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Use of various essential oils as bio preservatives and their effect on the quality of vacuum packaged fresh chicken sausages under frozen conditions

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ABSTRACT

The present study was undertaken to evaluate antimicrobial and antioxidant effect of essential oils on the quality of fresh chicken sausages. Total 15 essential oils (EOs) were screened for their antimicrobial activity; however only 9 EOs showed desired results in disc diffusion assay. It was found that four essential oils namely, clove oil (0.25%), holy basil oil (0.125%), cassia oil (0.25%) and thyme oil (0.125%) could pass sensory evaluation. Fresh chicken sausages incorporated with these EOs were vacuum packaged and stored at −18 ± 2 °C for 45 days. Control had significantly higher pH and TBARS than EO treated products and clove oil products showed least rate of increase of oxidation. Total phenolics and DPPH activity was significantly higher in treatment products than control. Treatment products showed slower rate of increase in microbial count than control and cassia oil products showed lowest microbial load at end of storage period. Regarding sensory attributes, cassia and holy basil products yielded comparably higher scores. Thus, present study indicates that all vacuum packaged frozen fresh chicken sausages were found quite acceptable even at the end of storage period of 45 days.

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1. Introduction

Poultry meat has become a very popular food commodity due to its high biological value animal proteins, essential amino acids and fatty acids, vitamins and other nutrients (Biesalski, 2005; Mulla et al., 2017). Poultry products have frequently been found to be contaminated with various micro-organisms and may serve as vehicles of some of these pathogens (Mor-Mur & Yuste, 2010). However, one difficulty associated with storage is oxidative rancidity which is one of the most important quality defects of chicken meat or meat products during storage. Since synthetic anti-oxidants which have been used for many years to extend the shelf life of meat and meat products are now accused with some carcinogenic and toxic properties (Sharma, Mendiratta, Agarwal, Kumar, & Soni, 2017). This has led many researchers to explore alternatives in the form of natural anti-oxidants. These natural additives should improve meat quality without leaving residues in product (Artiga-Artigas, Acevedo-Fani, & Martin-Belloso, 2017; Ghabraie, Vu, Tata, Salmieri, & Lacroix, 2016). Essential oils also called ethereal oils, are known for their bactericidal, virucidal and fungicidal properties (Burt, 2004). Before their use as “natural preservatives” (Nychas, 1995, pp. 58–89), careful evaluation is needed, so that when added to food product, latter appeals sensorially acceptable to consumer.

Cassia oil is obtained by steam distillation from stem bark of *Cinnamomum cassia* and is reported to possess antimicrobial, anti-tumorigenic, anti-inflammatory and anti-diabetic properties (Lee, Kim, & Kim, 2002). Cinnamon belongs to *Lauraceae* family and cinnamaldehyde is primary component of cinnamon oils (Verspohl, Bauer, & Neddermann, 2005). Thyme also possesses various beneficial effects as antiseptic, carminative, antimicrobial and antioxidative properties (Burt, 2004). Major phenolic compound present in Ajowan (*Trachyspermum ammi* or *Carum copticum)* and clove oil is thymol and eugenol, respectively. They have been

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reported to be germicidal, antispasmodic and antifungal agents. Holy basil (*Ocimum sanctum*) is known as tulsi and is reported to possess anti-bacterial and insecticidal properties (Aggarwal & Goyal, 2012). Essential oils from rosemary, lemon leaf, basil, oregano, ginger, basilica, balm, coriander, rosemary and clove have exhibited a better potential to be used as an antimicrobial agent in fresh meat, ground meat, sea food and their packaging/edible films (Alfonzo et al., 2017; El-Sayed, Chizzola, Ramadan, & Edris, 2017; Khaleque et al., 2016; Moradi, Tajik, Rohani, & Mahmoudian, 2016; Raeisi, Tabaraei, Hashemi, & Behnampour, 2016; Rodrigues et al., 2017). However, very few studies have been performed on the use of essential oils and/or blend of essential oils in emulsion based/ fresh meat products (Kumar, Mendiratta, Agarwal, Sharma, & Kumar, 2017; Sharma et al., 2017).

Considering the above mentioned facts that there is a need for research regarding use of natural additives or alternative methods in order to extend shelf life and/or improve food safety. The present study was attempted to firstly, screen out important essential oils which are able to exhibit antimicrobial activity in meat products as well as could pass sensory evaluation. Secondly, antimicrobial and antioxidant effect of essential oils was studied on the quality of fresh chicken sausages.

