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Natural antioxidants protecting irradiated beef burgers from lipid oxidation

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ABSTRACT

The effect of butylated hydroxytoluene/butylated hydroxyanisole blend (BHT/BHA), and rosemary and oregano extracts, added individually or in combination, on lipid oxidation and fatty acid composition was investigated on irradiated frozen beef burgers. Irradiation treatment was carried out using a ^{60}CO semi-industrial irradiator at doses of 6, 7 and 8 kGy, and then the treated meat samples were stored at −20 °C for 90 days. Lipid oxidation and fatty acid composition of beef samples were evaluated by measurement of TBARS and gas chromatography, respectively. The results of the experiment showed that rosemary extract, applied alone and in combination with either BHT/BHA or oregano extract, was more effective in maintaining a low oxidation level in the samples compared to oregano extract used individually or in combination with BHT/BHA. Results also showed no significant differences ($p > 0.05$) in fatty acid composition in all analyzed samples, although some changes in terms of decreased PUFA and MUFA, beside of slight increase of SFA content were observed. However, these differences do not correlate positively neither with the irradiation dose nor the type of antioxidant. Thus, there is a potential application of these spices as natural antioxidants in irradiated meats.

1. Introduction

With the growing interest in convenience foods, ready-to-cook products have become a popular category in the meat industry. Raw meats, however, are susceptible to microbiological contamination during the preparation process in the industry and these microorganisms may not be completely eliminated by heat treatment. The problem is further emphasized by the fact that treating carcasses with an organic acid spray proposed for sanitation can not reduce all pathogenic microorganisms, and these foods are commonly consumed raw or semi-cooked (Farkas, 1998). Additionally, lipid oxidation and fatty acid composition, such as high proportion of highly unsaturated fatty acids, are also important factors influencing quality and acceptability of meat and meat products due to its more or less susceptibility to degrading process (Ahn, Grün, & Mustapha, 2007).

Food irradiation is proven to be the best technology in eliminating disease-causing pathogens from raw meat. Only in the United States, the Center for Disease Control and Prevention (CDC) experts estimate that irradiating half of all ground beef, poultry, pork, and processed meat would reduce food poisoning by one million cases and prevent 6000 serious illnesses and 350 deaths caused by the main microorganisms involved in foodborne infections, such as *Escherichia coli* O157, *Campylobacter*, *Salmonella*, *Listeria*, and *Toxoplasma* (Tauxe, 2001). Even though irradiation is a prospective technology, its application causes physical-chemical and biochemical changes that may affect the nutritional value and sensory characteristics of irradiated food (Alfaia et al., 2007). The chemical changes in meat effected by irradiation, however, are of concern to consumers, and the meat industry is having difficulties in using the technology to achieve its food safety benefits (Nam et al., 2006). The chemical changes in irradiated meats, such as lipid oxidation or *cis*-trans isomerization, are initiated by the free radicals produced during the irradiation treatment. Lipid oxidation is a major cause of flavor deterioration in raw meats. Therefore, enhanced oxidative stability is needed for maintaining the safety and quality of meat.

As meat and meat products are some of the most important sources of dietary fat, modification of the lipid profile of such products can reduce their nutritional quality. Meat fats mostly comprise of monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs). The most ubiquitous fatty acids are oleic (C18:1), palmitic (C16:0), and stearic (C18:0) acids. Trans-fatty acids comprise about 1–2 g/100 g of total fatty acids across all types of meat (Valsta, Tapanainen, & Männistö, 2005). Typical composition

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of ground beef is about 18 g lipids/100 g total mass and its fatty acids content is divided into about 46 g/100 g SFA, 51 g/100 g MUFA, and 3 g/100 g PUFA (Giroux & Lacroix, 1998; Mensink, 2005). There are evidences that irradiation may increase trans-fatty acid content in irradiated fat-based food since irradiation is a process in which the products are exposed to radiant energy, including gamma rays, electron beams, and X-rays for a previously programmed time duration, and this exposition may cause trans isomerization of *cis*-double bond of fatty acids (Brito, Villavicencio, & Mancini-Filho, 2002; Yılmaz & Geçgel, 2006).

In order to inhibit the development of oxidative reaction in meat products, natural and synthetic antioxidants have commonly been used in the meat industry. Antioxidants are regarded as compounds that are able to delay, retard or prevent oxidation processes. Antioxidants with free radical scavenging activities may help to protect the irradiated beef burger from lipid oxidation (Ahn et al., 2007). However, due to concerns about toxicological safety of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), it may be desirable to replace these conventional antioxidants with natural antioxidative substances (Formanek, Kerry, Higgins, Buckley, & Morrissey, 2001).

Various extracts separated from natural sources (e.g. rosemary, oregano, sage, thyme) have proven to possess strong antioxidant activity due to their high content of phenolic compounds, and they are permitted for use in food to replace synthetic antioxidants such as BHT/BHA (Ahn et al., 2007). The antioxidant properties of spices are related mainly to their phenolic compounds, thus their antioxidant action is similar to synthetic phenolic antioxidants. The antioxidant activity of rosemary extract has been associated with the presence of several phenolic diterpenes such as carnosic acid, carnosol, rosmanol, and rosmaridiphenol, which break free radical chain reactions by electron donation and metal ion chelation (Georgantelis, Blekas, Katikou, Ambrosiadis, & Fletouris, 2007). Compounds responsible for the antioxidant activity of oregano were polyhydroxylbenzoic and cinnamic acids. In addition rosmarinic acid and its derivatives were found to be present along with protocatechuic and caffeic acids; a glucoside of protocatechuic acid was also identified (Shahidi, 2000). Rosmarinic acid has been identified not only in oregano, but also in rosemary extract. However, little is known about the biologically active compounds of oregano as antioxidant agents in foods (Cavero et al., 2006).

