 to 10% in raw beef burgers, would result in a decrease of cholesterol content ranging between 7.3 mg (TACO) and 9.5 mg (USDA table). The results of this study were consistent with the values indicated by the reference tables, since the low-fat burgers (100 g) had a mean cholesterol content reduction of 7.62 mg compared to the conventional burger.

Fat reduction showed significant effect in the cholesterol content of cooked products (Table 2). Cholesterol level of CN was significantly higher than those of the low-fat treatments (CT, PA, OC, PC), which showed a mean decrease of 9.15%. The same effect was found by Gök, Akkaya, Obuz, and Bulut (2011), who evaluated the use of ground poppy seed as a fat replacer on meat burgers and by Muguerza et al. (2001), which reported that products with 20, 25 and 30% of replacing level of pork back fat had significantly lower cholesterol content than the control.

3.2. Fatty acid profile

The treatment with addition of just pineapple by-product was not significantly different from the conventional and the low-fat control burgers regarding the fatty acids quantified, except for the palmitoleic acid, where PA differ from CT (Table 3). Since the pineapple by-product used here has a small amount of fat (1.32 g/100 g, data not shown), its addition was not enough to promote changes in the fatty acid profile of the low-fat beef burgers.

On the other hand, as expected, canola oil addition promoted significant alterations in the fatty acid profile of the reformulated products. According to the results, oil addition promoted a decrease in the miristic, palmitic and stearic acids compared to those found in CN and CT. The absence of miristic acid and the lower amounts of palmitic (4.30 g/100 g) and stearic acids (2.09 g/100 g) in the canola oil compared to the amount found in the pork fat (1.30 mg miristic acid, 23.8 palmitic acid, and 13.5 g stearic acid/100 g) (USDA, 2015), were responsible for these reductions in CO and PC, indicating that the fatty acid composition of the treatments reflected the characteristic lipid composition of the type of fat/oil used in the formulation. The reduction of the three major saturated fatty acids consequently resulted in CO and PC showing significantly lower SFA contents than the treatments without vegetable oil. Similar results were reported by Choi et al. (2010), who found a significant reduction in the total SFA and in the miristic, palmitic, and stearic acids of reduced-fat frankfurters with rice bran fiber and canola oil (10%) compared to the control. SFAs are known to increase the low density lipoproteins and hence, blood cholesterol level (Mattson & Grundy, 1985). Thus, regarding SFA, canola oil treatments showed nutritional advantages in relation to the other treatments.

Table 3
Fatty acid composition[1] (g/100 g fatty acids) of raw and cooked burgers (average ± standard deviation).

