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Addition of essential oils and inulin for production of reduced salt and fat ham

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Abstract

It has been estimated that approximately 75% of the human's sodium intake is added during industrial manufacturing. Processed meats can also contain relatively high amounts of saturated fats, and high levels of fat, associated with increased risk of obesity, diabetes and cancers, especially colon cancers. In contrast, dietary fiber intake (ex. inulin) has been associated with health benefits, including a suggested protective effect against colorectal cancer due to an inverse association with colorectal cancer risk. Nutritional and health concerns indicate that consumption of these two elements should be reduced. This study aimed to develop a ham with reduced levels of salt and fat. We added essential oils of oregano and rosemary to preserve the ham as a substitute for fat, for which inulin was also added. Six ham formulations were prepared (control, fat reduction + inulin, salt reduction, salt reduction + essential oils, salt reduction + no fat, and salt reduction + no fat + essential oils). The physical-chemical and microbiological quality parameters were evaluated. The addition of inulin did not interfere with the chemical composition of the formulated ham, presenting itself as a promising alternative for reducing the addition of fat in baked ham. The administration of essential oils in combination caused a significant decrease in native flora of cooked ham. These results suggest that a combination of essential oils and inulin may be a promising alternative in the production of meat products with lower salt and fat levels.

Keywords: meat product, natural preservative, food additives.

Introduction

Meat and its products are highly prone to microbial contamination because they are rich in essential nutrients and are perishable (Jayasena and Jo, 2013). *Enterococcus, Carnobacterium, Brochothrix thermosphacta*, *Pseudomonas, Enterobacter Lactobacillus* spp and yeast are microorganisms commonly known to dominate the spoilage microbiota of different meat products (Foulquié-Moreno et al., 2006; Nychas et al., 2008; Vasilopoulos et al., 2008). Additionally, foodborne diseases have emerged as important and growing public health and economic problems in many countries over the last few decades. In the Brazilian Legislation (Resolution RDC n. 12 of January 2, 2001, which establishes the microbiological standards for foods), tolerance limits are established for Coliform microorganisms at 45ºC/g, Coagulase positive Staphylococci per gram and Clostridium sulfite reductant at 46ºC of 10^3, $3x10^3$ and $5x10^2$ UFC/g, respectively, for cooked ham. For this reason, we have been concerned in studying the reduction of the native flora of cooked ham.

Sodium chloride, the main ingredient used in processed meats, is a major source of sodium (Horita et al., 2014) and is responsible for its flavour, preservation, and textural properties (Petracci et al., 2013). It has been estimated that approximately 75% of the sodium consumed is added during industrial manufacturing (Brown et al., 2009; Anderson, 2010). The largest contributors of sodium are processed meats (18%), bread and bakery products (13%), dairy products (12%), and sauces and spreads (11%) (Ni et al., 2010). A high intake of sodium is associated with a greater risk of high blood pressure, which is a major cause of cardiovascular disease and stroke (He et al., 2011). Processed meats can also contain relatively high amounts of saturated fats, associated with increased risk of obesity, diabetes and cancers, especially colon cancers (Aggett et al., 2005). Therefore, nutritional and health concerns indicate that consumption of these two elements should be reduced (Aaslyng et al., 2014; Greiff et al., 2015; Marchetti et al., 2015). Food technologists and nutritionists have been making great efforts to develop novel meat products with low fat, low sodium and antimicrobials, enriched with dietary fiber (Hygreeva et al., 2014). Consumers now prefer natural food additives that may also improve the quality and shelf life of meat products (Fratianni et al., 2010). Plant-derived essential oils (EOs) have shown remarkable

antioxidant properties and antimicrobial potency against spoilage and pathogenic microorganisms in meat and meat products (Jayasena and Jo, 2013; Pavelková et al., 2014). These oils are safe as food additives and certified as "Generally Recognized As Safe" (GRAS) (Lucera et al., 2012). The synergy of *Origanum vulgare* L. and *Rosmarinus officinalis* L. essential oils against bacteria has been reported previously. Azerêdo et al. (2011) suggested a synergistic effect of the combined application of these oils against *Listeria monocytogenes*, *Yersinia enterocolitica* and *Aeromonas hydrophilla*.
Dietary fibers such as inulin are functional ingredients that have been used to replace fat, resulting in increased water-binding capacity, and improved texture of meat products (Rodriguez Furlán et al., 2014). Inulin is is a prebiotic dietary fiber showing excellent properties as a carbohydrate-based fat substitute in relation to its ability to increase viscosity, form gels, provide mouthfeel and texture, and to increase water-holding capacity; thus presenting a good application potential in various food product formulations (Öztürk and Serdaroğlu, 2017). The gel obtained from inulin has a creamy and appropriate consistency, creating a mouthfeel of fat in low- fat food products (Alaei et al., 2018). Inulin is resistant to hydrolysis by animal digestive enzymes but are hydrolyzed and fermented by colon microbiota (Raninen et al., 2011). Dietary inulin is known to exert immune-modulatory effects and induces differentiation in several intestinal cell types to its effects on the gut flora (Barclay et al., 2010), and to inhibit development of colon cancers in animal models (Öztürk and Serdaroğlu, 2017). The aim of the present study was to evaluate the addition of essential oils and inulin in the production of ham with reduced levels of salt and fat.