Although irradiation has been studied for about 50 years and its beneficial effects demonstrated in fresh and cooked bovine, ovine, swine and poultry meat products (Ahn, Jo, Du, Olson, & Nam, 2000), limited information exists both on the use of natural antioxidants, which are directly added to raw meat before irradiation to avoid lipid oxidation as a secondary effect of ionizing radiation, as well as about the changes in fatty acid composition (Brito et al., 2002; Yılmaz & Geçgel, 2006). Therefore, this study aimed at evaluating rosemary and oregano extracts as natural antioxidants against changes on fatty acid profile and lipid oxidation caused by irradiation process in a ^{60}Co-radiation source at the maximum and high doses allowed commercially (6, 7 and 8 kGy).

2. Materials and methods

2.1. Chemicals

The leaves of oregano (*Origanum vulgare* L.) were purchased from a local market. Rosemary extract (Guardian™) and BHT/BHA blend (Grindox™) were obtained from Danisco S/A (São Paulo, BR). Pyrogallic acid, β-carotene, Linolenic acid puriss. pa. standard for GC, ≥98.5% (GC), Tween®, thiobarbituric acid (TBA), trichloroacetic acid, 1,1,3,3-tetraethoxipropane (TEP), and essential fatty acid standards were provided from Sigma-Aldrich Chemical Corporation (St. Louis, MO, USA); *N*-hexane p.a. from Merck (Darmstadt, Germany); ethylic ether p.a.; chloroform p.a.; methyl alcohol; ethyl alcohol 95%; sodium hydroxide (NaOH) 0.05 mol/l; anhydrous sodium chloride (NaCl); hydrochloric acid (HCl); esterification mixture. All these reagents were of analytical grade.

2.2. Samples manufacturing

Ready-to-cook beef burgers were prepared through adding an antioxidant, according to the industrial data used currently in a local processing industry in the city of São Paulo (Table 1). Aqueous extracts of oregano were obtained from sequential extraction using solvents with different polarities, beginning with diethyl ether, followed by ethyl alcohol and distillated water (Mancini-Filho, Van-Koiij, Mancini, Cozzolino, & Torres, 1998). For rosemary, a commercial extract (Guardian™) was used since it is available for food industry. Ground meats and ingredients were divided into seven batches. Concentrations of dry extract, where phenolic compounds are present, were calculated based on gravimetric analysis and expressed as mg/ml. The amount of extract added on the total mass used for each batch was calculated as milligram of dry residue of extract per kilogram of ground beef mass (mg/kg). Oregano extract alone, rosemary extract also alone (400 mg/kg) and in combination (200 mg/kg oregano plus 200 mg/kg rosemary), BHT/BHA alone (200 mg/kg), BHT/BHA in combination with oregano (100 mg/kg plus 200 mg/kg) and BHT/BHA in combination with rosemary (100 mg/kg plus 200 mg/kg) were added to six of the batches and the remaining batch was kept as a control sample (without antioxidant). Ground meats, antioxidant, and ingredients were mixed in a commercial mixer and molded in an industrial molder (which belongs to local industry). The beef burgers were then aerobically packaged in polyethylene bags and were held under frozen conditions (−18 to −20 °C) until the irradiation process.

Table 1
Addition of antioxidants for the preparation of experimental treatments of beef burgers.

Formulations	Beef (g/100 g)	Bovine fat (g/100 g)	Iced water (g/100 g)	Salt (g/100 g)	BHT/BHA (mg/kg)	Rosemary[a] (mg/kg)	Oregano[b] (mg/kg)
BHT/BHA	70.0	20.0	8.0	2.0	200	–	–
Rosemary extract	70.0	20.0	8.0	2.0	–	400	–
Oregano extract	70.0	20.0	8.0	2.0	–	–	400
Rosemary + oregano extract	70.0	20.0	8.0	2.0	–	200	200
BHT/BHA + rosemary extract	70.0	20.0	8.0	2.0	100	200	–
BHT/BHA + oregano extract	70.0	20.0	8.0	2.0	100	–	200
Control (without antioxidant)	70.0	20.0	8.0	2.0	–	–	–

[a] Commercial extract.
[b] Aqueous extract.

2.3. Gamma irradiation and storage

Gamma irradiation was carried out in a ^{60}Co semi-industrial irradiator, installed at the Institute for Energy and Nuclear Research (*Instituto de Pesquisas Energéticas e Nucleares* – IPEN), (São Paulo, Brazil). The applied radiation dose levels were 0 (control), 6, 7 and 8 kGy (one kGy more than the maximum dose allowed for frozen meat (7 kGy) simulating variation of the received dose within an irradiator source at industrial scale processing). The equipment operated at a dose rate of 3 kGy/h. During the time of irradiation, the samples were held frozen (−18 °C to −20 °C) in dry ice inside thermal boxes. Harwell Amber 3042 dosimeters were used to measure the radiation dose (±10%). To minimize the radiation variation in dose absorption due to distance from ^{60}Co source, the thermal boxes that contained the samples were turned 180° halfway through the procedure. After irradiation, the beef burgers were stored in a freezer at –20 °C for 0, 45 and 90 days.