Fatty acids	Treatments				
	CN	CT	PA	CO	PC
Raw burger					
Miristic C14:0	1.66 ± 0.08^{a}	1.54 ± 0.20^{a}	1.46 ± 0.20^{a,b}	1.25 ± 0.29^{b}	1.25 ± 0.24^{b}
Palmitic C16:0	21.92 ± 0.89^{a}	20.46 ± 0.23^{a}	20.95 ± 0.95^{a}	17.61 ± 1.39^{b}	17.54 ± 1.17^{b}
Palmitoleic C16:1	1.37 ± 0.32^{ab}	1.29 ± 0.29^{b}	1.42 ± 0.35^{a}	1.06 ± 0.37^{c}	1.10 ± 0.32^{c}
Stearic C18:0	9.08 ± 0.17^{a}	8.93 ± 0.40^{a}	9.01 ± 0.44^{a}	7.52 ± 0.76^{b}	7.40 ± 0.77^{b}
Oleic C18:1	44.82 ± 3.71^{b}	41.54 ± 1.70^{b}	42.51 ± 2.29^{b}	49.55 ± 2.19^{a}	49.00 ± 3.65^{a}
Linoleic C18:2	13.26 ± 1.65^{bc}	12.24 ± 1.73^{c}	12.38 ± 1.34^{c}	14.89 ± 0.98^{a}	14.64 ± 0.78^{a,b}
Linolenic C18:3	0.54 ± 0.11^{b}	0.53 ± 0.14^{b}	0.53 ± 0.15^{b}	1.97 ± 0.25^{a}	1.88 ± 0.27^{a}
ΣSFA	32.66 ± 0.75^{a}	30.93 ± 0.41^{a}	31.43 ± 1.43^{a}	26.38 ± 2.42^{b}	26.19 ± 2.12^{b}
ΣMUFA	46.20 ± 3.49^{b}	42.83 ± 1.55^{b}	43.92 ± 2.38^{b}	50.62 ± 2.10^{a}	50.10 ± 3.56^{a}
ΣPUFA	13.81 ± 1.70^{b}	12.77 ± 1.85^{b}	12.91 ± 1.44^{b}	16.86 ± 0.97^{a}	16.52 ± 0.81^{a}
P/S	0.42 ± 0.06^{b}	0.41 ± 0.06^{b}	0.41 ± 0.06^{b}	0.64 ± 0.08^{a}	0.64 ± 0.08^{a}
n-6/n-3	25.05 ± 5.15^{a}	23.76 ± 4.38^{a}	24.68 ± 6.61^{a}	7.64 ± 1.10^{b}	7.91 ± 1.16^{b}
AI	0.48 ± 0.02^{a}	0.48 ± 0.02^{a}	0.47 ± 0.02^{a}	0.34 ± 0.05^{b}	0.34 ± 0.04^{b}
TI	1.04 ± 0.04^{a}	1.06 ± 0.04^{a}	1.05 ± 0.03^{a}	0.68 ± 0.09^{b}	0.69 ± 0.09^{b}
Cooked burger					
Miristic C14:0	1.47 ± 0.17^{a}	1.57 ± 0.02^{a}	1.51 ± 0.15^{a}	1.61 ± 0.34^{a}	1.51 ± 0.22^{a}
Palmitic C16:0	20.98 ± 1.39^{a}	20.49 ± 1.71^{a,b}	20.83 ± 0.46^{a}	17.16 ± 0.88^{b}	17.31 ± 1.43^{b}
Palmitoleic C16:1	1.33 ± 0.30^{a}	1.36 ± 0.37^{a}	1.36 ± 0.31^{a}	1.17 ± 0.34^{b}	1.15 ± 0.34^{b}
Stearic C18:0	9.01 ± 0.57^{a}	8.56 ± 1.52^{a}	8.86 ± 0.37^{a}	8.47 ± 0.93^{a}	8.42 ± 0.62^{a}
Oleic C18:1	43.48 ± 3.72^{a,b}	39.20 ± 8.99^{b}	41.61 ± 5.31^{a,b}	47.86 ± 5.36^{a,b}	51.74 ± 3.29^{a}
Linoleic C18:2	12.41 ± 1.01^{b,c}	11.70 ± 0.74^{c}	11.66 ± 1.18^{c}	14.11 ± 1.25^{a}	13.52 ± 0.41^{a,b}
Linolenic C18:3	0.46 ± 0.07^{b}	0.54 ± 0.09^{b}	0.57 ± 0.11^{b}	1.66 ± 0.33^{a}	1.64 ± 0.17^{a}
ΣSFA	31.46 ± 1.78^{a}	30.62 ± 3.20^{a}	31.20 ± 0.49^{a}	27.82 ± 0.57^{a}	27.25 ± 2.07^{a}
ΣMUFA	44.81 ± 3.63^{a,b}	40.55 ± 8.84^{b}	42.97 ± 5.05^{a,b}	49.20 ± 5.13^{a,b}	52.89 ± 3.15^{a}
ΣPUFA	12.87 ± 1.03^{a}	12.25 ± 0.79^{a}	12.23 ± 1.27^{a}	15.77 ± 1.24^{b}	15.16 ± 0.38^{b}
P/S	0.41 ± 0.06^{b}	0.41 ± 0.07^{b}	0.39 ± 0.04^{b}	0.56 ± 0.05^{a}	0.56 ± 0.05^{a}
n-6/n-3	27.34 ± 4.14^{a}	21.93 ± 3.67^{a}	20.66 ± 2.90^{a}	8.76 ± 1.98^{b}	8.28 ± 0.96^{b}
AI	0.46 ± 0.01^{a}	0.51 ± 0.05^{a}	0.49 ± 0.04^{a}	0.38 ± 0.03^{b}	0.35 ± 0.04^{b}
TI	1.05 ± 0.02^{a}	1.11 ± 0.04^{a}	1.08 ± 0.06^{a}	0.78 ± 0.05^{b}	0.72 ± 0.08^{b}

