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Thiol oxidation and protein cross-link formation during chill storage of pork patties added essential oil of oregano, rosemary, or garlic

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ABSTRACT

The effect of two levels (0.05% and 0.4%) of essential oil of rosemary, oregano, or garlic on protein oxidation in pork patties was studied during storage under modified atmosphere (MAP: 70% O_2: 20% CO_2: 10% N_2) or under aerobic conditions (AE) at 4 °C. The oxidative stability of the meat proteins was evaluated as loss of thiols for up to 9 days of storage, and as formation of myosin cross-links analyzed by SDS-PAGE after 12 days of storage. Protein thiols were lost during storage to yield myosin disulfide cross-links. Essential oils of rosemary and oregano were found to retard the loss of thiols otherwise resulting in myosin cross-links. Garlic essential oil, on the contrary, was found to promote protein oxidation, as seen by an extreme loss in thiol groups, and elevated myosin cross-link formation compared to control.

1. Introduction

Lipid and protein oxidation in meat products is of principal concern for preservation of meat quality during storage. Whereas the influence of lipid oxidation on quality traits such as odor and taste is well recognized, the impact of protein oxidation on meat quality requires further attention. Protein oxidation has been defined by Schacter (2000) as the covalent modification of a protein induced either directly by reactive oxygen species (ROS) or indirectly by secondary byproducts of oxidative stress. Protein oxidation proceeds, in the presence of ROS, by free radical chain reactions similar to those of lipid oxidation, including initiation, propagation and termination stages (Davies & Dean, 1997). For decades, the effect of protein oxidation has challenged manufacturers of surimi products, as the production involves extensive cellular disruption resulting in imbalance in the oxidative status of the cells (Parkington et al., 2000). Oxidative reactions occur also during manufacture or storage of meat, and meat products, such as burger patties, are highly susceptible to oxidation as mincing, cooking, and salt addition promote the formation of ROS (Ladikos & Lougovois, 1990). During these processes, meat proteins are modified by oxidized lipids as well as by metal- or enzyme-catalyzed oxidative reactions or other chemical and biological processes, which together reduce the eating quality of the meat and may affect the nutritional value (Xiong, 2000).

Oxidative damage of proteins leads to textural alterations, which affects the gelation, emulsification, viscosity, solubility, and rehydration properties of meat proteins (Xiong, 2000). The chemical mechanisms leading to oxidative deterioration of meat proteins were recently reviewed (Lund, Heinonen, Baron, & Estévez, 2011). However, little is known about the relative significance of different protein oxidation products and their adverse effects on meat quality, and of the effectiveness of different antioxidants against protein oxidation. It has been reported that the texture of fresh pork is negatively affected by oxidizing proteins during chill storage in high-oxygen atmosphere packaging (Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007), the same tendency has been demonstrated for beef (Zakrys-Waliwander, O'Sullivan, O'Neill, & Kerry, 2012) and most recently for lamb (Kim, Bødker, & Rosenvold, 2012).

The use of natural preservatives to control oxidation in meat products is promising and widely used since a large range of substances of vegetable origin show antioxidant properties (Nieto, Huvaere, & Skibsted, 2011; Xiong, 2000). In this sense, many aromatic plants and spices have been shown to be effective in hindering the process of lipid peroxidation (Kulisic, Radonic, Katalinic, & Milos, 2004; Nieto, Diaz, Bañón, & Garrido, 2010; Nieto et al., 2011). Compounds from essential oils have also been shown to prevent oxidation and color loss in red meat packaged under modified atmosphere (Djenane, Sánchez-Escalante, Beltrán, & Roncalés, 2002; Sanchez-Escalante, Djenane, Torrescano, Beltran, & Roncales, 2001).