2.4. 2-Thiobarbituric acid reactive substances values (TBARS measurement)

Using the method of Turner et al. (1954), thiobarbituric acid (TBA) values of the beef burgers were measured during storage. A 5 g sample was homogenized in a 50 ml centrifuge tube with a 25 ml extracting solution (7.5 g/100 ml trichloroacetic acid, and 92.5 g/100 ml distillated water) in a Ultraturax homogenizer for approximately 2 min at high speed and after total homogenization this mixture was filtered into a volumetric flask. The volume was filled up to 25 ml with distilled water. A 5 ml aliquot of this solution was transferred into another tube and was mixed with 5 ml of TBA solution 0.05 mol/l. The mixture was heated at 50 °C in boiling water for 10 min so that the reaction between reactive substances and 2-thiobarbituric acid could occur. Following heating, the mixture was cooled and the absorbance at 532 nm was measured by conventional spectrophotometry (Spectrometry® 20 Genesys™). The concentration (mg/kg sample on the basis of wet weight) of malondialdehyde (MDA) equivalents was calculated by using a determination curve elaborated with 1,1,3,3-tetraethoxipropane (TEP) as standard reagent and expressed as mgTBARS/kg of sample.

2.5. Fatty acid composition

Fatty acid methyl esters (FAMEs) were prepared by alkaline hydrolysis extraction according to the AOAC method (2001). FAMEs were analyzed using a GC-17A Shimadzu Gas chromatograph, equipped with a flame ionization detector. The derivatives were separated on a SP-2560 (biscyanopropil polysiloxane) fused-silica column (100 m long and 0.25 mm internal diameter). The injector and detector temperatures were held at 250 °C and 260 °C, respectively. Oven temperature was maintained at 240 °C. The flow rate of carrier gas (He) was set at 1.0 ml/min. Identification of FAMEs was based on retention times of reference compounds from Sigma-Aldrich Chemical Corporation (St. Louis, MO, USA). Fatty acid composition was expressed as percentage of total FAMEs on basis of total mass. The quantification of PUFA, MUFA, and SFA was carried out by using tridecanoic acid ($C_{13:0}$) as an internal standard. Results are expressed as g/100 g beef burger. All analyses were performed in triplicate.

2.6. Statistical analysis

The means and standard deviations (SD) from three measurements within a batch were obtained from all analytical experiments. Results from the experiments were used as variables and analyzed by using a one-way analysis of variance (ANOVA) from GraphPad® software in order to assess the effect of the addition of the antioxidants on the oxidative stability of beef burgers and differences between fatty acid compositions among the samples. The study of the effect of refrigerated storage on the oxidative deterioration of beef burgers was carried out by using a *t*-student test for dependent variables. When statistically significant differences were found, Tukey tests were performed. Statistical significance was set at $p \leq 0.05$.

3. Results and discussion

3.1. Irradiation dose effects and storage time on TBARS values

Histograms showing oxidation levels in non-irradiated and irradiated samples at doses 6, 7, and 8 kGy during frozen storage of 0, 45, and 90 days are shown in Fig. 1. When foods are irradiated in an industrial irradiator and inside a bag or box (*e.g.* on an industrial-scale production), it is possible that they receive different doses due to the position and the distance that these packages are placed from the irradiation source. Then, it is necessary to assure that these differences do not intensively affect the chemical compounds of foods. For this reason, the present study investigated the level of oxidation produced by minimal variations between different irradiation doses up to 1 kGy. For this purpose, maximum doses allowed for frozen meat were applied: 7 kGy, and assuming a variation of 1 kGy, doses of 6 and 8 kGy were also applied. Accurate dosimetry was used during the whole irradiation treatment to assure that each batch would only receive the desired dose.

Our objective was to evaluate the antioxidant activity of the extracts capable of protecting the samples from oxidative deterioration caused by storage time and ionizing radiation. Since they are distinct forms leading to oxidation, two groups were used as controls, one non-irradiated (0 kGy) for evaluating the effects of irradiation and another with no added antioxidant (named control) for evaluating the effects of the different types of antioxidants. The antioxidative effects of the treatments, as measured by TBARS at the time zero of this experiment (day 0) are shown in Fig. 1. TBARS values in the control sample rapidly increased upon irradiation, and this effect was more evident in samples formulated with oregano extract and the control samples. Non-irradiated samples presented fewer differences among different types of antioxidants used, but it was observed that rosemary extract, either alone or in combination with BHT/BHA and oregano extract retarded the oxidation reaction similar to BHT/BHA. Also, the control samples, even at day 0, showed higher TBARS values as effects of rapid auto-oxidation in processed products. It is observed that all formulations showed similar TBARS values (~ 1.0 mg/kg) in the three doses applied (6, 7 and 8 kGy), except for the control samples and oregano samples, whose values raised to approximately 2 mg/kg. Oregano extract demonstrated to be less efficient in avoiding oxidation in the irradiated samples, even so, TBARS values were below 3.0 mg/kg in the three doses employed until the 45th day, whereas control samples showed approximately 4.0 mg/kg (Fig. 1). TBARS values after 45 and 90 days of storage time are shown in Fig. 1. Overall, all formulations exhibited oxidation levels similar to the initial period (day 0). It must be emphasized that even after 90 days of storage, rosemary extract alone or in combination with BHT/BHA or oregano extract maintained lower oxidation levels, whereas the oxidation of the control samples remained increased values during storage. Also, all extracts enhanced protection against lipid oxidation in the non-irradiated samples (0 kGy), which showed TBARS values below 2.0 mg/kg, whereas control samples showed 2.7 mg/kg and 2.5 mg/kg after 45 and 90 days, respectively (Fig. 1).

