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Antimicrobial and antioxidant activity of liquid smoke and its potential application to bacon

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ABSTRACT

The aim of the present study was to evaluate the liquid smoke antioxidant and antimicrobial capabilities and its implementation potential on bacon, due to its oxidizing stability. The liquid smoke presented an inhibitory effect on the *Escherichia coli*, *Salmonella choleraesuis*, *Staphylococcus aureus* and *Listeria monocytogenes* microorganisms with minimal bactericidal concentration ranging from 7.5 to 15%. The liquid smoke's antioxidant activity presented 0.24 mg/mL of IC_{50}. Regarding the fatty acids, there has been a significant increase ($p < 0.05$) of oleic acid and a decrease of the linoleic one after storage and consequently forming hydro peroxides, malonaldehyde and hexanal, especially when a greater dilution of liquid smoke was applied (1:2, smoke: water, v/v). Hexanal had not been detected up until 60 days of storage and it was noted that there was a progressive increase from 75 to 90 days of storage, correlated to the peroxides degradation ($p < 0.05$). The use of liquid smoke provided a 43% cooking time reduction (hours) in relation to the conventional system, reducing considerably the production processing costs.

Industrial relevance: The liquid smoke is an alternative to conventional smoking of meat products. The liquid smoke presented an inhibiting effect on *Escherichia coli*, *Salmonella choleraesuis*, *Staphylococcus aureus* and *Listeria monocytogenes* bacteria, showing also an antioxidant effect. In addition, liquid smoke reduced the processing time (hours) in about 43%, factor that can reduce significantly the costs related to the bacon productive process. With this kind of information, it will be possible to produce safer, economical and uniform smoked meat products using liquid smoke.

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1. Introduction

The progress on traditional smoking of foods has been focusing on factors such as; the control of the smoke composition, the implementation of engineering principles of heat and mass transfer in order to shorten the processing period, minimize product weight loss, on quality assurance, on equipment upgrading and on smoke treatment, intended to avoid environmental pollution (Sheard, 2010).

Over the last few years, liquid smoke has been used in meat products as an alternative process derived from the smoke treatment after the burn of sawdust or wood chips, followed by either the condensation or polymerization stages (Sheard, 2010). Improved hygiene, a decrease on processing time, lower environmental pollution and less smoke varieties, are among the advantages of such technology, obtaining products with distinct organoleptic characteristics (Guillén, Sopelana, & Partearroyo, 2000; Stolyhwo & Sikorski, 2005), with antimicrobial properties (Lingbeck et al., 2014) as well as the possibility to eliminate poly aromatic hydrocarbons - PAH_S (Guillén et al., 2000; Visciano, Perugini, Conte, & Amorena, 2008; Aaslyng, Vestergaard, & Koch, 2013).

Liquid smoke is traditionally applied to meats, fish and poultry and it has also been used to add flavor to items such as cheese, tofu and even pet foods (Sheard, 2010). It is viable for use on marinades, sauces or brines and typically on processed meats such as bacon due to the concentration of smoke flavor. Bacon is an industrialized meat product obtained from the thoracic-abdominal pig cut (belly), with or without ribs, with or without skin, adding ingredients (sodium chloride, nitrite and sodium nitrate, sugar and erythorbic) submitted to the appropriate smoking thermal process (Brasil, 2000).

Since bacon contains an abundance of saturated and unsaturated fatty acids in its composition, it becomes a product prone to oxidations that occur mainly by the degradation of poly-unsaturated fatty acids entailing the occurrence of unpleasant odors and flavors. Degradation of fatty acids may reduce the food's nutritional value and decrease the storage period of many foods (Ordoñes, 2005).

In this context, the aim of the present study was to evaluate the liquid smoke's antioxidant and antimicrobial capabilities *in vitro* and its potential implementation on bacon, aiming at oxidative stability.

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2. Materials and methods

2.1. Samples preparation

The cuts of bacon (pig belly) have derived from pig carcasses (n = 20 carcasses) of Largewhite and Landrace breeds, weighting 84 kg on average per carcass, obtained from and prepared at a slaughterhouse plant located in the north of the Rio Grande do Sul State, Brazil. The bacon samples have been prepared in pieces (84 pieces, 20 cm × 15 cm, with thickness varying between 4 and 5 cm) of pig belly adding curing salts (water, sodium chloride, nitrite and sodium nitrate, sugar and erythorbic). Then, the cuts went through a massaging process (tumbling) and remained in the curing solution for approximately 12 h. Afterwards, the cuts were submerged in commercial liquid smoke (Smokez 8168, BKG ADICON) following a 2^2 factorial design, varying the liquid smoke's dilution fraction (1:0–1:2 smoke: H_2O, v/v) and the period of contact (60–180 s), undergoing baking oven cooking (72–74 °C for about 5 h).

The samples cooked were wrapped in Nylon Poli packages (100 cm^3 O_2/m^2/day permeability, 23 °C) with vacuum (~460 mm Hg) and stored at 25 °C for 90 days.

The oxidative stability of the bacon samples smoked with liquid smoke was evaluated based on the concentration measurements of the substances reactive to thiobarbituric acid - TBARS, peroxides, hexanal and the fatty acids composition (palmitic, stearic, oleic, linoleic and linolenic) on 1, 30, 60, 75 and 90 days of stored at 25 °C.

The antimicrobial capability (*Escherichia coli*, *Salmonella choleraesuis*, *Staphylococcus aureus* and *Listeria monocytogenes*) has been evaluated *in vitro* of liquid smoke concentrations from 0.65 to 20% and the oxidant activity was evaluated using the DPPH method (2.2-diphenyl-1-picryl hydrazyl).