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The acceptance among consumers for the use of essential oils is growing, and more than 150 essential oils are listed as GRAS (generally recognized as safe) by the US Food and Drug Administration. However, the protecting effects of natural antioxidants, such as essential oils, against protein oxidation has only been sparsely investigated as recently recognized by Zhou and Elias (2011) calling for in-depth investigations exploring the mechanisms behind the protection of protein against oxidation by natural antioxidants.

The quantification of protein thiols (the sulfhydryl group (SH) of a cysteine residue) combined with the detection of protein disulfide cross-link formation by SDS-PAGE enables detailed investigation of protein oxidation. In biological systems the mechanism behind thiol oxidation and formation of disulfide bonds involves a series of thiol/disulfide exchange reactions. Thiol groups may be oxidized in the presence of transition metal ions by radical-mediated reactions to yield thiyl radicals (RS•), which may react to form cross-linked structures (Hawkins & Davies, 2001). Thiol oxidation and protein disulfide formation have been found to be directly linked to alterations in meat texture (Kim et al., 2012; Lund, Hviid, & Skibsted, 2007; Zakrys-Waliwander et al., 2012).

Accordingly, the objective of the present study was to investigate the progression of protein oxidation in pork patties added two different levels of each of three essential oils extracted from ingredients commonly used in the Mediterranean cuisine (oregano, rosemary, and garlic) during storage under high oxygen MAP or under aerobic conditions. The pork patties were evaluated by loss of protein thiols and correlated with the degree of protein cross-linking during storage, and the observed effects were discussed in relation to the content of active components in each of the three essential oils.

2. Materials and methods

2.1. Chemicals

Tris(hydroxymethyl)-aminomethane (TRIS), and L-cysteine of analytical grade were obtained from Merck, Darmstadt, Germany. Propylgallate, 2,4-dinitrophenylhydrazine (DNPH), and 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) all of analytical grade were obtained from Fluka, Stenheim, Germany. Dithiothreitol (DTT) and sodium dodecyl sulfate (SDS) were obtained from AppliChem GmbH, Darmstadt, Germany. Bovine album serum (BSA) was obtained from Sigma, Missouri, USA. NuPAGE® Novex 3–8% TRIS–acetate gels, LDS (lithium dodecyl sulfate) sample buffer, SDS TRIS–acetate running buffer, and Molecular Probes SYPRO® Ruby Protein Gel Stain were obtained from Invitrogen, CA, USA. Precision Plus Protein Standard All Blue was obtained from Bio-Rad Laboratories, Inc., CA, USA. Dithiothreitol (DTT) was obtained from AppliChem GmbH, Darmstadt, Germany. Water was prepared using a Millipore-Milli-Q purification system (Milli-Q Plus, Millipore Corporation, Bedford, MA).

2.2. Essential oils

The essential oil of oregano (*Origanum vulgare* L.; ref. F70900L) with a density of 0.938 g/ml at 20 °C was obtained by steam extraction of leaves. The essential oil of rosemary (*Rosmarinus officinalis* L.; ref. F71371R) with a density of 0.909 g/ml at 20 °C was obtained by steam distillation of the entire plant. Garlic essential oil (*Allium sativum* L.; ref. F2110-758) with a density of 1.088 g/ml at 20 °C was obtained by steam distillation. All the essential oils were purchased from Ravetllat Aromatics (Barcelona, Spain) and rosemary and oregano essential oils were previously characterized (Viuda-Martos, Ruiz-Navajas, Fernandez-Lopez, & Angel Perez-Alvarez, 2007). Between the 32 components identified in the oregano essential oil, accounting for 89.5% of the oil, the major components were carvacrol (61.21%), p-cymene (15.12%) and γ-terpinene (4.80%), terpinolene (3.63%), β-caryophyllene (2.62%), and α-pinene (2.34%). Between the 39 components identified in the rosemary essential oil, accounting for 89.5% of the total, the major constituents were α-pinene (36.42%), camphor (15.05%), 1,8-cineole (12.02%), camphene (11.08%), borneol (4.00%), β-pinene (3.67%), p-cymene (2.14%), and γ-terpinene (0.18%). The most abundant sulfur compounds in garlic essential oil have previously been determined to be γ-glutamyl-S-2-propenyl cysteine (0.5%, w/w, based on fresh weight of garlic), allicin (0.31%, w/w, based on fresh weight of garlic), γ-glutamyl-S-(*E*,*Z*)-1-propenyl cysteine (0.36%, w/w, based on fresh weight of garlic), and methyl 2-propenyl thiosulfinate (0.12%, w/w, based on fresh weight of garlic) (Block, 2010). The structures of the dominating compounds in the three essential oils are shown in Scheme 1.

