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Antioxidant and antibacterial properties of *Citrus paradisi* barks extracts during turkey sausage formulation and storage

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ABSTRACT

Antioxidant and antibacterial properties of *Citrus paradisi* fruit barks (CPFB) extract was evaluated in turkey sausage formulation and storage. The CPFB water extract contained a high amount of total phenolics and flavonoids (118 ± 4 mg GAE g^{-1} dried extract and 794 ± 8.7 mg QE g^{-1} dried extract, respectively) and showed important antioxidant and antibacterial activities in all assays used. The capacity of the water extract of CPFB to delay lipid oxidation in sausages was evaluated at the level of 2.5 g kg^{-1} turkey sausage. Water extracts from CPFB showed a higher antioxidant activity than sodium lactate. Microbial growth was monitored regularly during 14-day storage of sausages at 4 °C. The number of microorganisms decreased significantly ($p < 0.05$) when CPFB water extract was added to the sausage. Our results show that the addition of herbal extracts can minimize lipid oxidation and improve microbiological stability during the formulation and storage of turkey sausage.

1. Introduction

Free radical oxidation of food lipid components due to the chain reaction of lipid peroxidation constitutes a major strategic problem for food manufacturers. Due to undesirable influences of oxidized lipids on food quality, it is essential to protect them from these phenomena. Lipid oxidation can be effectively prevented by using synthetic antioxidants such as butyl hydroxyanisole (BHA). However, the use of chemical compounds has begun to be restricted because of their induction of DNA damage and their toxicity (Sasaki et al., 2002). Eliminating chemically synthesized additives from foods is a current worldwide demand.

The use of natural antioxidants has the advantage of being readily accepted by the consumer, considered as safe, and no safety tests are required when the food component is "Generally Recognized As Safe" (Pokorny, 1991). Plant extracts are of growing interest in the food industry because of their antioxidant and antimicrobial properties, which make them useful as natural additives in foods to protect the latter from oxidation and/or to prevent the proliferation of microorganisms (Pattnaik et al., 1997; Tauchen et al., 2015; Stefanović et al., 2015; Martin-Puzon and Rivera, 2015). Furthermore, antioxidants are able to neutralize the chemically active products, such as free radicals that can damage the body by various diseases believed to be associated with oxidative stress. Many researchers have indicated that lipid oxidation in meat products can be controlled or minimized by adding commercial synthetic or natural antioxidants (Dey and Dora, 2010; Kim et al., 2011; Suchandra et al., 2012).

The use of natural antioxidants such as those from rosemary (Sebranek et al., 2005), black pepper (Martínez et al., 2007) as well as tea (Bañón et al., 2007), herbal extract mixture and different oils (Hanczakowska et al., 2015), plant extracts combined with vitamin E (Gobert et al., 2010); fresh leaves of crown daisy (*Chrysanthemum coronariumvar*. spatiosum), and the flower heads of broccoli (*Brassica oleracea* L. var. italica Plenck) (Kim et al., 2013), plant extract (PE) from Lippia (Rossi et al., 2014) can be advantageous because natural products do not require extensive safety testing prior to use. However, natural products are often more expensive and less effective than synthetic ones. As a result, special attention has been focused on the extraction of antioxidants from inexpensive or residual sources from agricultural industries, such as potato peel (Singh and Rajini, 2008), grape seeds (Brannan, 2009)

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and carrot waste (Eim et al., 2008).

To our knowledge, no investigation has addressed the use of *Citrus paradisi* extracts as readily available and inexpensive antioxidants sources for stabilization of meat and meat by products. The aim of this work was to determine the main polyphenolic compounds of various *C. paradisi* extracts and to study their antioxidant and antibacterial effects during the formulation and the storage of turkey sausage.

2. Materials and methods

2.1. Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), thiobarbituric acid (TBA), ethylenediamine tetraacetic acid (EDTA), β-carotene, linoleic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). All solutions were freshly prepared in distilled water.

2.2. Plant material

C. paradisi (grapefruit) is well known as an effective pharmacological remedy and for its nutritional properties in many parts of the world (Owira and Ojewole, 2010). The grapefruits (*C. paradisi*) with uniform coloration of skin, free of cuts, were obtained from a local fruit market. The fruits were washed with distilled water and barks were separated, ground, and then dried at 80 °C for at least 5 h to obtain *C. paradisi* fruit bark (CPFB) powder. The dried preparation was ground further to obtain a fine powder, and then stored in glass bottles at room temperature.

