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Effects of grape seed extract on the oxidative and microbial stability of restructured mutton slices

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ABSTRACT

The antioxidant and antimicrobial efficacy of grape seed extract (GSE) was studied in restructured mutton slices (RMS) under aerobic and vacuum packaging conditions during refrigerated storage. The RMS treated with grape seed extract (GSE) had significantly ($P < 0.05$) lower thiobarbituric acid reactive substance (TBARS) values and free fatty acids (FFA) % compared to control (C) and butylated hydroxy anisole (BHA) treated RMS during storage at 4 ± 1 °C. Addition of GSE significantly ($P < 0.05$) reduced the total psychrophilic and coliform counts in RMS during refrigerated storage. The GSE treated mutton slices recorded significantly ($P < 0.05$) superior scores of color, flavor, juiciness and overall palatability than C and BHA treated RMS. The TBARS values, FFA % and microbial counts increased significantly ($P < 0.05$) during storage. It can be concluded that GSE has excellent antioxidant and antimicrobial properties compared to control and BHA treated RMS during refrigerated storage under aerobic and vacuum conditions.

1. Introduction

Cooked meat is more susceptible to lipid oxidation than raw meat during refrigerated and frozen storage due to the heating process. This susceptibility of precooked and restructured meat products to lipid oxidation has challenged the meat technologist, processor and distributor to come up with techniques to extend the shelf life of these products and much attention has been focused on packaging (both vacuum and modified atmosphere) and addition of antioxidants during processing, cooking or packaging (Kingston, Monahan, Buckley, & Lynch, 1998).

An antioxidant is a substance that delays oxidation by inhibiting initial free radical formation or by preventing them from producing more free radicals which perpetuate the reaction (Fennema, 1996). Several synthetic phenolic antioxidants *i.e.* butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butyl hydroquinone (TBHQ) and propyl gallate and combination of these with citric acid and alpha tocopherol and ascorbyl palmitate were used successfully to control discoloration and prevent/delay lipid oxidation in restructured meat products (Boling, Graham, Marriott, & Kelly, 1990; Chastain, Huffman, Hsieh, & Cordray, 1982). Consumer preferences for "natural" products have resulted in increased interest in the use of natural antioxidants including rosemary, sage, aloe vera, mustard, tea catechins, whey protein concentrate, and cottonseed meals (Rababah, Hettiarachchy, & Horax, 2004; Rhee, Ziprin, & Calhoun, 2001). Several types of natural plant derived antioxidants have been studied in various raw and cooked meat systems, including rosemary extract, sage, thyme, rice bran, white peony, red peony, sappanwood, moutan peony, rehmania or angelica, sedge, marjoram, wild marjoram, caraway, basil extract, ginger, plum concentrates, aloe vera, mustard, and tea catechins (Armitage, Hettiarachchy, & Monsoor, 2002; El-Alim, Lugasi, Hóvári, & Dworschák, 1999; Fiorentino et al., 2008; Han & Rhee, 2004; Nunez de Gonzalez et al., 2008; Tsen, Ameri, & Smith, 2006).

Grape (*Vitis vinifera*) seeds from grape juice and wine processing can be separated, extracted, dried and purified into grape seed extract (GSE) which contains proanthocyanidins and phenolic compounds (Lau & King, 2003). Furthermore, grape seed extracts are industrial derivatives from whole grape seeds that have a high concentration of vitamin E, flavonoids and linoleic acid. From a health perspective, GSE has been shown to be active against HIV by inhibiting virus expression and replication (Nair, Kandaswami, & Mahajan, 2002), anticarcinogenic (Roy et al., 2002) and as a cardio-protective agent (Shafiee, Carbonneau, Urban, Descomps, & Leger, 2003). Clinical data has shown that the antioxidant potential of grape seed is twenty and fifty fold greater than vitamins E and C respectively (Shi, Yu, Pohorly, & Kakuda, 2003), arising from increased levels of polyphenol proanthocyanidins and oligomers of flavan-3-ol units, especially catechin and epicatechin in GSE (Yilmaz & Toledo, 2004).

