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ORIGINAL ARTICLE

Soft cheese supplemented with black cumin oil: Impact on food borne pathogens and quality during storage

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Abstract Black cumin seed oil (BCSO) was tested for its inhibitory effect against some pathogenic bacteria (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Listeria monocytogenes* Scott A and *Salmonella enteritidis* PT4) in Domiati cheese during cold storage. Physical, chemical and sensorial changes in cheese during storage were recorded. Pasteurized milk was inoculated before renneting with a mixed culture of bacteria at *ca.* 4 log CFU mL^{-1}. *In vitro* and *in situ* supplementation with BCSO showed antimicrobial impact on the growth of *S. aureus*, *E. coli*, *L. monocytogenes* and *S. enteritidis* inoculated into media and cheese samples. Supplementing of cheese with BCSO (0.1% or 0.2%, w/w) significantly reduced the counts of the inoculated pathogens by *ca.* 1.3 log and 1.5 log CFU g^{-1} after 21 days of storage. In addition, BCSO controlled the development of titratable acidity, limited the changes in ripening indices, flavor components and kept considerable physicochemical and sensorial properties of cheese.

1. Introduction

Soft cheese is one of the most appreciated cheeses in Middle Eastern countries. The cheese is a pickled cheese (salt 2–15%), although it may be sold fresh. This type of cheese is produced either by enzymatic or acidic coagulation of fresh milk (buffalos' or cows' milk) or reconstituted skim milk powder with oils (Abou-Donia, 1986). It also has been made with or without the addition of starter cultures to cheese milk. Starter cultures govern the flavor and texture of the cheese, and help to suppress the growth of spoilage bacteria.

Spices are being sought for their medicinal value as antioxidants and as antimicrobials (Frankel et al., 1996; El-Ghorab et al., 2010). Black cumin seeds have a strong and hot peppery taste and have been used in coffee, tea, salads and breads.

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Seeds are also used as a natural remedy for asthma, hypertension, diabetes, inflammation, cough, bronchitis and influenza (Ramadan, 2007). Studies were conducted on biological properties of the essential oils and thymoquinone of black cumin on antioxidant activity (Burits and Bucar, 2000; Luther et al., 2007; Lutterodt et al., 2010) and as antimicrobial (Hanafy and Hatern, 1991). Black cumin oil is rich in linoleic and oleic acids as well as sterols and tocols (Ramadan et al., 2003; Ramadan and Moersel, 2002; Ramadan, 2007).

Cold-pressing technology for oil production involves no heat treatment or chemical treatments (solvent extraction). The consumption of new and improved products such as cold-pressed oils may improve human health and may prevent certain diseases (Lutterodt et al., 2010). Common spices and flavors added to cheeses include red and green peppers, black peppercorns, horseradish, thyme, cloves, cumin, caraway, parsley, tarragon, nutmeg, basil, onion/garlic, and sundried tomatoes. Other flavors include liquid and natural smoke, soot/ashes, and nuts like almonds and walnuts. Levels of the additives are typically less than 1% of the curd (Hayaloglu and Farkye, 2011).

Foodborne pathogens are considered as major concerns of food safety (Buzby and Roberts, 1996). The infection risk is high because the infective dose of *Escherichia coli* O157:H7 is low such as 10–20 CFU/g (Bolton et al., 1996). Plant extracts rich in bioactive compounds may serve as natural antimicrobial agents (Luther et al., 2007). *Listeria monocytogenes*, *Salmonella enteritidis* and *E. coli* have the ability to survive and grow up to 16 days in raw and pasteurized milk kept at 4 °C (Mahgoub et al., 2011, 2013). Hence, the aims of this contribution were to: (1) delineate the potential of BCSO as antibacterial and antioxidant agent during manufacture and storage of cheese prepared with starter cultures containing contaminating bacteria as well as artificially inoculated bacterial pathogens (*Staphylococcus aureus* ATCC 6538, *E. coli* ATCC 8739, *L. monocytogenes* Scott A and *S. enteritidis* PT4), and (2) evaluate the physicochemical and sensory attributes of cheese supplemented with BCSO. The obtained results will be utilized to develop novel cheese rich in bioactive molecules with a desirable shelf life.