Different letters in the same row differ significantly ($P < 0.05$) by the Tukey's test.
AI: atherogenic index; TI: thrombogenic index.
CN: conventional, with 20% fat; CT: control, with 10% fat; PA: with 10% fat and 1.5% of pineapple by-product; CO: with 10% fat and 5% of canola oil; PC: with 10% fat, 1.5% of pineapple by-product and 5% of canola oil.
[1] The fatty acid profile of the beef burgers was determined during the first 15 days of frozen storage.

In cooked burgers, among the saturated fatty acids, just palmitic acid was significantly lower in the canola oil treatments compared to CN and PA, and consequently, no significant difference was observed in the SFA content among treatments. The different mechanisms that occur during heat treatment, such as water and fat loss, hydrolysis, oxidation, and polymerization of the triacylglycerol molecule (Takeoka, Full, & Dao, 1997), may have promoted some alterations in the fatty acid profile of the products, leading to different behaviors regarding the SFA content of raw and cooked burgers. This is in agreement to previous studies that indicated that cooking process affects the fatty acid composition of meat products (Poon, Durance, & Kitts, 2001; Scheeder et al., 2001).

In raw burgers of CO and PC, MUFA levels increased compared to the values found in treatments without canola oil incorporation. Despite the significantly lower content of palmitoleic acid in these two treatments, the expressive content of oleic acid in canola oil (61.74 g/100 g) (USDA, 2015) was certainly the factor that caused this increment in the amount of MUFAs. MUFAs are hypocholesterolemic and among the unsaturated fatty acids, they display more beneficial effects than PUFA regarding the increase in the HDL-cholesterol, which is an important factor for the prevention of cardiovascular diseases (Mattson & Grundy, 1985). Increase in monounsaturated fatty acids was also reported by Pelser et al. (2007), evaluating the use of canola oil in low-fat Dutch style fermented sausages.

In cooked burger, a slight increase in the content of MUFA was also observed, however it was not significant. Differences in the behavior of the MUFA content between raw and cooked burgers, as previously discussed, were probably a result of the different events that occurs during cooking, which affected the fatty acid composition of the cooked burgers. In this case an important factor that may have caused the reduction of MUFA in cooked products is the initiation of the lipid peroxidation of unsaturated fatty acids during the heat treatment (Bilek & Turhan, 2009).

The partial substitution of animal fat by canola oil resulted in higher PUFA contents in raw and cooked burgers. Both linoleic and linolenic acids increased in treatments with vegetable oil addition, and according to the results, this PUFA increase was mainly caused by the linolenic acid, since its amount was raised approximately 3.61 and 3.15 times in raw and cooked burgers, respectively, compared to the other treatments. As MUFAs, PUFAs are also known to reduce plasma cholesterol, acting positively in the prevention of cardiovascular diseases (Mattson & Grundy, 1985). These results are similar to the findings of Asuming-Bediako et al. (2014), who reported higher levels of C18:2 and C18:3 in sausages with rapeseed oil compared to the formulation with back fat.