Results and discussion

Effect of inulin and essential oils on protein, ash, moisture and fat content in cooked ham

The moisture value showed significant differences ($p<0.05$) in ham formulations compared to control. The ham with reduced salt, fat and added essential oils exhibited an 8.67% moisture reduction compared to the commercial product, which may result in a longer shelf life. This may be explained due to the diluent effect of fibre and water binding in the inulin chains that allow easy absorption. Furthermore, inulin acts as a reductant of moisture due to osmotic pressure, reducing the water available in food preparations (Gomes et al., 2007). Huang et al. (2011) showed that with increased level of inulin, the moisture content of the sausage samples was decreased, which was consistent with the results of our study.
Lower values were found for lipid in the formulations in which inulin was added. The formulation with reduced salt content and total inulin replacement of fat exhibited a 44.9% reduction compared to the control. Reducing the amount of fat and substituting it with inulin were the main reasons for the decreased level of fat in the final product. This makes the product healthier and places it in the reduced-fat category. Menegas et al. (2013) showed that the addition of inulin reduced the fat content, compared to the control group, similar to results obtained in our study. Silva-Vazquez et al. (2018) suggest that animal fat could be replaced with inulin as a strategy for producing stable, more health-friendly meat batters. Alaei et al. (2018) showed that the use of inulin as a fat substitute could improve physicochemical, textural, color, and sensory properties of chicken sausages.
According to Oliveira et al. (2017), the use of essential oil (clove and /or rosemary) has potential to maintain /improve the quality (lipid content, pH and color) of the meat during its useful life. Resolution 234 made on 21/05/1996 by the Ministry of Health determines that a "reduced fat product" provides a minimum fat reduction of 25% fat compared to the conventional and low-fat product when the fat content is less than 3% in solids.

Effect of essential oils on survival of autochthonous microflora in cooked ham

The effect of salt reduction, with or without inulin or the essential oils of *O. vulgare* and *R. officinalis* (in mixture), on the count of mesophilic bacteria, *Enterobacteriaceae*, psychrotrophic bacteria, fungi, *S. aureus* and coliform is shown in Table 1. Reducing salt without the presence of essential oils caused increase in count of mesophilic, psychrotrophic and Enterobacteriaceae bacteria by approximately 2 logarithmic cycles during the 120 hours of refrigerated storage ($p \leq 0.05$). Moreover, the scores of all microorganisms in the ham receiving essential oils were much lower than those found in other ham formulations ($p \leq 0.05$). According to the results of this work, application of mixture of essential oils caused a significant decrease in these groups (or family) of microorganisms. This behavior was also evidenced by Van Haute et al. (2016), who investigated effect of cinnamon, oregano and thyme essential oils on the microbial shelf life of marinated fish and meat products. They found that these essential oils increase the shelf life of meat products and decrease microbial counts.
Some researchers revealed that the application of oregano essential oil in meat effectively inhibited *S.enteritidis* and *E.coli*, with a significant reduction in *E. coli* and *S. enteritidis* viable counts after 6 h of drying at 55 ºC with 2 mL (0.038 mL L^{-1} air) and 1.5 mL (0.028mLL^{-1} air) of oregano essential oil, respectively. Additionally, these autors recommended that a concentration of 0.75-1.5 mL of essential oil should be tested in combination with other natural preservatives such as organic acids, essential oils, lysozyme, nisin, conventional preservatives or physical methods according to the hurdle technology concept (Hernández et al., 2017). Pesavento et al. (2015) observed that only 0.5% concentration of the three tested EOs (Rosmarinus, Thymus and Oregano) could be used as food additives because it does not substantially alter the flavour of the food but is able to act as a bacteriostatic or bactericidal agent against high and low ($<10^2$ CFU/g) pathogen concentrations, respectively. In this work, even though the concentration of the oregano essential oils was very low (0.03125%), when combined with rosemary essential oil at a concentration of 0.5%. A synergistic response was occurred capable of significantly reducing the microbial counts.
Azerêdo et al. (2011) found a synergistic effect of the combined application of OVEO and ROEO against the bacteria associated with minimally processed vegetables.

