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Challenge of green tea essential oil in mitigation of *Candida albicans* contamination in minced beef

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ABSTRACT

The meat industry has continuously adjusted its technology to create meat products that are healthier and/or more functional in response to consumer knowledge of the impact of food on health. *Candida albicans* (*C. albicans*) is one of the opportunistic yeasts that cause spoilage of meat and meat products and might cause foodborne diarrhea. Natural essential oils, which have antifungal activities, is one way to find a solution. This study investigated the antifungal activities and sensory contribution of green tea essential oil (*Camellia sinensis*) against *C. albicans* experimentally inoculated to minced meat at infected dose 10^5 cfu/g and then mince divided into five groups, first group not inoculated or treated (control negative), second group not treated (control positive), third, fourth and fifth groups treated with green tea essential oil at concentrations 0.5%, 1% and 1.5%, All samples chilled at (4°C) for 12 days. Candida count and sensory evaluation were detected at zero, 3, 6, 9, 12 days of storage. The obtained results indicated that green tea essential oil at all tested concentrations significantly reduced *C. albicans* counts in the experimentally inoculated beef mince. The used green tea essential oil concentrations could enhance the sensory attributes of the minced meat and extend the shelf life to 12 days compared to the untreated samples which spoiled on the 6th day of cold storage. Therefore, it is highly recommended to use green tea essential oil to extend the shelf life of the minced meat and to reduce *C. albicans* count.

1. INTRODUCTION

The consumption of red meat and meat products has increased due to population growth, higher per capita wealth, and a preference for its high nutritional content (Mohammed and Hamza, 2017). It is distinguished by a significant digestibility factor, which has been estimated to be 94% (Al-Ali et al., 2015). The inherent biological and chemical properties of meat and its products make them very vulnerable to microbial proliferation and fat oxidation during storage, both of which have a significant impact on the meat's quality. Increased microbial activity, particularly fungal contamination of meat and meat products has led to meat spoilage and the emission of several compounds that induce foodborne illness, as well as financial setbacks (Andres et al., 2014; Yolmeh et al., 2014).

Candida albicans *(C. albicans)*, a member of the ascomycete fungi family, has adapted to exist as a commensal organism. Due to its several attributes that render it highly suitable for opportunistic pathogenicity, it primarily impacts those with impaired immune systems. There are many different surfaces on which *C. albicans* can grow. The study conducted by Deorukhkar et al. (2014) suggested that the pathogen has the potential to spread across several ecological niches. Consequently, managing the infection becomes challenging once it has infected the host, as indicated by Whittington et al. (2014). Candida spp. is the predominant yeast species responsible for nosocomial infections and foodborne diarrhea making it a subject of increasing interest in the field of human disease research (Martins et al., 2015). Candidiasis is known to cause gastrointestinal symptoms, particularly in immune-compromised patients. The most common symptoms observed in these patients were belching, bloating, indigestion, nausea, diarrhea, and gas.

The emergence of fungal resistance to synthetic fungicides has led to more stringent regulations and stricter control over the application of chemical substances (Kim et al., 2018; Tetz et al., 2019). Researchers are currently prioritizing the development of new, reliable techniques that are less susceptible to antimicrobial resistance. They are doing this by utilizing biological substances with antimicrobial properties instead of chemical fungicides, bactericides, and pesticides to ensure food safety and preservation. Due to the growing interest in biological food additives, the scientific community has been compelled to create innovative techniques for alternative food preservation. Natural preservatives generated from plants, microorganisms, and algae have been proven to effectively prevent foodborne infections due to their antimicrobial properties (Dubey et al., 2017; Mtibaa et al., 2019).

In response to consumer awareness of the relationship between food and health, the meat industry has consistently adapted its technology to produce meat products that are both healthier and more functional (de Medeiros et al., 2020). A potential substitute for chemical preservatives in food products could involve the utilization of naturally existing compounds that possess antifungal properties (Martins et al., 2014). In addition, the use of natural chemicals in food, such as phenolic acids and flavonoids, enhances its nutritional content, reduces fungal spoilage, and

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prolongs the shelf life of various food items, including meat (Zhang et al., 2016). One method to find a remedy is by utilizing natural essential oils, which possess antibacterial, antifungal, and antioxidant properties (Sośnicka, 2019).
Camellia sinensis, the plant from which green tea is derived, is rich in phenolic compounds that have antibacterial and antioxidant characteristics. This technology's technical application in food has yielded promising results. The technological implementation of this method in the food industry has demonstrated encouraging outcomes (Lorenzo and Munekata, 2016; Aziz and Karboune, 2018). Phytochemical screening of green tea leaves revealed the presence of various polyphenols, particularly catechins, These compounds play significant roles in plant physiology and offer potential health advantages for humans, such as acting as antioxidants, anti-inflammatory agents, anti-cancer agents, antihypertensive agents, and antimicrobial agents (Rahardiyan, 2018).
Thus, the objective of this study was to evaluate the efficacy of green tea essential oil in prolonging the storage duration of minced beef. The study also examined the antifungal properties and sensory impact of green tea essential oil.