2.2. Analytical measurements

2.2.1. Antimicrobial activity of liquid smoke

Four bacterial strains have been used in order to determine the liquid smoke's minimal bactericidal activity: *Escherichia coli* (ATCC 25922), *Salmonella choleraesuis* (ATCC 107008), *Staphylococcus aureus* (ATCC 25923) and *Listeria monocytogenes* (ATCC 7644), derived from the American Type Culture Collection.

The measurement of the minimal bactericidal concentration has been done *in vitro* following the methodology described by Smith-Palmer, Stewart, and Fyfe (1998) with some modifications. Firstly, successive dilutions (20; 15; 10; 7.5; 5; 3.75; 2.5; 1.87; 1.25; 0.935 and 0.625%) were done for the liquid smoke in LB medium. The concentrations used were based on commercial use recommendations (up to 75%). After that, 900 μL of the solutions were inoculated with 100 μL of the bacteria medium (10^8 UFC/mL. Only the bacteria have been inoculated on the control test, without any smoke. After 10 min homogenization, 100 μL were removed from the dilution, added to solidified Luria-Bertani Agar (LBA) medium and incubated at 37 °C for 24 h. The minimal bactericidal concentration was found to be the one with the lowest concentration of liquid smoke capable to prevent the microbial growth in culture media.

2.2.2. Antioxidant activity of liquid smoke

The measurement of the liquid smoke's antioxidant activity has been done using the DPPH assay (2.2′-diphenylpicrylhydrazyl) according to methodology described by Miranda and Fraga (2006) with some modifications. It consists of in incubation for 30 min, 500 μL of 0.1 mM DPPH (2.2-diphenyl-1-picryl hydrazyl) in ethanol with 500 μL of solutions containing growing concentrations of liquid smoke in ethanol. The control solution was obtained replacing 500 μL of liquid smoke for 500 μL of ethanol, without DPPH. Absorbance measurements were then taken at 517 nm. The measurement of antioxidant activity on commercial additives BHA (butylhydroxyanisol) and BHT (butylhydroxytoluene) were used as controls. The capturing activity of the liquid smoke radicals was expressed as a DPPH inhibiting percentage. The IC_{50} was established upon regression analysis, corresponding to the quantity needed to capture 50% of the DPPH free radical.

2.3. Bacon oxidative stability

2.3.1. Fatty acids composition

First of all, the lipids were extracted from the sample in accordance with Bligh and Dyer (1959) methodology. For the fatty acid analysis, a lipids aliquot of approximately 200 mg was esterified according to methodology proposed by Hartman and Lago (1973), which employs methanol as the esterificant agent.

The fatty acids have been determined by gas chromatography (Shimadzu 2010 - Plus). The employed chromatographic conditions on establishing the methyl esters from fatty acids (DIN EN14103 standard from the European Standardization Committee). Chromatographic separations were performed with Rtx-wax fused silica capillary columns (30 m × 0.25 mm i.d., film thickness 0.25 μm). Carrier gas was helium. A total of 1 μL of the sample was injected with a split ratio of 50:1. Injector and detector temperatures at 250 °C. The oven temperature at 120 °C for 2 min (increased at a rate of 10 °C/min), at 180 °C for 3 min (increased at a rate of 5 °C/min) and at 230 °C for 2 min. The fatty acids identification was done by comparing the fatty acids' methyl esters retention time from the samples with known fatty acids methyl esters standards (Methyl oleate, methyl estearate, methyl linolenate, methyl palmitate and methyl linoleate). The results were expressed in grams per 100 g of sample.

2.3.2. Peroxides index

The peroxide index was established according to IAL (1985) methodology, on the cold-pressed fat fraction (Bligh & Dyer, 1959). Initially, a 30 mL solution of acetic acid - chloroform was added to 5 g of fat fraction. After the homogenization, a 0.5 mL of saturated potassium iodide solution had been added, resting for approximately 1 min. Subsequently, 30 mL of distilled water and 0.5 mL of a 2% starch solution were added and titrated with a 0.01°N sodium thiosulfate solution. The peroxides index was expressed in milliequivalents per kilogram of sample.

2.3.3. TBARS - substances reactive to thiobarbituric acid

Lipid oxidation (TBARS) was measured spectrofotometrically (Parkin Elmer model Lambda EZ150) at 531 nm using a standard curve with tetraethoxypropane – TEP (1.10^{-8} to 1.10^{-7} mol/mL) with thiobarbituric acid reactive substances (TBARS) following the methodology Raharjo, Sofos, and Schmidt (1993), modified by Wang, Pace, Dessai, Bovell-Benjamin, and Philips (2002). This analysis constitutes an estimation of lipid oxidation, since it determines the reactive substances to thiobarbituric acid. Results were expressed in milligrams of malonaldehyde (MA) per kilogram of sample.

2.3.4. Hexanal

The hexanal was extracted from the sample using the solid stage micro-extraction technique (SPME - headspace method) according to methodology described by Fernando, Berg, and Grün (2003b), with some modifications.

For the solid state micro-extraction (headspace method) assays, it was used a Carboxen/polydimethylsiloxane (PDMS) 65 μm fiber (Supelco, Bellefonte, PA), 10 mL vials, sealed with Teflon faced rubber septa. Approximately 5 g of sample and 4 mL of milli-Q water were put in vials and kept at 65 °C water bath on the surface of a magnetic stirrer (Fisatom, 752 A) for 30 min. After 10 min, the fiber was exposed and kept in that state for 20 min. Subsequently, the fiber was retrieved into the syringe and submitted to gas chromatography with FID detector, employing the following conditions: Rtx-wax fused silica capillary columns (30 m length, 0.25 mm internal diameter and 0.25 μm thickness); injector and detector with temperatures kept at 250 °C and

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