Table 1. Formulations of cooked ham, with or without inulin and with or without essential oils.

Sample	Formulation				
	Meat (g)	% Salt	% Fat	% Inulin	% Oil
Control	3000	1	10	-	-
Fat reduction and inulin	3000	1	5	5	-
Salt reduction	3000	0.8	10	-	-
Salt reduction and essential oils (FIC)	3000	0.8	10	-	In mixture OVEO: 0.3125 µL/mL; ROEO: 5 µL /mL
Salt reduction and no fat	3000	0.8	-	10	-
Salt reduction + no fat + essential oils	3000	0.8	-	10	In mixture: OVEO: 0.3125 µL /mL; ROEO: 5 µL /mL

Table 2. Count (log of cfu/g) of the microflora in cooked ham exposed to O. *vulgare* and *R. officinalis* (in mixture) essential oils and inulin, with or without salt on days 01 (0 h) and 05 (120 h) of cold storage.

Treatment	Microorganisms Mesophilics		Enterobacteriaceae		Psychrotrophic		Moulds and Yeasts		*S. aureus*		Thermotolerant coliforms	
	0	120 h	0	120 h	0	120 h	0	120 h	0	120 h	0	120 h
Control	3.5 (± 0.2) ^{a}	3.88 (± 0.2) ^{b}	< 1.0 ^{a}	1.27 (± 0.2) ^{a}	< 1.0 ^{a}	1.79 (± 0.2) ^{a}	5.82 (± 0.2) ^{b}	6,73 (± 0.2) ^{a}	2.02 (± 0.2) ^{b}	3.54 (± 0.2) ^{b}	< 1.0 ^{a}	< 1.0 ^{a}
Fat reduction + inulin	< 1.0 ^{d}	1.17 (± 0.4) ^{d}	< 1.0 ^{a}	< 1.0 ^{b}	< 1.0 ^{a}	< 1.0 ^{b}	7.03 (± 0.3) ^{a}	7.47 (± 0.2) ^{a}	< 1.0 ^{c}	< 1.0 ^{c}	< 1.0 ^{a}	< 1.0 ^{a}
Salt reduction + essential oils	2.07 (± 0.3) ^{b}	1.52 (± 0.4) ^{d}	< 1.0 ^{a}	< 1.0 ^{b}	< 1.0 ^{a}	< 1.0 ^{b}	2.15 (± 0.3) ^{d}	1.95 (± 0.2) ^{c}	1.53 (± 0.3) ^{b}	< 1.0 ^{c}	< 1.0 ^{a}	< 1.0 ^{a}
Salt reduction	4.08 (± 0.2) ^{a}	6.53 (± 0.2) ^{a}	< 1.0 ^{a}	2.25 (± 0.3) ^{a}	< 1.0 ^{a}	2.67 (± 0.3) ^{a}	4.40 (± 0.2) ^{c}	4.95 (± 0.1) ^{b}	4.50 (± 0.1) ^{a}	5.40 (± 0.2) ^{a}	< 1.0 ^{a}	< 1.0 ^{a}
Salt reduction + no fat + essential oils	1.15 (± 0.3) ^{b}	< 1.0 ^{d}	< 1.0 ^{a}	< 1.0 ^{b}	< 1.0 ^{a}	< 1.0 ^{b}	1.38 (± 0.4) ^{d}	< 1.0 ^{d}	< 1.0 ^{c}	< 1.0 ^{c}	< 1.0 ^{a}	< 1.0 ^{a}
Salt reduction + no fat	3.53 (± 0.2) ^{a}	5.28 (± 0.2) ^{a}	< 1.0 ^{a}	2.20 (± 0.3) ^{a}	< 1.0 ^{a}	2.06 (± 0.3) ^{a}	3.43 (± 0.2) ^{c}	4.78 (± 0.2) ^{b}	3.39 (± 0.2) ^{a}	4.66 (± 0.2) ^{a}	< 1.0 ^{a}	< 1.0 ^{a}

Means (±SE) in the same column with different superscript capital letters are significantly different ($p<0.05$) according to the Duncan test.

According to these authors, the increased antimicrobial activity caused by a mixture of OVEO and ROEO could be partially explained by considering the different compounds found in each essential oil. This leads us to suggest that artificial mixtures might be prepared by adding other main constituents present in the EOs to increase the biological activity or verify the contribution of such pure compounds to the final activity (Fratini et al., 2014). Thus, application of essential oils in food systems is an interesting and growing area for researchers whose results could end up having a great use for food industries (Fernández-López and Viuda-Martos, 2018).