As expected, all non-irradiated samples show similar behavior concerning oxidation during storage. Although the sample

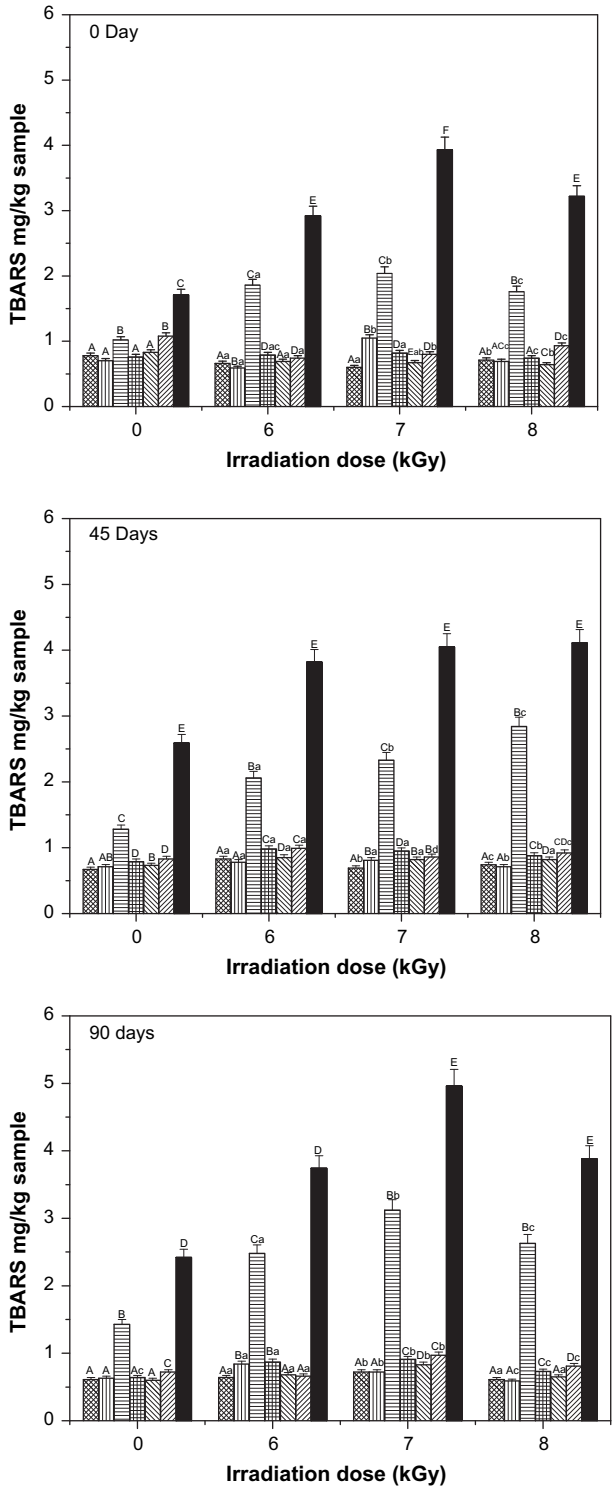

Fig. 1. TBARS values of irradiated and non-irradiated beef burger samples formulated with different types of antioxidants. (▨) BHT/BHA (▥)Rosemary (▤)Oregano (▦) Rosemary + Oregano (▧) BHT/BHA + Rosemary (▨) BHT/BHA + Oregano (■) Control. Data represents means ± respective standard errors. Bars with different capital letters (A–F) within the same irradiation dose differ significantly ($p < 0.05$); different small letters (a–c) means significant differences ($p < 0.05$) among three doses employed for the same formulation.

formulated with oregano extract was more susceptible to lipid oxidation than that of BHT/BHA and rosemary extract preparations, the rate of TBARS formation was lower when compared to samples without antioxidants, demonstrating its antioxidant efficiency. The irradiation caused a marked increase of TBARS values as soon as the samples were submitted to it as seen at day 0 of storage (Fig. 1) where the TBARS values reached 3.0 mg/kg. Also, a subtle decrease of TBARS values from the 45th to 90th days of storage is observed in some samples, except for the control and oregano samples when they were irradiated with 7 kGy. Probably, this phenomenon may be due to hydroperoxide decomposition rate being higher than the rate at which it is formed (Georgantelis et al., 2007).