A preliminary study was conducted to determine the concentration levels to be applied. Pork patties were cooked in a microwave oven at high power (700 W) for 4 min. A panel of seven judges experienced in pork evaluation was used for sensory analysis. The panelists were asked to evaluate taste and odor intensities of cooked samples. Acceptability of odor and taste was estimated using an acceptability scale ranging from 5 to 0, with 5 corresponding to a most liked sample and 0 corresponding to a least liked sample. A score of 3.5 was taken as the lower limit of acceptability. The concentration levels applied in the present study were based on this sensory evaluation of the taste and odor intensity of cooked samples.

2.3. Total phenolic content

The amount of total phenolics in essential oils was determined according to the Folin–Ciocalteu method. Samples (200 μl, two replicates) were mixed with 1.0 ml of Folin–Ciocalteu's reagent (diluted 1:10 with water) and 0.8 ml of a 7.5% solution of sodium carbonate was added. The absorption at 765 nm was measured after 30 min with a Cary 3 UV–vis spectrophotometer (Varian Techtron Pty. Ltd., Mulgrave, Victoria, Australia). The total phenolic content is expressed as gallic acid equivalents (GAE) in mg/l of essential oil.

2.4. Preparation and packaging of pork patties

Fresh, semi-boneless pork meat shoulders (Boston butts) were purchased from a local meat supplier. Upon arrival, the Boston butts were processed in a cool room at ~6 °C to yield one single batch of meat. Fat and lean tissues were manually separated and the connective tissue discarded. Fat and lean meat were grounded separately with a meat grinder (Braher International, San Sebastian, Spain) using 5 mm orifice plates, and then mixed for 10 min using an RM-60 Mixer (Mainca, Granollers, Spain). Meat batter with target fat percentage of 30% was obtained by mixing fat and lean meat after determination of fat percentage in each by HFT-2000 fat analyzer (Data Support Co., Inc., Encino, CA, USA).

Seven lots of 2 kg meat batter, each prepared in duplicate, were prepared. Pork patties were prepared from six lots of meat batter (each 2 kg) with rosemary (R), oregano (O), or garlic (G) essential oils added at two different levels: 0.05% or 0.4%: Lot 1 and lot 2 (addition of rosemary at 0.05% or 0.4%), lot 3 and lot 4 (addition of oregano at 0.05% or 0.4%), lot 5 and lot 6 (addition of garlic at 0.05% or 0.4%). The seventh lot (2 kg) without addition of essential oil was used for preparation of control (C) pork patties. The total number of samples was 280 (20 pork patties per lot × 7 different lots × 2 replicates each). Twenty pork patties (100 g each) were prepared using a conventional burger-maker before being packaged in polystyrene trays (B5-37, AERpack, Spain). The trays were sealed using modified atmosphere packaging (MAP) with the gas composition 70% O_2/20% CO_2/10% N_2 in BB4L bags of low gas permeability (8–12 $cm^3\ m^{-2}$ per 24 h) (Cryovac, Spain) using a discontinuous INEINI packer (Pack Multifunction, Spain), or overwrapped with oxygen-permeable polyvinyl chloride (PVC) film (650 $cm^3\ m^{-2}\ h^{-1}$ at 23 °C) for storage under aerobic conditions (AE). The pork patties were stored in a