2.3. Preparation of CPFB extracts

The CPFB dried powder (50 g, each) was extracted sequentially (with a maceration method; Hajji et al., 2009) by adding solvents with increasing polarity: ethyl acetate, methanol and water. The powder was first extracted by stirring with 500 ml of ethyl acetate at 30 °C for 24 h. The extract was filtered through Buchner funnel with Whatman No.1 filter paper (Sigma-Aldrich Chemical Co.). The filtrate was evaporated to dryness under reduced pressure using a rotatory vacuum evaporator (EYELA N1000, Tokyo, Japan) at 40 °C. The remaining residues were successively extracted by ethyl acetate, methanol and water under the same conditions. The water extract was freeze-dried. The dried sample of each extract was weighed and the yield of soluble constituents was determined. The dried extracts were kept in dark at −80 °C for further analyses.

2.4. Microbial strains

Antibacterial activities of plant extracts were tested against seven strains of bacteria: *Staphylococcus aureus* (ATCC 25923), *Micrococcus luteus* (ATCC 4698), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 13883), *Bacillus cereus* (ATCC 11778) and *Enterococcus faecalis* (ATCC 29212).

2.5. Sausage preparation

Turkey sausage was formulated using mechanically separated turkey (MST) meat obtained from local processors (Chahia, Sfax, Tunisia). MST meat was produced from turkey after meat cutting. Approximate chemical composition of MST was 65% water, 14% proteins, 20% fat and 1% ash. Food grade NaCl, $NaNO_2$, ascorbic acid and sodium tripolyphosphate (TPP) were used.

MST meat was ground in a commercial food processor (Universo, Rowenta, Germany), equipped with a 14-cm-long blade, for 2 min at the highest speed. The addition of ingredients took less than 5 min and final temperature of batters varied between 10 and 12 °C. The batters were manually stuffed in collagen reconstituted casing (27 mm diameter) and hand-linked to form approximately 8-cm-long links. The sausages were then heat-processed in a temperature-controlled water-bath (Haake L, Haake Buchler Instruments, Karlsruhe, Germany) maintained at 90 °C until a final internal temperature of 74 °C was reached. Temperature was measured using a type-T (copper–constantan) thermocouple inserted into the center of a link, and the time/temperature data were recorded. Then, samples were cooled immediately in an iced-water-bath and stored for 13 days at 4 °C. The antioxidant and antibacterial activities were studied by adding 2.5 g of CPFB water extracts per kg of sausage. A negative control was performed in the absence of natural extract.

2.6. Total phenolics content

The total phenolics content (TPC) of each extract was determined by the Folin–Ciocalteu method (Slinkard and Singleton, 1977). A 0.5 mL aliquot of diluted extract solution was mixed with 0.5 mL of Folin–Ciocalteu's reagent. After 5 min homogenization, 0.5 mL of 20% (w/v) sodium carbonate solution was added and the mixture was shaken once again for 1 min. Finally, the solution was brought up to 5 mL by adding distilled water. The control reaction contained all reagents except the extract. The reaction mixture was then incubated in the dark at 25 °C for 90 min, and the absorbance of the resulting color was measured at 760 nm against a distilled water/sodium carbonate blank. Gallic acid monohydrate was used as standard for the calibration curve. TPC was expressed as mg gallic acid equivalent (GAE) per gram extract. The values presented are the average of three measurements.

2.7. Total flavonoids content

The flavonoid content of CPFB was determined as described by Zhishen et al. (1999). Briefly, 250 μL of each sample were mixed with 1 mL of distilled water and subsequently with 150 μL of 15% (w/v) sodium nitrite solution. After 6 min incubation, 75 μL of a 100 g L^{-1} aluminum chloride solution was added, and the mixture was allowed to stand for an additional 5 min before 1 mL of 4% (w/v) NaOH solution was added. The mixture was immediately made up to 2.5 mL with distilled water and mixed well. The absorbance of the mixture was then measured at 510 nm. The total flavonoid content was expressed as mg quercetin equivalent (QE) g^{-1} dried extract. The values presented are the average of three measurements.

2.8. Determination of DPPH radical-scavenging activity

The antioxidant activity of CPFB extracts was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable DPPH as a reagent according to the method described by Kirby and Schmidt (1997). A volume of 500 μL of diluted extract at different concentrations (25–200 μg mL^{-1}) was added to 375 μL of methanol and 125 μL of DPPH solution (0.2 mM in methanol) as a free radical source. Absorbance measurements were read at 517 nm after 60 min of incubation time at room temperature and in the dark. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control. BHA was used as a positive control. Scavenging activity was measured by monitoring the decrease in absorbance at 517 nm. In its radical form, DPPH has an absorption band at 517 nm which disappears upon reduction by an antiradical compound. Lower absorbance of the reaction mixture indicated higher