Gray and Pearson (1987) reported that rancid flavor is initially detected in meat products with TBARS values between 0.5 and 2.0, what is also emphasized by Campo et al. (2006) who reported that a TBARS value of around 2.0 could be considered the limiting threshold for the acceptability of oxidized beef. Thus, efforts must be made by food manufacturers to avoid oxidation of their products, to maintain TBARS values below 2.0 mg/kg during the storage period and to protect the products during any process to which they may be submitted and that may cause oxidative deterioration (for example, irradiation) and consequently, diminish the products shelf-life. For this purpose, synthetic (*e.g.* BHT, BHA, TBHQ tert-butylhydroquinone) and natural antioxidants such as extracts of herbs have commonly been used in the food formulations (Shahidi, 2000). The use of antioxidant systems to accomplish a reduction in oxidation is also not a new concept, and studies up to the mid-1980s that included vegetable extracts, citrus juice concentrates and oilseed products were reviewed by Rhee, Ziprin, and Ordóñez (1987) and Pokorny (1991). Other characteristcs of natural and herb-derived antioxidants and their general applications are reviewed in the recent paper by Balasundram, Sundram, and Samman (2006). Moreover, over the past years, increasing consumer demand for more natural, "preservative-free" products has led the food industry to consider the incorporation of natural antioxidants in a range of products. The use of natural antioxidants has the advantage of being more acceptable to the consumers as they consider these substances to be "non-toxic". In addition, they do not require safety tests before being used (Fasseas, Mountzouris, Tarantilis, Polissou, & Zervas, 2007).

Rosemary extracts are probably the most widely investigated natural antioxidant systems used in meat products, but many others have been investigated as well, such as oregano extracts (Fasseas et al., 2007; Govaris, Botsoglou, Papageorgiou, Botsoglou, & Ambrosiadis, 2004). Lee et al. (2005) investigated the combined effects of gamma irradiation and rosemary extract on the shelf-life of hamburger steak under anaerobic conditions and found that the differences in the type and concentration of the antioxidants were not statistically significant. However, their results may have been underestimated by the other ingredients that were added to the samples, and also by the fact that under anaerobic condition lipid oxidation did not occur. For this reason, in our experiment no other additives were applied in the samples. Sebranek, Sewalt, Robbins, and Houser (2005) compared rosemary extract and BHT/BHA in pork sausage and found that rosemary extract, at concentrations of 1500 mg/kg, was capable of maintaining TBARS values below 1.5 mg/kg throughout the 112 days of the study, whereas the TBARS values of the control (without antioxidants) and BHT/BHA-treated (200 mg/kg) sausages exceeded 2.0 mg/kg by 42 days. Georgantelis et al. (2007), also found that rosemary extract maintained TBARS values below 2.0 in beef burgers up to 90 days, increasing more intensively only over 120 days of frozen storage. The average levels of lipid oxidation of beef burger found in the present study during storage time and antioxidant capacity of natural extracts such as rosemary and oregano were in agreement with the results of previous study reported by other researchers, and confirms the great potential to their applications in meat and meat products in replacement of synthetic antioxidants.

Table 2
Fatty acid composition (g/100 g) of beef burgers formulated with different types of antioxidants and submitted to different irradiation doses (0, 6, 7 and 8 kGy) and storage time (45 days or 90 days).