Unlike the results observed for SFA and MUFA, there was no change in the PUFA behavior of raw and cooked burgers, since in both ways PUFA was significantly higher in OC and PC. According to Ono, Berry, and Paroczay (1985), this may be explained by the fact that more saturated than unsaturated fatty acids are lost during heating, because the latter have a greater association with structural components and thus are not readily released during the cooking process.

Besides the evaluation of the fatty acids, the nutritional quality of the fatty acid profile can be evaluated using different indexes, such as the PUFA/SFA, and n-6/n-3 ratios, and the atherogenic, and thrombogenic indexes (Table 3). Considering the improvement in the fatty acid profile of raw and cooked burger with canola oil incorporation, these treatments showed better ratios than the results found in the other formulations. It is recommended that PUFA/SFA ratio should be higher than 0.45, since lower ratios in the diet may increase the incidence of cardiovascular disease (Cifuni, Napolitano, Riviezzi, Braghieri, & Girolami, 2004). PC and CO showed a significant increase in the PUFA/SFA compared to the other treatments, resulting in higher ratios (0.56 to 0.64) than the recommended value, while treatments without vegetable oil incorporation showed levels below 0.42. In agreement with the results present here, the use of flaxseed oil in beef patties (Bilek & Turhan, 2009), rapeseed oil in UK-style sausages (Asuming-Bediako et al., 2014), and olive, corn, canola and soybean oil in frankfurters (Choi et al., 2010) increased the PUFA/SFA ratios to values higher than 0.45.

According to Simopoulos (2002), high n-6/n-3 ratios are associated with the occurrence of some health problems, including cardiovascular, inflammatory and autoimmune diseases, whereas lower ratios exert suppressive effects. For prevention of cardiovascular disease, the recommendation is to reduce the value to less than 4 (Salcedo-Sandoval, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2014). Considering that some meats naturally have the n-6/n-3 ratio higher than this value (Wood et al., 2004), the addition of 5% of canola oil emulsion was not enough to reduce the characteristic high ratio of meats to the recommended level, but its incorporation in raw and cooked beef burgers significantly reduced the ratios from 20.66–27.34 in the treatments CN, CT and PA, to values between 7.64–8.76.

Atherogenic (AI) and thrombogenic indexes (TI) are also used to measure the propensity of a diet or food to influence the incidence of coronary heart disease and the differences of these indexes for the PUFA/SFA and n-6/n-3 ratios are based on the fact that they consider the different promoting and suppressive effects of the fatty acids regarding these two processes. The SFAs that are thrombogenic are not quite the same as those that are atherogenic and unsaturated fatty acids present different degrees of protection against atheroma and thrombosis (Ulbricht & Southgate, 1991). Similarly to the other ratios, the use of canola oil in the two formulations of beef burger (CO and PC) significantly decreased the AI and TI of raw and cooked burgers compared to the other treatments. Rodríguez-Carpena et al. (2012) reported AI values of 0.41 for control pork patty (with pork back fat) and 0.25, 0.23 and 0.24 for patties with avocado, sunflower and olive oils, which are in agreement with the results presented here. Reduction in TI was observed in the study of López-López, Cofrades, Ruiz-Capillas, and Jiménez-Colmenero (2009), who found values of 0.85 for control and 0.57 for frankfurters with olive oil.

3.3. Oxidative stability

The oxidative stability of the samples was evaluated by measuring the malonaldehyde, which was determined and quantified by its reaction with the thiobarbituric acid. MA is one of the major secondary products of the lipid oxidation process, being formed by the hydroperoxides decomposition (Bonnes-Taourel, Guérin, & Torreilles, 1992).

According to the results, there were significant effects of the formulation, storage time, and interaction between both factors in raw burgers.

Overall, there was no effect of the formulations on the malonaldehyde content of raw burgers after one day of frozen storage (Fig. 1). This result was probably because the development of lipid oxidation after just one day of the burger manufacture was not enough to show the possible effects of the fat reduction and partial fat substitution by pineapple by-product and/or canola oil on the oxidative stability of the products.