2. MATERIAL AND METHODS

This research was approved by the Institutional Animals Care and Use Committee of Faculty of Veterinary Medicine, Benha University (approved number BUFVTM 03-12-23).

2.1. Sampling:
Accurately 3 kg of fresh beef was purchased from a butcher shop in El-Qalubia governorate and was directly minced and transferred without delay to the laboratory in pre-cooled insulated containers with ice packs under sterile conditions.

2.2. Green tea essential oil:
Green tea (*Camellia sinensis*) was purchased at 100% pure concentration from the Unit of Extracting & Pressing Natural Oils, National Research Center, Dokki, Cairo, Egypt.

2.3. Preparation of inoculums:
To achieve a stock culture that was approximately 10^9 according to Krifors et al. (2023), strain of *Candida albicans* ATCC10231 was prepared. In brief, Candida albicans ATCC10231 (were sub-cultured once into Sabouraud's dextrose agar (SAD) and incubated for 24h at 37°C. Five colonies were transferred to sterile distilled water to make inoculum (5 mL). After fungal growth, the cells were collected by centrifuging the conical tubes at 1,200 ×g for 5 min at room temperature. Discard the supernatant and add 10 mL of PBS. Resuspend the cells and centrifuge again at 1,200 ×g for 5 min at room temperature. Repeat the PBS wash and centrifugation twice. After the third wash, resuspend the cells in 5 mL (according to the pellet size) of 2X RPMI-1640 medium. To ensure homogeneity, the suspensions were mixed for 15 seconds then adjusted cell density with a spectrophotometer by adding sufficient sterile saline or sterile water to match the turbidity of a 0.5 McFarland standard (APHA, 1985; Krifors et al., 2023).

2.4. Experimental Study:
Meat samples were assigned into five groups (n = 3/group, each sample weighing 200 g). Group 1 was regarded as a negative control that was not inoculated with *C. albicans*, nor treated with the green tea essential oil. Group 2 was regarded as a positive control that was inoculated with *C. albicans* but not treated with the green tea essential oil. Group 3 was inoculated with *C. albicans* at 10^5 cfu/g and treated with the green tea essential oil 0.5%. Group 4 was inoculated with *C. albicans* at 10^5 cfu/g and treated with the green tea essential oil 1%. Group 5 was inoculated with *C. albicans* at 10^5 cfu/g and treated with the green tea essential oil 1.5%. Meat samples were cold stored at 4°C.

2.5. Preparation of samples:
To confirm the inhibitory effects of the green tea essential oil on *C. albicans* count, twenty- five grams of each examined minced meat were homogenized with 225 ml of 1% peptone water in a stomacher for 1 minute, and serial dilutions were prepared (APHA, 1985).

2.6. Candida albicans count:
One milliliter of selected dilutions was put into petri dishes and Sabauroud's dextrose agar was poured onto them. The plates were then incubated at a temperature of 30°C for 3-5 days. The presence of *Candida albicans* (colony-forming units per gram) was assessed on the zero, 3rd, 6th, 9th, and 12th days of storage at a temperature of 4°C, using the methodology described by Cruickshank et al. (1975). Yeast counts were converted into $\log_{10}$ cfu/g.

2.7. Sensory evaluation:
Staff members in the Animal Health Research Institute (Benha branch) examined the inoculated samples on days 0, 3, 6, 9, and 12 of cold storage. Each member was asked to evaluate levels of odor, color, texture (toughness or juiciness), and appearance. Samples were coded with random numbers and the judges were not informed about the experimental approach, samples of the various treatments were presented in covered small porcelain bowls to each member in a separate area where distractions, noises, and odors were avoided to determine a score indicating the overall acceptance of each sample (color, odor, texture, and appearance). A nine-point descriptive scale (9 = Excellent, 8 = Very very good, 7 = Very good, 6 = Good, 5 = Medium, S = spoiled) for the evaluation of the overall acceptability (Horwitz, 2020).

2.8. Statistical analysis:
Statistical analysis was conducted using analysis of variance (one-way ANOVA) followed by Tukey's Kramer HSD test using SPSS software. (Sabine and Brian, 2014). Individual variances were estimated for each comparison, and the significance level was set at $p < 0.05$. The mean values were reported with their corresponding standard deviations (SD).

3. RESULTS

Table (1) showed the impact of green tea essential oil on *C albicans* count in minced beef. Results stated that the control groups exhibited a high count of *C. albicans* of 6.85 ± 0.02 $\log_{10}$ cfu/g which increased to 7.74 ± 0.02 on the 3rd and the 6th days. The yeast counts ($\log_{10}$ cfu/g) were significantly reduced by green tea essential oil 0.5% to 5.86 ± 0.02, 3.54 ± 0.02, and 1.89 ± 0.02 on the zero, 3rd and 6th day of storage respectively. Whereas yeast counts were recorded to non-detectable levels on the days 9th and 12th. Samples treated with green tea essential oil 1% recorded a decreasing pattern in the count of yeast to 5.71 ± 0.01, 2.22 ± 0.01, 0.62 ± 0.01 on zero, 3rd, and the 6th day, respectively. Green tea essential oil 1.5% was the most effective in decreasing the count of *C. albicans* as the count recorded 5.59 ± 0.01, 1.18 ± 0.01, 0.58 ± 0.01 on the zero, 3rd, and the 6th day, respectively with complete inhibition of *C. albicans* growth by the 9th and 12th

days after treatment with the green tea essential oil as declared in Table (1).