2. Materials and methods

2.1. Experimental design

Based on literature, 15 important essential oils (Oregano, Thyme, Holy basil, Carvacrol, Lemon grass, Clove, Ginger, Beetle, Ajowan, Cassia, Rosemary, Coriander, Cinnamon, Garlic and Citrus oil) were evaluated for their anti-microbial activity along with determination of minimum inhibitory concentration (MIC). Nine essential oils were selected on the basis of production of visible zone of inhibition around each disc. This was followed by screening of essential oils for incorporation in meat products on the basis of sensory evaluation. Finally, four essential oils were choosen for selected/ amined study that were exhibiting anti-microbial activity (visible zone of inhibition) as well as could pass the sensory evaluation at minimum base level concentration of 0.125%. Several preliminary trials were conducted to standardize the optimum level of incorporation of essential oils in fresh chicken sausages. Thereafter, effect of incorporation of various essential oils on anti-microbial and anti-oxidant activity was studied using a completely randomized design (CRD). Chicken sausages were incorporated with four different treatments viz, clove oil (0.25%), holy basil oil (0.125%), cassia oil (0.25%) and thyme oil (0.125%); vacuum packaged and stored at -18 ± 2 °C. Physico-chemical characteristics, anti-oxidant activity, sensory attributes (cooked product) and microbiological characteristics were assessed on 0, 15, 30 and 45 days in vacuum packaged products.

2.2. Chicken meat and casings

Chicken carcasses were procured from Central Avian Research Institute (CARI), Izatnagar within 4 h of slaughter. These carcasses were manually deboned; tendons, body fat were trimmed off in experimental abattoir of LPT Division, IVRI, Izatnagar. Thereafter, it was packaged in clean low density polyethylene bags (50 μm) and shifted to deep freezer (Blue Star, FS345, Denmark) for storage at -18 ± 2 °C till further use. Cellulose casings (C17 × 84 ft) were purchased from Euromate Food Tech. Pvt. Ltd., New Delhi. LDPE-Nylon-LDPE coextruded multilayer films (150 μm thickness) for vacuum packaging of products were procured from M/s Hitkari Industries Ltd., New Delhi. Fresh chicken sausages were prepared by mixing following ingredients in a mixer (Russell Hobbs RMG550, Delhi, India): Chicken meat-92%, Ice flakes- 6%, Sodium chloride-1.6%, Sodium tripolyphosphate- 0.4% and Sodium nitrite-150 ppm. Processing of fresh chicken sausages is displayed in Fig. 1.

2.3. Essential oils and other chemicals

Food grade essential oils were purchased from reputed commercial suppliers. Refined salt (Tata Chemicals Ltd., Mumbai) was purchased from local market of Bareilly. Food additives were of food grade quality and procured from reputed firms i.e., sodium nitrite (Merck Specialities Pvt. Ltd., Mumbai), sodium tripolyphosphate (Central Drug House Pvt. Ltd., New Delhi). All chemicals, reagents and media for laboratory analysis were of analytical grade viz, Qualigens, GS chemicals, Sigma, CDH, Merck and Himedia® Laboratories, Mumbai.

2.4. In vitro anti-microbial activity of essential oils and determination of MIC

15 essential oils were evaluated for their *in vitro* anti-microbial activity against seven food borne pathogens and spoilage organisms viz, *E.coli* (MTCC standard strain MTCC-443), *Proteus* sp. (isolated field strain P-1), *Pseudomonas aeruginosa* (MTCC standard strain MTCC-4999), *Salmonella* enterica (MTCC standard strain MTCC-3219), *Listeria monocytogenes* (MTCC standard strain MTCC-657), *Staphylococcus aureus* (MTCC standard strain MTCC-96) and *Bacillus cereus* (MTCC standard strain MTCC-430), by disc diffusion method (Bauer, Kirby, Sherris, & Turck, 1966). The strains used in the study were mostly standard MTCC strains available from MTCC (Microbial Type Culture Collection and Gene Bank), IMTECH, Chandigarh or field isolates. Essential oils showing broad spectrum anti-microbial activity against above mentioned organisms were selected for further studies. Minimum inhibitory concentration (MIC) of selected nine essential oils was determined by disc dilution method. Disc with highest dilution of EOs showing visible zone of inhibition was considered as MIC of essential oil against test organism.

2.5. Initial screening of EOs for incorporation into meat products

A series of preliminary trials were conducted to ascertain the lowest concentration of EOs which could pass sensory evaluation. For this, 0.125% (5 times of base concentration) of individual EOs was incorporated in fresh chicken sausages and sensory evaluation was carried out using 8-point hedonic scale. Among these, four most preferred essential oils which could pass sensory evaluation at 0.0125% level were selected for detailed experiments.

2.6. Analytical methods

2.6.1. Chemical composition

pH of chicken sausages was recorded by immersing combined glass electrodes of digital pH meter (Model LI-120) in homogenate (Troutt et al., 1992). Product yield (%) was obtained by measuring weight of chicken sausages for control and treatments and calculating the ratio of cooked weight and raw weight. Moisture content of fresh chicken sausages was determined by standard methods using hot air oven (Acumen labware Pvt Ltd., Haryana, India) as per AOAC (2005).

2.6.2. Thiobarbituric acid reacting substances (TBARS) number

Lipid oxidation was evaluated in fresh chicken sausages by measuring TBARS by using distillation method described by Tarladgis, Watts, Younathan, and Dugan (1960). O.D. was recorded using spectrophotometer (Model: Beckman DU 640, USA) and

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