There is increasing evidence demonstrating the ability of GSE to retard lipid oxidation in meat during storage, likely due to the fact that GSE is a rich source of polyphenolic compounds especially proanthocyanidins (Weber et al., 2007). In raw meat, GSE has been shown to be effective in reducing the amount of primary lipid oxidation products (*i.e.* lipid hydroperoxides and hexanal) and secondary lipid oxidation products (*i.e.* thiobarbituric acid reactive substances

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(Kulkarni, Desantos, Kattamuri, Rossi, & Brewer, 2011) in pork (Carpenter, O'Grady, O'Callaghan, O'Brien, & Kerry, 2007). The antimicrobial properties of GSE against Gram positive and Gram negative bacteria have been reported (Jayaprakasha, Singh, & Sakariah, 2001). Antimicrobial activity of GSE is mainly due to damage to cell membranes which might be due to low-pH exposure that can cause sublethal injury to cell membranes, causing disruption of proton motive force, and loss of H^+-ATP ase (Lin, Labbe, & Shetty, 2004). GSE is generally regarded as safe as a food additive (Ahn, Grun, & Mustapha, 2004) and effectively reduced the numbers of *Escherichia coli* and *Salmonella typhimurium* and retarded growth of *Listeria monocytogenes* in cooked ground beef (Ahn, Grun, & Mustapha, 2007).

The objective of the present investigation was to evaluate the antioxidant and antimicrobial efficacy of GSE compared to synthetic antioxidants in both aerobic and vacuum packed precooked restructured mutton slices under refrigerated storage (4 ± 1 °C).

2. Materials and methods

2.1. Meat and other ingredients

Heavy weight rams (Nellore Brown) above 4 years of age were procured from Livestock Experimental Station, College of Veterinary Science, Rajendranagar, Hyderabad and slaughtered by Halal method in the Department slaughter house at different intervals as per the experimental requirements. The carcasses were deboned manually within 3 h of slaughter and fat was separated. The muscles of shoulder, intercostal muscles, flank region thin muscles, neck and back region muscles and long bone trimmings from each carcass were separated and packed in low density polyethylene (LDPE) bags and chilled at 4 ± 1 °C for about 24 h. Later, the meat was portioned, packed in LDPE bags and transferred to −18 ± 1 °C until further processing. The meat was thawed at 4 ± 1 °C for 16 h before utilization. Refined salt, refined wheat flour (maida), cane sugar, spice mix ingredients, onion and garlic were procured from a local market in Hyderabad city. Fine pastes of onion and garlic in the ratio 3:1 were made in a mixer. Food additives incorporated in the formulations were procured of food grade quality from reputable firms *i.e.* sodium tripoly phosphate (Central Drug House (P) Ltd, New Delhi, India), sodium nitrite and calcium carbonate (E-Merck (India) Ltd, Mumbai, India), sodium ascorbate (Himedia Laboratories Pvt.Ltd, Mumbai), butylated hydroxy anisole (Qualigens Fine Chemcals, Mumbai) and grape seed extract (Dakshina Laboratories, Hyderabad). Chemicals and reagents (AR grade) were procured from commercial companies.

2.2. Preparation of RMS

The processing and preparation of RMS (C) by a hot-set binding system were as follows: thawing of frozen mutton at 4 ± 1 °C for 16 h then mincing the mutton portions using a 25 mm blade, adding curing ingredients (salt @1.5%, sodium tripolyphosphates @ 0.4%, sodium nitrite @ 150 ppm, sodium ascorbate @ 500 ppm, sugar @ 1%) then curing the formulation for about 12 h at 4 ± 1 °C. Continuous vacuum tumbling of sample was then done for about 1 h with ice flakes (6%) and then maida (2.5%), spicemix (0.5%) and onion garlic paste (2%) were added. Then massaging continued until the samples became tacky.