Antioxidants	Fatty acid	45 Days				90 Days			
		0 kGy	6 kGy	7 kGy	8 kGy	0 kGy	6 kGy	7 kGy	8 kGy
BHT/BHA	∑SFA	10.07 ± 0.31^{ad}	10.11 ± 0.23^{ad}	9.98 ± 0.18^{ad}	9.71 ± 0.14^{ad}	10.22 ± 0.19^{ad}	10.04 ± 0.13^{ad}	9.91 ± 0.07^{ad}	10.07 ± 0.07^{ad}
	∑MUFA	7.35 ± 0.25^{ad}	7.35 ± 0.14^{ad}	7.60 ± 0.16^{ade}	7.85 ± 0.20^{ae}	7.44 ± 0.17^{ad}	7.67 ± 0.20^{ad}	7.73 ± 0.11^{ad}	7.59 ± 0.10^{ad}
	∑PUFA	0.93 ± 0.26^{ad}	0.94 ± 0.14^{ad}	0.75 ± 0.01^{ad}	0.74 ± 0.04^{ad}	0.70 ± 0.03^{ad}	0.62 ± 0.04^{bd}	0.67 ± 0.06^{ad}	0.65 ± 0.04^{ad}
	∑Trans	0.71 ± 0.02^{adf}	0.67 ± 0.02^{ad}	0.73 ± 0.03^{adf}	0.77 ± 0.03^{ad}	0.69 ± 0.03^{ad}	0.74 ± 0.04^{ad}	0.75 ± 0.04^{ad}	0.73 ± 0.05^{ad}
Rosemary	∑SFA	10.21 ± 0.34^{ad}	9.24 ± 0.42^{ae}	9.74 ± 0.13^{adf}	10.18 ± 0.08^{adf}	9.86 ± 0.18^{ad}	9.82 ± 0.02^{ad}	9.87 ± 0.03^{ad}	9.90 ± 0.00^{ad}
	∑MUFA	7.39 ± 0.16^{ad}	7.86 ± 0.27^{aef}	7.87 ± 0.15^{aef}	7.58 ± 0.04^{adf}	7.87 ± 0.31^{bd}	7.78 ± 0.00^{ad}	7.80 ± 0.04^{ad}	7.78 ± 0.00^{ad}
	∑PUFA	0.67 ± 0.16^{ad}	1.17 ± 0.27^{ae}	0.57 ± 0.01^{ad}	0.49 ± 0.02^{ad}	0.58 ± 0.01^{ad}	0.65 ± 0.02^{bd}	0.57 ± 0.02^{ad}	0.52 ± 0.00^{ad}
	∑Trans	0.80 ± 0.04^{ad}	0.79 ± 0.02^{ad}	0.88 ± 0.03^{ad}	0.81 ± 0.06^{ad}	0.82 ± 0.06^{ad}	0.80 ± 0.01^{ad}	0.82 ± 0.01^{ad}	0.86 ± 0.00^{ad}
Oregano	∑SFA	10.83 ± 0.03^{ad}	10.05 ± 0.03^{ae}	9.88 ± 0.11^{ae}	10.24 ± 0.18^{ae}	10.03 ± 0.15^{bd}	10.00 ± 0.05^{ad}	9.79 ± 0.15^{ad}	10.34 ± 0.23^{ae}
	∑MUFA	7.00 ± 0.04^{ad}	7.69 ± 0.05^{ae}	7.73 ± 0.15^{ae}	7.47 ± 0.22^{ae}	7.67 ± 0.19^{bd}	7.65 ± 0.04^{ad}	7.88 ± 0.22^{ad}	7.40 ± 0.15^{ae}
	∑PUFA	0.54 ± 0.01^{ad}	0.55 ± 0.05^{ad}	0.60 ± 0.01^{ad}	0.57 ± 0.07^{ad}	0.63 ± 0.03^{ad}	0.61 ± 0.02^{ad}	0.58 ± 0.06^{ad}	0.52 ± 0.02^{ad}
	∑Trans	0.70 ± 0.02^{ad}	0.77 ± 0.03^{adf}	0.85 ± 0.05^{aef}	0.78 ± 0.01^{adf}	0.74 ± 0.03^{ad}	0.79 ± 0.01^{ad}	0.81 ± 0.01^{ad}	0.80 ± 0.07^{ad}
Rosemary + Oregano	∑SFA	10.70 ± 0.31^{ad}	10.28 ± 0.12^{ad}	10.32 ± 0.12^{ad}	10.43 ± 0.22^{ad}	10.57 ± 0.03^{ad}	10.30 ± 0.04^{ad}	10.33 ± 0.10^{ad}	10.42 ± 0.22^{ad}
	∑MUFA	6.86 ± 0.31^{ad}	7.25 ± 0.06^{ad}	7.29 ± 0.12^{ad}	7.01 ± 0.08^{ad}	7.03 ± 0.05^{ad}	7.31 ± 0.07^{ad}	7.22 ± 0.12^{ad}	7.12 ± 0.31^{ad}
	∑PUFA	0.66 ± 0.15^{ad}	0.63 ± 0.05^{ad}	0.60 ± 0.02^{ad}	0.69 ± 0.17^{ad}	0.56 ± 0.04^{ad}	0.53 ± 0.03^{ad}	0.57 ± 0.00^{ad}	0.52 ± 0.04^{ad}
	∑Trans	0.85 ± 0.06^{ad}	0.91 ± 0.04^{ad}	0.85 ± 0.01^{ad}	0.94 ± 0.03^{ad}	0.89 ± 0.01^{ad}	0.92 ± 0.01^{adf}	0.93 ± 0.02^{adf}	1.00 ± 0.07^{aef}
BHT/BHA + Rosemary	∑SFA	10.29 ± 0.47^{ad}	9.62 ± 0.19^{ae}	9.96 ± 0.11^{ade}	10.23 ± 0.22^{ad}	10.15 ± 0.10^{ad}	9.89 ± 0.04^{ad}	10.11 ± 0.07^{ad}	10.27 ± 0.09^{ad}
	∑MUFA	7.23 ± 0.45^{ad}	7.79 ± 0.13^{ae}	7.61 ± 0.06^{ade}	7.44 ± 0.12^{ade}	7.50 ± 0.13^{ad}	7.74 ± 0.08^{ad}	7.50 ± 0.08^{ad}	7.37 ± 0.14^{ad}
	∑PUFA	0.65 ± 0.08^{ad}	0.83 ± 0.10^{ae}	0.67 ± 0.05^{ade}	0.54 ± 0.09^{ad}	0.57 ± 0.03^{ad}	0.58 ± 0.02^{bd}	0.58 ± 0.02^{ad}	0.49 ± 0.03^{ad}
	∑Trans	0.90 ± 0.06^{ad}	0.82 ± 0.01^{ad}	0.82 ± 0.02^{ad}	0.85 ± 0.04^{ad}	0.85 ± 0.00^{ad}	0.85 ± 0.01^{ad}	0.87 ± 0.01^{ad}	0.94 ± 0.10^{ad}
BHT/BHA + Oregano	∑SFA	10.28 ± 0.00^{ade}	9.77 ± 0.09^{ad}	9.98 ± 0.03^{ade}	10.48 ± 0.33^{ae}	9.41 ± 0.37^{bd}	10.04 ± 0.15^{ae}	9.81 ± 0.05^{ade}	9.83 ± 0.13^{bde}
	∑MUFA	7.40 ± 0.00^{ade}	7.75 ± 0.08^{ad}	7.83 ± 0.11^{ad}	7.15 ± 0.34^{ae}	8.20 ± 0.42^{bd}	7.54 ± 0.14^{ae}	7.77 ± 0.05^{ade}	7.83 ± 0.12^{bde}
	∑PUFA	0.68 ± 0.00^{ad}	0.77 ± 0.06^{ad}	0.57 ± 0.09^{ad}	0.65 ± 0.03^{ad}	0.65 ± 0.07^{ad}	0.67 ± 0.07^{ad}	0.64 ± 0.03^{ad}	0.55 ± 0.04^{ad}
	∑Trans	0.70 ± 0.00^{ad}	0.77 ± 0.00^{ad}	0.69 ± 0.05^{ad}	0.78 ± 0.07^{ad}	0.80 ± 0.03^{bd}	0.81 ± 0.02^{ad}	0.84 ± 0.02^{bd}	0.84 ± 0.03^{ad}
Control without antioxidants	∑SFA	10.06 ± 0.03^{ade}	9.63 ± 0.03^{ad}	9.94 ± 0.01^{ade}	10.40 ± 0.45^{ae}	10.03 ± 0.12^{ad}	9.74 ± 0.06^{ad}	9.91 ± 0.05^{ad}	9.90 ± 0.17^{ad}
	∑MUFA	7.66 ± 0.02^{ad}	8.10 ± 0.01^{ae}	7.72 ± 0.02^{ade}	7.45 ± 0.39^{ad}	7.66 ± 0.13^{ad}	7.93 ± 0.03^{ad}	7.77 ± 0.04^{ad}	7.78 ± 0.14^{ad}
	∑PUFA	0.62 ± 0.00^{ad}	0.55 ± 0.00^{ae}	0.57 ± 0.02^{ade}	0.46 ± 0.05^{af}	0.56 ± 0.02^{ad}	0.57 ± 0.02^{ad}	0.57 ± 0.03^{ad}	0.51 ± 0.01^{ad}
	∑Trans	0.73 ± 0.04^{ad}	0.78 ± 0.04^{ad}	0.83 ± 0.03^{ae}	0.75 ± 0.01^{ad}	0.81 ± 0.02^{ad}	0.82 ± 0.02^{ad}	0.81 ± 0.02^{ad}	0.88 ± 0.05^{ad}