Even after 30 days of storage, treatments were not significantly different. This result could be due to the use of frozen storage, to the fact that the samples were raw and to the short storage time that do not favor the lipid oxidation, resulting in treatments with similar malonaldehyde contents.

From 60 days until the end of the storage time, treatments showed significant difference. After 60 days, CO was the most oxidized sample, while after 90 and 120 days PC-burgers showed the highest MA values. In the last three evaluation times (60, 90 and 120 days), the same treatments (CN, CO and PC) were the most susceptible to the occurrence of lipid oxidation. PA and CT were the two treatments that had the lowest amounts of MA throughout the storage time, which, after 120 days, showed values of 0.65 and 0.88 mg MA/kg sample, respectively.

For cooked burgers, there was significant effect of the treatments, storage time, and interaction between them. As observed for raw products, cooked burgers, even after heat treatment, did not show significant

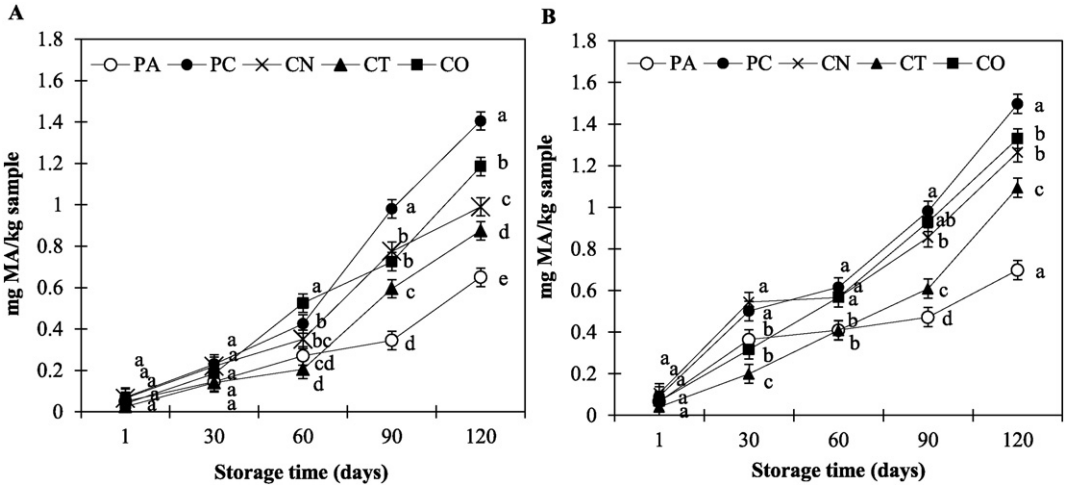

Fig. 1. MDA content (mg/kg) of raw (A) and cooked (B) burgers. Different letters among treatments, in the same storage period, differ significantly ($P < 0.05$) by the Tukey's test CN: conventional, with 20% fat; CT: control, with 10% fat; PA: with 10% fat and 1.5% of pineapple by-product; CO: with 10% fat and 5% of canola oil; PC: with 10% fat, 1.5% of pineapple by-product and 5% of canola oil.

difference among treatments one day after the burger manufacture (Fig. 1). In general, cooked treatments showed the same trend observed in raw samples. With 30 days of storage, CN and PC showed higher MA content than the other treatments, and CT had the lowest value. After 60 and 90 days, full-fat burgers and the two formulations with canola oil were more oxidized than the low-fat control and the treatment with pineapple by-product. Finally, at the end of the storage time, the treatment with addition of canola oil in combination with pineapple by-product presented significantly higher MA content than all the other treatments. CN and CO showed intermediate oxidation values, followed by CT and PA, which had the lowest MA content.