2. Material and methods

2.1. Materials

BCSO was obtained from local market in Zagazig (Egypt). Standards used for sterol characterization were purchased from Supelco (Bellefonte, PA, USA). Standards used for tocopherols (α-, β-, γ- and δ-tocopherol) were purchased from Merck (Darmstadt, Germany). Bacterial strains of *S. aureus* ATCC 6538 and *E. coli* ATCC 8739 were from Egyptian Culture Collection (MERCIN, Ain Shams University, Cairo, Egypt). *Salmonella enterica subsp. enterica* seriovar Enteritidis PT4 and *L. monocytogenes* ScottA were kindly obtained from Prof. George-John Nychas (Agriculture University of Athens, Greece). Starter cultures including *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Lactobacillus lactis* susbp. *lactis*, were obtained from Chr-Hansen's Laboratories (Copenhagen, Denmark). Raw bovine milk was collected from a private farm located in Sharkiah governorate (Egypt) and handled within 1 h after milking. Powder animal rennet was obtained from Chr-Hansen's Laboratories, (Copenhagen, Denmark). Rennet was diluted with distilled water to a standard rennet solution before use.

2.2. Methods

2.2.1. Analysis of BCSO

Chromatographic analysis of BCSO including gas chromatography analysis of fatty acid methyl esters (FAME) and sterols as well as normal phase high performance liquid chromatography (NP-HPLC) separation, identification and quantification of tocopherols was carried out according to Ramadan et al. (2010).

2.2.2. Phenolic compounds in BCSO

Aliquots of BCSO were dissolved in *n*-hexane (5 mL) and mixed with 10 mL methanol–water (80:20, v/v) in a glass tube for two min in a vortex. After centrifugation at 3000 rpm for 10 min, the hydroalcoholic extracts were separated from the lipid phase using a Pasteur pipet then combined and concentrated *in vacuo* at 30 °C until a syrup consistency was reached. The lipidic residue was re-dissolved in 10 mL methanol–water (80:20, v/v) and the extraction was repeated twice. Hydroalcoholic extracts were re-dissolved in acetonitrile (15 mL) and the mixture was washed three times with *n*-hexane (15 mL each). Purified phenols in acetonitrile were concentrated *in vacuo* at 30 °C then dissolved in methanol for further analysis. Aliquots of phenolic extracts were evaporated to dryness under nitrogen. The residue was re-dissolved in 0.2 mL water and diluted (1:30) Folin–Ciocalteu's phenol reagent (1 mL) was added. After 3 min, 7.5% sodium carbonate (0.8 mL) was added. After 30 min, the absorbance was measured at 765 nm using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). Gallic acid was used for the calibration and the results of triplicate analyses are expressed as parts per million of gallic acid.

2.2.3. Minimal inhibitory concentration (MIC) test

The agar disk diffusion method was conducted for the determination of antimicrobial activities of BCSO against *L. monocytogenes*, *S. enteritidis*, *S. aureus* and *E. coli*. Briefly, 0.1 mL from 8 log CFU/mL bacterial suspension was spread onto Mueller Hinton Agar (MHA) plates. Filter paper disks (6 mm in diameter) impregnated with 10 μL of the undiluted oil were placed on the inoculated plates. The plates, after remaining at room temperature in laminar flow for 2 h, were incubated at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimeters. All tests were performed in triplicate.

2.2.4. Preparation of inoculum

L. monocytogenes, S. enteritidis, S. aureus and *E. coli* strains were maintained frozen in broth until use. Prior to use, the cultures were activated by three successive transfers in tryptic soy broth (Difco Laboratories) at 37 °C for 24 h. Cells were harvested by centrifugation (10000*g* for 10 min at 4 °C), washed three times and re-suspended in Ringer's solution (Lab M, Bury, UK). The resulting pellet was washed once with Ringer's solution (LAB, Merck) to remove residual organic material, re-centrifuged, and then re-suspended in Ringer's to a final volume of 10 mL. A final inoculum was prepared by serially diluting in Ringer's solution to reach a final level of 5 log CFU/mL. Aliquot of 0.1 mL of each pathogen was inoculated into pasteurized milk before manufacturing cheese in the second experiment, so the final count of each becomes *ca.* 4 Log CFU/g in cheese samples.