Mean values, ± standard deviation, ($n = 3$).
Means in a row followed by a different superscript lowercase letters are significantly different ($p < 0.05$).
Fatty acids included in SFA (14:0; 15:0; 16:0; 17:0; 18:0) – MUFA (14:1; 16:1; 17:1; 18:1) – PUFA (18:2; 18:3; 20:4; 22:5) – Trans (18:1 t; 18:2 t).
[a–c] Superscript lowercase letters in the same line means comparison between different storage periods in the same irradiation dose (0, 6, 7 or 8 kGy).
[d–f] Superscript lowercase letters in the same line means comparison between different irradiation doses in the same storage period (45 days or 90 days).

3.2. Fatty acid composition

Another important characteristic of the nutritional and functional quality of meat and meat products is the fatty acid composition. Generally, the composition of ground beef is about 18–20 g lipid/100 g total mass and its fatty acids content is divided into 46 g/100 g SFA, 51 g/100 g MUFA and 3 g/100 g PUFA (Valsta et al., 2005). Beef burger samples with 20 g lipid/100 g of total mass were used in our experiments, and total saturated, mono-unsaturated, polyunsaturated and trans-fatty acids (g/100 g) found in all tested samples are shown in Table 2. Since polyunsaturated fatty acids are oxidized rapidly, precautions must be taken during the irradiation treatment, such as maintaining a constant low temperature, a well established fat content, and minimizing the variation of the radiation dose applied since oxidative and non-oxidative changes can occur as consequences of these variations. Ionizing radiation causes radiolysis of water present in a great extent in meat, which generates free radicals such OH^-, hydrated electron and H^-. These compounds react with food constituents (Giroux & Lacroix, 1998) causing oxidation and loss of food quality. Merritt and Angelini (1978) reported that the amounts of radiolysis sub-products vary as a function of nutrient composition (e.g. fat content and fat composition) and also as a function of temperature during irradiation and the irradiation dose. Furthermore, additional biochemical changes may be related to irradiation effects as reported by Brito et al. (2002) who showed increase of trans-fatty acids and lipid oxidation as being one of the important factors that can be considered in the irradiation process.

Here, we analyzed the effects of irradiation at different doses and the addition of different types of antioxidants on the fatty acid composition. Our results showed that fewer changes were found, although some were statistically significant ($p \leq 0.05$) as shown in Table 2. However, these changes do not represent a positive correlation with irradiation dose or storage time since the values found are very low. Only irradiation and storage variables were considered for statistical analyses, that is, independent differences between each type of antioxidants were not analyzed. All samples presented a mean of 10, 7.5, 0.6 and 0.8 g/100 g for SFA, MUFA, PUFA and TFA, respectively. As reported in Table 2, although the concentration of trans-fatty acids (TFA) increased significantly ($p \leq 0.05$) in the samples formulated with BHT/BHA, oregano, rosemary plus oregano and the control samples, it does not show a positive correlation with irradiation nor storage time. It is important to emphasize that when the samples were submitted to a higher irradiation dose (8 kGy), no difference in trans-fatty acids when compared to non-irradiated samples was observed, this includes samples without antioxidants, what demonstrates that there is no specific effect of irradiation on these compounds. Only a slight increase of TFAs was observed, but it is not possible to attribute this phenomenon only to irradiation or storage. The most expressive values in TFAs are observed in the samples formulated with rosemary plus oregano and rosemary plus BHT/BHA which showed 1.0 g/100 g and 0.94 g/100 g of TFAs upon irradiation dose of 8 kGy and after 90 days of storage, respectively. Our results differ from other previous studies that analyzed the effects of ionizing radiation on trans-fatty acid formation in meat, for example, Brito

