Green Lemon Essential Oil Antimicrobial Activity Against *Listeria monocytogenes* Inoculated in Chicken Meat

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Abstract
The provision of safe and healthy food is one of the industry's most significant obstacles. As a result, this cannot be accomplished without a variety of processes and chemicals. The food industry uses a variety of synthetic preservatives to delay spoilage, and the development of pathogenic microorganisms in order to increase the safety and shelf life of products. Contrarily, the preference of consumers to consume food products with natural additives encouraged the food industry to produce preservatives with a natural basis. On the growth of *Listeria monocytogenes* CCM 4699 on chicken breast meat, the impacts of vacuum packaging, storage temperature, and green lemon essential oil (GLEO) were investigated. Slices of raw breast chicken were vacuum-packed with green lemon essential oil, and then inoculated with a strain of *Listeria monocytogenes* CCM 4699. Following treatment, the slices were stored for 5 days while *L. monocytogenes* growth and microbial shelf life were monitored. Enterobacteriaceae family, total viable counts (TVC), and microbial populations of inoculated *Listeria monocytogenes* in poultry meat were also observed during storage's first 0 days, 1 day, 3 days, and 5 days. Green oil or no green oil vacuum packaging greatly reduced microbial populations. The combination of vacuum-packaging and green oil was also found to be highly successful in preventing the growth of Enterobacteriaceae in ground chicken breast meat. With green lemon essential oil added to chicken breast meat, vacuum packaging's antimicrobial effects can be strengthened against some food pathogens.

Keywords: green lemon essential oil, *Listeria monocytogenes*, antimicrobial activity, chicken meat

1. Introduction

It is projected that in 2023, poultry meat production, including chicken, will be more than 130.7 million tons. This would make the poultry industry the largest sector in meat production [1]. The main issues facing the chicken industry are the limited shelf life of chicken meat and the danger of pathogen contamination. Unsaturated fatty acid oxidation is to responsible for the low shelf life of chicken meat.

In particular, *Listeria monocytogenes* contamination is common in slaughterhouses [2], and is associated with the transmission of infection in humans after the consumption of infected meat [3]. The content of *L. monocytogenes* in chicken meat is relatively high [4].

One of the oldest, most popular plant species is the genus *Citrus* (Rutaceae). Its cultivation has been recorded far as back as 2100 before Christ [5]. Citrus fruits are grown all over the world because of their numerous health advantages. Citrus fruits are consumed fresh as desserts, and are also used to make jam and juice. Vitamins, particularly vitamin C, are rich in them [6]. Interestingly, the most important by-product of citrus processing is essential oil (EO) [7].

Due to their extensive spectrum of biological activities, which include antibacterial and antioxidant properties, citrus EOs are used as food preservatives. Terpenes, flavonoids, carotenes, and coumarins are suggested to be responsible for the

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plant's strong antibacterial and antioxidant properties. Citrus essential oils are also utilized in air fresheners, household cleansers, fragrances, cosmetics, and medications due to their pleasant, reviving aroma and flavor [8, 9].

The goal of the research was to determine the effects of vacuum packaging, storage temperature, and green lemon essential oil application to the growth of *Listeria monocytogenes* CCM 4699 on chicken breast meat.

2. Materials and methods

2.1 Essential oil

Green lemon essential oil (GLEO) was purchased from Hanus s.r.o. (Nitra, Slovakia). EO was produced by cold-pressing fresh pericarp of green lemons, and the major components were limonene 67.3 %, β-pinene 12.3 %, γ-terpinene 5.8 %, and citral 2.8 %.

2.2 Bacterial strain

Listeria monocytogenes CCM 4699 culture was obtained from the Czech Collection of Microorganisms (CCM, Brno, Czech Republic). *L. monocytogenes* was infused into Muller Hinton broth, and kept there for 24 hours at 35 °C. After incubation, 1 mL of bacteria culture (approximately 10^6 CFU/mL) was added to ground chicken breast samples and blended for 1 min.

2.3 Chicken breast meat samples

The chicken's breast meat without skin was purchased from a legitimate retailer in Slovakia. The chicken meat samples were delivered to the microbiological laboratory in a clean refrigerator under hygienic conditions, where they were kept at 4 °C until the analysis was completed. Within 30 minutes, samples were moved from the authorized shop to the lab. Using a vacuum packer (Concept, Choce, Czech Republic), diced chicken meat samples measuring 5 g were treated with 1.0 % solutions of green lemon essential oil (GLEO) dissolved in rapeseed oil. All samples were inoculated with bacteria *L. monocytogenes*.

The samples were prepared as follows:

1. Meat samples were packed in polyethylene bags under aerobic condition and kept at 4 °C in the control aerobically packaged group (CG);
2. Meat samples were packed in polyethylene bags under aerobic condition and kept at 4 °C in the control aerobically packaged group with (CGGLEO);
2. Vacuum-packaged control group: Fresh chicken breast samples were placed in polyethylene bags and kept at 4 °C in anaerobic storage (VPCG);
4.1 % essential oil of GLEO vacuum-packaged: Meat samples were packaged, and vacuum sealed in polyethylene bags, then kept in anaerobic conditions at 4 °C after being soaked in a rapeseed oil solution containing 1 % GLEO (VPGLEO);

2.4 Microbial analyses

On days 0., 1., 3., and 5. of holding at 4 °C, microbiological tests were conducted. With 45 mL of 0.1 % sterile saline solution, five grams of materials were diluted. For 30 minutes, the materials were homogenized in a shaker (GFL 3031, Burgwedel, Germany).