Sensory evaluation and overall acceptance:

The overall acceptability results of the minced beef samples kept at 4 °C showed that on the 6th day of cold storage, the control samples were spoiled. Interestingly, ~~by~~ the addition of the green tea essential oil maintained the whole acceptability of sensory attributes and extended the shelf-life in comparison to the untreated samples during the entire cold storage period (12 days) (Table, 2). Samples treated with green tea essential oil 1.5% had firmer textures and brighter colors in comparison to the concentrations 0.5% and 1%.

Table 1 Candida albicans count (Log_{10} cfu/g) in the experimental meat samples during the storage period at 4°C

Samples	0	3rd	6th	9th	12th
Control positive	6.85±0.02 [b]	7.74 ± 0.02 [a]	7.74 ± 0.02 [a]	6.86 ± 0.02 [b]	5.63± 0.02 [d]
Green tea Eo. 0.5%	5.86±0.02 [c]	3.54 ± 0.02 [e]	1.89 ± 0.02 [f]	-	-
Green tea Eo. 1.0%	5.71±0.01 [c]	2.22 ± 0.01 [d]	0.62 ± 0.01 [e]	-	-
Green tea Eo. 1.5%	5.59 ±0.01 [c]	1.18 ± 0.01 [d]	0.58 ± 0.01 [e]	-	-

Significant differences were observed between values with different letters in the same row (P <0.05).

Table 2 Sensory analysis of the minced beef samples both treated and untreated, kept in cold storage at 4ºC.

Samples	0	3rd	6th	9th	12th
Control negative	9	6	5	S	S
Control positive	9	6	5	S	S
Green tea Eo. 0.5%	9	7.5	6.5	5.5	5
Green tea Eo. 1%	9	8	7	6	5.5
Green tea Eo. 1.5%	9	8.5	7.5	6.5	6

*Score system: 9 = Excellent. 8 = Very very good. 7 = Very good. 6 = Good. 5 = Medium. S = spoiled.

4. DISCUSSION

Recently, essential oils have gained ~~a~~ significant interest as possible natural substitutes for conventional synthetic fungicides (Elshafie et al., 2015). Green tea extracts containing high concentrations of epicatechin can serve as bio-preservative agents for meat and animal products (Demir, 2021). Catechins, found in *Camellia sinensis*, are the main components responsible for its antimicrobial capabilities (Renzetti et al., 2020). During the 12-days storage period at a temperature of 4 °C, the count of *C. albicans* was noticeably decreased in all of the green tea essential oil-treated meat samples in the experimental groups. A higher concentration of green tea essential oil (Eo) at 1.5% was found to be more effective in lowering the count, followed by green tea Eo at 1%, and then a concentration of 0.5%. This aligns with the findings of Monte et al. (2020), who conducted an *in vitro* study on the plant extract derived from *Camellia sinensis* (green tea). They discovered that the extract had antifungal properties against all strains of Candida, both in individual cells and in biofilm form. Also, strains of *C. albicans* that were treated with the extract had a lower count than the control group that wasn't treated. The polyphenolic EGCG found in green tea has a wide range of antimicrobial effects because it forms cross-links with different proteins, which could potentially harm the lipids and proteins in the microbial cytoplasm. Studies have shown that *Camellia sinensis* has significant levels of catechins, which possess antimicrobial characteristics and can effectively combat various illnesses (Rahardiyan, 2018).

Table (2) demonstrated that the sensory characteristics of the treated minced beef samples were enhanced by the application of green tea Eo during cold storage (4 °C) and improved when compared to the control samples. The use of green tea essential oil resulted in improved sensory characteristics, including color, odor, and texture, across three different concentrations (0.5%, 1%, and 1.5%). Notably, samples treated with a concentration of 1.5% green tea Eo exhibited a greater enhancement compared to samples treated with concentrations of 1% and 0.5%. Likely, Mosavinezhad et al. (2020) discovered that the application of green tea to chicken and duck meat has a preservative effect. They saw that samples that were treated with 0.5% *Camellia sinensis* had better physicochemical properties, fewer signs of oxidative rancidity, microbial breakdown, and changes in how the samples felt. These findings indicate that applying green tea extract to these meats can serve as an effective preservative. Besides, according to a study conducted by Suradi et al. (2019), a 5% concentration of green tea showed excellent preservation properties against microbial deterioration in duck meat. The study specifically examined green tea's antibacterial activity in preserving duck meat's physicochemical characteristics.

5. CONCLUSIONS

Based on our findings, it can be inferred that green tea Eo is efficacious in reducing the count of *C. albicans* in minced beef cold stored at a temperature of 4 °C. The results indicated the inclusion of green tea essential oil (Eo.) preserved the overall sensory acceptability of the samples in comparison to the untreated samples and extended the shelf life of the chilled minced meat to the 12th day.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest**.**

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