et al. (2002) analyzed several doses of irradiation (0 up to 7 kGy) and verified that storage time did not increase TFA values, but a dose of 1 kGy of irradiation produced two times more TFAs than the initial values. Yılmaz and Geçgel (2006) also irradiated ground beef with 1, 3, 5 and 7 kGy and observed that increases of TFA values had a positive correlation with the irradiation dose. It is believed that the temperature during the irradiation process plays an important and principal role since when products like meat are irradiated under chilled conditions, more effects of irradiation on water molecules are observed, also more free radicals are produced, and the mobility of these compounds along the chain of fatty acid provide favorable conditions to TFA formation. In frozen conditions, this effect does not occur since there is not enough mobility of molecules to provide a great exposure of the chain and hydrogen bond to ionizing radiation and free radicals. Our results are in accordance with other researches that also reported no great changes in fatty acids composition in irradiated samples up to 10 kGy (Baggio & Bragagnolo, 2006; Chen et al., 2007). Raddy, Maxwell, Wierbicki, and Phillips (1988) reported no significant differences ($p > 0.05$) in total saturated and unsaturated fatty acids when compared to irradiated (1, 3, 6 kGy) and non-irradiated frozen (−20 °C) chicken muscle. Considering that the World Health Organization (WHO) recommends that diets should provide a very low intake of TFA (Hunter, 2005), any process that increases TFAs content in food must be avoided.

No detailed data about specific types of fatty acids within each formulation are shown here, but some observations about the most important known fatty acids are made. Palmitic (16:0) and stearic (18:0) acids were less sensible to irradiation or storage effects in all formulations and their concentrations did not differ significantly ($p > 0.05$). Palmitoleic acid (16:1) showed a significant increase ($p \leq 0.05$) in the formulations with BHT/BHA and oregano after 45 days of storage, but a decrease ($p \leq 0.05$) after 90 days of storage. Samples formulated with a combination of oregano and rosemary presented a decrease of palmitoleic acid after 45 days of storage although this was statistically significant ($p \leq 0.05$) only for those irradiated at 8 kGy. Interestingly, in samples without antioxidants, the most evident reduction of palmitoleic acid was observed in samples irradiated at 8 kGy, even though this was not statistically significant ($p > 0.05$). Oleic acid (18:1) showed increase only in the samples formulated with rosemary and irradiated at 8 kGy ($p > 0.05$). A significant decrease ($p \leq 0.05$) of α-linolenic acid (18:3) was observed in the samples submitted to 8 kGy, except for the BHT/BHA and rosemary samples, whose values were similar independently of the irradiation dose employed. Arachidonic acid (20:4), a polyunsaturated fatty acid present at very low concentrations in meat, but which is very sensible to oxidation since it contains four double bonds along its chain, was also investigated in this experiment and was found to be reduced as irradiation dose and storage time increase, though this reduction was not significant ($p > 0.05$). The values of other important fatty acids found in meat and that play important roles in metabolism, such as myristic acid (C14:0) which is the most artherogenic acid and whose cholesterol rising effect is four times higher compared to palmitic acid (C16:0), were not increased as a consequence of irradiation. Overall, saturated fatty acids are well known compounds that have influence in the total and low-density lipoprotein (LDL) cholesterol, whereas polyunsaturated fatty acids are thought to have beneficial effects on health. Also, recent interest in trans-fatty acids (TFAs) was sparked off by epidemiological evidence linking trans-fatty acids to higher plasma total cholesterol and low-density lipoprotein (LDL) cholesterol and increased incidence of coronary heart disease (CHD) (Valsta et al., 2005; Yılmaz & Geçgel, 2006). A few studies about biochemical changes in specific molecules, such as fatty acids, caused by ionizing radiation are reported in the literature. Free radical-mediated actions are importantly influenced by several factors, such as fat content and fat composition, water activity of the product, apart from the temperature and irradiation dose (Giroux & Lacroix, 1998). All variables described above are important factors which must be considered and well established in any treatment which employs ionizing radiation.

4. Conclusion

The results indicate that both rosemary and oregano extracts possess antioxidant capacity on beef burgers which have a mixture of several compounds, such as lipid, protein and carbohydrates, and have been submitted to a lipid oxidation acceleration process like ionizing radiation. Among the natural products tested here, the highest antioxidant capacity was obtained from rosemary extract. As expected, the antioxidant capacity of natural extracts decreased and lipid oxidation increased with storage time and also with increase in the irradiation dose. Irradiation did not cause broad changes in the fatty acid composition, although small differences such as increases of SFA and/or decreases of PUFA were noted. There was no significant formation of trans-fatty acids in the irradiated beef burger analyzed during the storage period.

The food industry has been encouraged to intensify efforts to develop products targeting or maintaining low levels of trans-fatty acid and to replace synthetic antioxidants with natural antioxidative substances. Therefore, the combined use of natural extracts and ionizing radiation is recommended to control microbiological and quality changes in beef burger during storage.

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