For each sample, appropriate series decimal dilutions in 0.1 % saline solution were made. Plate count agar (PCA, Oxoid, UK) was covered with 1 mL of successive dilutions for the purpose of counting total viable organisms (TVC). After being incubated for two days at 30 °C, they were tallied. Coliform bacteria were identified using Violet Red Bile Lactose Agar (VRBL; Oxoid, Basingstoke, UK) that were cultured at 37 °C for 24 to 48 hours. With 1 mL of the material, an Oxford agar was inoculated with the Oxford supplement. The incubation took place for 24 hours at 37 °C.

3. Results and discussion

One of the most crucial issues in the food industry is food safety. Consumers, food producers, and governmental agencies are expressing worries about pathogenic microbes that cause foodborne diseases [10, 11]. Consequently, the food industry strives to create nutritious and secure food [12, 13]. As a result, research efforts have always included a portion devoted to advancing knowledge about the creation of safe foods and the creation of novel techniques used to increase their safety [14–18].

The presence of *Listeria* spp. in meat and meat products is a serious problem in the meat industry due to the ability of this organism to grow in both raw and cooked meat during refrigerated storage, and among the food products, contaminated meat products are known to be one of the main sources for *L. monocytogenes* infections [19–21].

The number of microorganism showed Tables 1, 2, and 3. On day zero, the total viable count (TVC) was 4.85±0.4 log CFU/g. The highest number of the total viable count was found in 5. day in control group packaging aerobically.

On day zero, coliform bacteria was zero. The number of coliform bacteria growth by the day of storage. Number of *L. monocytogenes* in zero day was 5.96 log CFU/g.

Table 1. Total viable count number in log CFU/g in different packaging systems with/without the presence of 1 % GLEO stored in refrigeration condition

Day of storage	CG	CGGLEO	VP	VPGLEO
0.	4.85±0.4	4.85±0.5	4.85±0.7	4.85±0.5
1.	5.50±0.6	5.30±0.3	5.70±0.4	5.00±0.4
3.	6.50±0.4	6.10±0.3	6.00±0.3	5.40±0.5
5.	7.50±0.5	7.00±0.5	6.40±0.5	5.60±0.6

CG-control group samples stored in air, CGGLEO-control group samples with GLEO, VP-vacuum packaging samples, VPGLEO-vacuum packaging samples with GLEO

Table 2. The number of coliform bacteria in log CFU/g in different packaging systems with/without the presence of 1 % GLEO stored in refrigeration condition

Day of storage	CG	VPCG	VP	VPGLEO
0.	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
1.	1.53±0.3	1.42±0.4	1.54±0.3	1.25±0.3
3.	1.76±0.2	1.67±0.4	1.68±0.2	1.48±0.4
5.	2.50±0.5	1.96±0.5	1.74±0.7	1.68±0.3

CG-control group samples stored in air, CGGLEO-control group samples with GLEO, VP-vacuum packaging samples, VPGLEO-vacuum packaging samples with GLEO

Table 3. The number of *Listeria monocytogenes* in log CFU/g in different packaging systems with/without the presence of 1 % GLEO stored in refrigeration condition

Day of storage	CG	CGLEO	VP	VPGLEO
0.	5.96±0.5	5.96±0.5	5.96±0.5	5.96±0.5
1.	6.18±0.3	6.09±0.4	6.21±0.3	6.32±0.3
3.	6.36±0.2	6.25±0.2	6.30±0.4	6.65±0.4
5.	6.22±0.3	6.18±0.3	6.56±0.1	6.36±0.3

CG-control group samples stored in air, CGGLEO-control group samples with GLEO, VP-vacuum packaging samples, VPGLEO-vacuum packaging samples with GLEO

Numerous studies have demonstrated the effectiveness of essential oils derived from clove and cinnamon in inactivating *L. monocytogenes* to lengthen the shelf life of meat, which is consistent with the results of the current research [22–24].

In order to increase the safety of ground chicken products, it would be beneficial to use green limon EOs to control *L. monocytogenes* in chicken breast flesh. De Oliveira et al. [25] found comparable experimental findings showing that cinnamon EOs had lower inhibitory activity in chilled meat.

This research looked at the combined effects of vacuum packaging, modified atmosphere (MAP) (20 % CO_2/80 % N_2), and bay essential oil (0.5 % v/w) on ground chicken breast meat stored at 4° C. *Listeria monocytogenes* AUFE 39237 was inoculated into poultry meat, and *Escherichia coli* and total viable counts (TVC) were also observed during storages first 0 days, 1. days, 3. days, and 5. day. With or without bay oil, MAP packaging substantially (p 0.01) increased the reductions of microbial populations. The combo of vacuum packaging and bay oil was also discovered to be highly efficient (p 0.01) against *E. coli* in ground chicken meats. By adding bay essential oil to poultry meats, MAP and vacuum packaging can more effectively prevent the growth of some food pathogens [26].

4. Conclusions

Application of green lemon essential oil (1 %) inactivated *Listeria monocytogenes* in chicken breast meat within by day of exposure, regardless of storage temperatures. Hence, the use of green lemon EO can provide an adequate degree of protection against foodborne pathogens in chicken breast meat.

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