These results are indicative that the addition of canola oil, with or without pineapple by-product, negatively affected the oxidative stability of raw and cooked burgers. This behavior can be attributed to the increased susceptibility of unsaturated fatty acids to lipid oxidation (double bonds are reactive sites in the molecule), when animal fat was partially replaced by the vegetable oil. Similarly to the present study, Choi et al. (2010) reported higher TBARS values of reduced-fat frankfurters with addition of canola oil than those of the control, containing no added vegetable oil.

Despite the enhanced lipid oxidation, at the end of the storage time (120 days), CO and PC showed MA values of 1.19 and 1.41 mg/kg of raw burger, respectively and 1.33 and 1.50 mg/kg of cooked burger, respectively. These values are below the MA threshold for acceptability, since according to Trindade, Mancini-Filho, and Villavicencio (2009), 2 mg MA/kg is believed to be the threshold that can indicate loss of sensory quality and perception of oxidation by consumers.

Regarding the high MA values found in the burgers of the conventional treatment, although animal fat is high in saturated fat, which is more stable to oxidation, pork back fat has 56.3% of unsaturated lipids (45.1% MUFA and 11.2% PUFA) (USDA, 2015). As CN has twice the amount of fat of the other treatments, it was expected that the lipid oxidation of these burgers would occur at a higher speed, resulting in high MA content.

The lipid oxidation of low-fat control and the treatment with pineapple by-product was retarded in raw and cooked burgers compared to the other treatments due to the reduced amount of animal fat in both formulations and, in the case of PA, probably because pineapple by-product has phenolic compounds (3.78 mg gallic acid equivalent/g pineapple by-product, data not shown), with antioxidant activity (DPPH: 5.76 μmol Trolox/g pineapple by-product; ABTS: 13.46 μmol Trolox/g pineapple by-product, data not shown) that could have helped to protect the burgers against the lipid oxidation.

MA content of beef burgers of all treatments increased throughout the storage time. In raw burgers, the values started the storage with 0.05 mg MA/kg of sample and ended the 120 days of frozen storage with 1.02 mg MA/kg of sample. In cooked burgers, as expected, these values were slightly higher, beginning and ending the storage time with 0.08 and 1.18 mg MA/kg of sample, respectively. The development of oxidative rancidity occurs even during the frozen storage because while the rate of the deteriorative reactions (microbiological and enzymatic) can be inhibited by low temperatures, lipid oxidation is slowed, but not completely prevented (Grau, Guardiola, Boatella, Barroeta, & Codony, 2000). López-López et al. (2010), studying low-fat beef burger with addition of olive oil and wakame (seaweed), also observed increase in MA values of beef burger during frozen storage, which started with values ranging from 0.43 to 0.53 mg MA/kg raw burger at the beginning of the experiment (4 days) and showed values between 0.73 to 0.87 mg MA/kg raw burger at the end of the storage time (152 days). The initial oxidation values found by these authors are much higher than those present here. The causes for these differences can be related to the time of storage, type of vegetable oil used, the burger formulation, the oxidative condition of the raw material (beef, fat, and oil), but in this case, it could be also due to the method used to evaluate the lipid oxidation. López-López et al. (2010) used the traditional TBARS method (colorimetric), which is known for its lack of specificity, leading to overestimated results, while the present study used the MA quantification by HPLC that is a more sensitive and specific method.

4. Conclusions

The use of canola oil in combination with pineapple by-product as partial fat replacers leads to healthier cooked burgers as a result of a cholesterol content reduction and an improvement in the nutritional quality of the fatty acid profile, observed through an increase in the PUFA/SFA ratio and a decrease in the n-6/n-3 ratio and in the atherogenic and thrombogenic indexes.

Despite the higher MA values found in CO and PC, canola oil incorporation did not reduce the shelf life of beef burgers in terms of lipid oxidation, since 120 days of storage, their values were lower than the MA threshold for consumer's acceptance.

Thus, the results of this study highlight the application of canola oil and pineapple by-product as food ingredients in the development of healthier beef burgers.

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