Antioxidants (butylated hydroxyl anisole (BHA) @ 0.01% and grape seed extract (GSE) @ 0.1% were added, based on total weight of the finished product) were dispersed in salt (1.5% of finished product weight) as a carrier. GSE is a light brown colored fine powder containing 95.71% w/w procyanidins on a dry basis with 0.5 to 0.75 g/cc bulk density. The meat batter was filled in round stainless steel moulds and then steam cooked for 45 min (core temperature of the product reached to 72 ± 1 °C). After chilling at 4 ± 1 °C for about 12 h the cooked product was made into slices (5 mm thick) with the help of mechanical slicer (Sirman Mod.No:Auto m, 300 VV, Italy). The slices were packed separately in LDPE film by both aerobic packaging and vacuum packaging (Boxer 42 X, LBA, Henkelman Vacuum Systems, Netherland) and stored at 4 ± 1 °C. At 7 days intervals samples were removed for analysis of oxidative and microbial stability. The analysis was continued for 14 and 28 days in aerobic and vacuum packaged RMS respectively.

2.3. Analytical procedure

2.3.1. pH

The pH of muscle was determined using a Thermo Orion pH meter Model 420A+ (Orion Research, Inc. Beverly, MA, USA) after calibration the pH meter at pH 4, 7 and 9.2.

2.3.2. TBARS value

TBARS value was determined based on the procedure of Witte, Krause, and Bailey (1970). Trichloroacetic acid (TCA) extract of the restructured mutton slices was prepared by homogenizing 4 g of sample with 20 ml of pre cooled 20% TCA solution for 2 min in an ultra turrex homogenizer. The contents were allowed to extract for 10 min and then centrifuged at 3000 *g* (CPR-24, Remi Instruments, Mumbai, India) for 10 min.

Three ml of supernatant was mixed with an equal volume of 0.1% TBA reagent. The mixture was boiled in water bath for 30 min, cooled and the absorbance measured at 532 nm using a UV–VIS spectrophotometer (model: UV-1700 PharmaSpec, SHIMADZU, Japan) and the TBARS values were calculated using a TBA standard (1,1,3,3-Tetra Methoxy Propane standard) and expressed in mg malonaldehyde/kg. For the blank, the same procedure was followed except that 3 ml of 20% chilled TCA solution was added instead of TCA extract.

2.3.3. Free fatty acid %

Free fatty acids percent was determined according to Koniecko (1979). Exactly 5 g of restructured mutton slices was blended for 2 min with 30 ml of chloroform in the presence of about 5 g anhydrous sodium sulfate. Then it was filtered through Whatman no. 1 filter paper into a 150 ml conical flask. Two to three drops of 0.2% phenolphthalein indicator was added to the chloroform extract and titrated against 0.1 N alcoholic potassium hydroxide till a pale pink color was obtained. The quantity of potassium hydroxide consumed during the titration was recorded. Free fatty acid content was calculated and expressed as a percentage as follows.

Free fatty acids (% oleic acid) are as follows: 0.1 × ml of 0.1 N alcoholic KOH × 0.282/weight of sample (g) × 100.

2.4. Microbiological analysis

Bacterial counts were determined by the pour plate method. Meat samples (10 g) were homogenized with 90 ml, 0.1% sterile peptone water. Serial 10-fold dilutions were prepared by diluting 1 ml of homogenate in 9 ml of 0.1% peptone water. Appropriate serial dilutions were duplicate plated with plate count agar for total psychotropic (PPC) and violet red bile agar (VRBA) for coliform counts. Plates were incubated at 37 °C for 48 h for coliforms and 7 °C for 10 days for PPC (ICMSF, 1983). For enumeration of anaerobic counts, about 20 ml of anaerobic agar (M228) melted and maintained at 44–46 °C, was poured gently onto Petri plates. The plates were incubated at 37 ± 1 °C for 48 h. The anaerobic bacterial colonies with a white color were counted and expressed as log cfu/g. For counting of *Lactobacilli* (LAB) bacteria, about 20 ml of MRS agar (M6411) melted and maintained at 44–46 °C (with 1 ml glycerol/100 ml media) was poured gently. The plates were incubated at 37 ± 1 °C for 48 h. The white colored colonies were counted and expressed as log cfu/g. For enumeration of yeast and mold counts, about 20 ml of Potato Dextrose agar (M096) melted and maintained at 44–46 °C was poured gently. The plates were