Materials and methods

Essential oils and inulin

OVEO (*Origanum vulgare* L. Essential Oil - density at 20ºC: 0.90; refractive index at 20ºC: 1.47) and ROEO (*Rosmarinus officinalis* L. Essential Oil - density at 20ºC: 0.94; refractive index at 20ºC: 1.51) were purchased from Ferquima Ind. Com. Ltd. (São Paulo, Brazil). The OVEO and ROEO emulsions were prepared in brain heart infusion broth - BHI (Himedia, India) at a range of concentrations (80 -0.312 mL/mL) using Tween 80 (1%, v/v; Sigma -Aldrich, USA) as an emulsifier (Carvalho et al., 2015, Monte et al., 2014). Inulin was acquired from Clariant S.A. (São Paulo, Brazil).

Treatments

Six treatments were conducted to test the replacement of pork fat and salt with 5 and 10% inulin and OVEO (0.3125 µl/mL) + ROEO (5 µl/mL), respectively. The formulations/treatments were: control (no salt and fat reduction); Fat reduction and inulin (5% inulin + 5% fat); Salt reduction (0.8% salt); Salt reduction and essential oils (0.8% salt + OVEO: 0, 0.3125 µl/mL + ROEO: 5 µl/mL); Salt reduction and no fat (0.8% salt + 10% inulin); Sal reduction and no fat and essential oils (0.8% salt + 10% inulin + OVEO: 0, 0.3125 µl/mL + ROEO: 5 µl/mL).

Sample preparation and cooked ham

Pork meat was acquired from the Meat Technology Laboratory - Federal Institute of Education, Science and Technology of Pernambuco (Brazil). The pH of the ham muscles was monitored in order to exclude material outside the normal range (5.5-5.7). The meat and pork fat (lard) were vacuum-packed and stored at −18°C until required for ham production. The meat was boned and cut into cubes of approximately 5 cm^2. After this step, grinding was performed, with subsequent addition of the other curing ingredients (antioxidant (1%), starch (0.5%), ham condiments (1%), sodium glutamate (0.5%), and cochonied carmine dye (0.001%). The meat and other ingredients were weighed according to the formulations shown in Table 2. After 20 h of drainage in a cold room (-4ºC) for ripening, the meat was wrapped in “cook-in” packaging and, soon after, placed into metal pans. These metal pans, in loaded and closed forms, were placed in the cooking tank with hot water (75°C), where they remained for approximately 2 hours, which is long enough for the temperature in the thermal centre of the product to reach 72°C, causing coagulation of proteins, destruction of micro-organisms, flavour development and flavour and colour fixation of the product. A total of 3 presses were performed during the 2 hours of cooking. The ham was then sealed into laminated plastic bags of polyamide/polyethylene and chilled overnight at 4°C. After overnight of cooling, the product was kept at 4 °C.

Effect of inulin and essential oils on physical-chemical parameters in cooked ham

Moisture, protein, fat and ash content were determined according to the AOAC (2005) methods. All chemical determination was performed in duplicate. The Kjeldahl method was used to measure protein concentrations. Ash content was determined using a muffle furnace, and moisture content determination was performed by drying at 105°C ± 2 until the samples reached a constant weight.

Effect of essential oils on survival of bacteria in cooked ham (0 and 120 h of cold storage)

Twenty-five g samples of ham were aseptically transferred into a sterile stomacher bag containing 225 mL of sterile peptone water (1 g/L) and homogenized for 60 s. Subsequently, a decimal dilution series (10^{-2} – 10^{-5}) was performed in the same diluent and enumeration of the natural flora by pour-plating 1 mL of the appropriate sample dilutions on Plate Count Agar (Himedia, India) at 37°C (24–48 h) for total mesophilic bacteria and at 6°C (7 d) forpsychrotrophic bacteria. Enterobacteriaceae was spread-plated 0.1 mL onto Eosyne-Metilen-Blue agar (Himedia, India) at 37°C (24 h), Baird Parker agar (Himedia, India) supplemented with 50 mL/L of the egg yolk emulsion containing potassium tellurite (3.5%) (Himedia, India) to count viable S. aureus and fungi were spread-plated on Sabouraud agar (Himedia, India) at 28°C (48–72 h). The results were expressed as log of cfu/mL (Lopéz-Galvéz et al., 2010). Control flasks containing sterile distilled water were tested in the same way.

Statistical analysis

All assays were made in triplicate on three separate occasions, and the results were expressed as average of the assays. Statistical analysis was performed to determine significant differences (p≤0.05) by ANOVA followed by Duncan test. For statistical analysis, the SigmaStat 3.5 computer programme was used.

Conclusion

The results showed that inulin may be a promising alternative to reduce the added fat in cooked ham because, in the present study, this material did not significantly interfere with the physical and chemical attributes of the product. The mixture of oregano and rosemary essential oils was effective in reducing the native flora microorganism counts during the storage period.

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