RESEARCH ARTICLE

Clostridium perfringens in Meat and Meat Products: A minireview on the Incidence, Public Health Significance, and the Effects of Essential Oils

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Abstract

Anaerobic spore formers, particularly Clostridium perfringens, are an important cause of food poisoning outbreaks worldwide, which is mainly related to the consumption of contaminated meat and its products. C. perfringens is a commensal inhabitant in the intestinal tract of animals and humans. C. perfringens is a ubiquitous Gram positive, anaerobic spore former which produces many toxins and is related to various diseases in humans such as food poisoning, necrotic enteritis, diarrhea, enterotoxemia and gangrene. The occurrence of multidrug resistant C. perfringens in meat and its products raises public health concerns. The ability of C. perfringens to grow as a biofilm favors its survival in the environment. Therefore, the bacteriological quality and safety of commercially processed meat products is important for the consumers and public health worldwide. The prevention of growth of C. perfringens is best achieved by following the food service practices by addition of some natural essential oils such as Thyme (Thymus vulgaris) and Marjoram (Origanum majorana) essential oil.

Keywords: C. perfringens, Essential oils, Food poisoning, Meat products.

Introduction

Clostridium is a Gram positive, anaerobic, spore-forming, and rod-shaped pathogen which frequently exists in humans and animals’ guts and present in the contaminated environment by feces [1]. Clostridium perfringens is classified into five toxin types from A to E according to the four major lethal toxins produced (alpha, beta, epsilon, and iota [2-4]. Recently, two other types were reported (F and G) based on the detection of C. perfringens enterotoxin (CPE) and necrotic enteritis B-like toxin (NetB) [5]. In spite of the pathogenicity of all C. perfringens strains for animals, only types A and C strains are dangerous to humans and type A is the most form isolated from poultry meat [6]. C. perfringens type A is incriminated in numerous human diseases such as food poisoning and gastrointestinal illness, including food poisoning and non-foodborne diarrhea, gas gangrene (Clostridial myonecrosis), and enterocolitis [3, 7]. In addition, C. perfringens is a fast-growing bacterium that harbored more than 20 toxins related to intestinal illness in humans and animals [8]. The symptoms of C. perfringens include watery diarrhea and severe abdominal cramps; these symptoms appear within six to twenty-four hours after consumption of foods containing C. perfringens [9]. C. perfringens produces several toxins; the principal toxins are enterotoxin (CPE) and b2-toxin (CPB2). The CPE is encoded by the cpe gene which causes food poisoning [10], while, alpha toxin is a necrotizing toxin that is present in all C. perfringens strains; this toxin can cause severe acute pulmonary diseases, as well as hemolysis, vascular leak, liver damage and thrombocytopenia [11].

To minimize the economic losses caused by these bacterial infections, several antimicrobials have been used preventively in the livestock industry in different countries [12]. Therefore, antimicrobial resistance of C. perfringens to these antibiotics has significantly increased along the last three years [13]. Biofilm is structured communities of the bacterial cell that provides an increased resistance to
environmental stresses; it protects *C. perfringens* from the contact with the atmospheric oxygen and the high antibiotics concentrations [14]. Biofilm formation is a defensive reaction against the effect of antibiotics because minimal concentrations of antibiotics could act as stress signals. The biofilm matrix is the main actor that can act as the primary physical barrier influencing antibiotics penetration [15].

There is an urgent need to control food poisoning outbreaks associated with meat and meat products. There are many compounds derived from spices have antibacterial activities against wide ranges of bacteria including Clostridium species. Essential oils are volatile oily liquids which are obtained from plant ingredients, such as seeds, fruits and leaves [16]. The action of these substances is mostly on the cytoplasmic membrane of the cell and the presence of a hydroxyl group is associated with the enzyme’s deactivation. This group leads to loss of the cell component, changes in phospholipids and fatty acids, in addition to it prevents the synthesis of genetic material and energy metabolism [17].

Therefore, in this review, we explore the features of *C. perfringens* food poisoning public health impact including contamination of meat and meat products by this important pathogen, virulence factors, antimicrobial resistance profiles and biofilm formation. Finally, we highlight on the antibacterial effect of some essential oils on *C. perfringens*.

**Classification of Clostridium**

Genus *Clostridium* belongs to family Clostridiaceae that contains 152 species in Nomenclature. The most species of the genus Clostridium are saprophytic bacteria that are found in many environmental places [18, 19]. Clostridium species are non-motile, anaerobic, and spore forming encapsulated bacteria. The vegetative cells are pleomorphic occur in short chains or pairs and rod shaped [2]. Clostridium genus was initially identified and isolated as a new pathogen by William H in 1891[20].

**Meat and meat products as potential sources of C. perfringens**

Several studies reported that more than 90% of *C. perfringens* outbreaks were related to poultry and meat products [21-23]. Beef and poultry meat and their products are the main reservoirs for *C. perfringens* foodborne disease, despite its presence on herbs, spices, and in processed and raw feed [24]. There is a relation between meat contamination and poor personal hygiene; food handlers may be the source of meat contamination either carriers of pathogen or through poor hygienic practices, therefore, improper handling, poor personal hygiene, and storage of food in addition to poor knowledge of food handlers about the public health significance of foodborne pathogens are the main risk factors of meat contamination [25]. Foodborne diseases including foodborne infection and intoxication are the situations that are generally transmitted via ingested food. In case of intoxication food poisoning the bacterium multiply in food and form a toxin from the bacterium cell leading to food poisoning syndromes when the toxin is ingested with food [26]. *C. perfringens* food poisoning isolates have the capability to procedure spores which are extremely resistant to several stress factors including high pressure, high temperature, radiation, and toxic chemicals [27]. These high resistance properties enable the survival of *C. perfringens* spores in food vehicles, as they germinate and return to vegetative cells [28]. When *C. perfringens* spores present in meat and meat products, they are not destroyed by cooking temperature and can survive at 71°C for two hours [29]. *C. perfringens* food poisoning infection can arise through eating of improperly cooked and stored foods [24] as food is generally cooked in a large container and placed in the cooler. Rapid cooling is impossible because of the container’s size. Hence, the germination of the spores may occur and grow to large numbers that can survive high heat and can cause food poisoning faster than other bacteria [19, 30]. *C. perfringens* is associated with food service as it needs a free air environment, strict nutrient supply, a storage time, and high temperatures. These situations are frequently seen in the improperly stored meat [24].

**Public health impact of C. perfringens infection**

From the period of 1983 to 1997 in the United States, *C. perfringens* was the third confirmed outbreak causing foodborne illness [31]. The Center of Disease Control (CDC)
reported a total of 965,958 acquired cases annually in the U.S., second to Salmonella implicated with foodborne illness [24]. Fifty-one outbreaks were annually reported from 2001 to 2005 in the U.S., however, 34 outbreaks included 1,880 cases were reported in 2006 at U.S., and in one outbreak from 50 to 100 people are affected [32, 33]. Each year in the United States, ninety-four million cases of foodborne disease were reported, and 10% (1 million) of these cases result from C. perfringens poisoning [34]. In Ghana at the Eastern region, a suspected C. perfringens food poisoning outbreak occurred and the older and young people were the victims of C. perfringens poisoning and the immunocompromised people are at a higher hazard of the disease severity than the others [19, 35]. Various studies reported that between 20% and 30% of healthy persons and their families in the hospital carry this pathogen in their feces, and the losses after two weeks may be ranged from 50% to 88% [2, 36]

C. perfringens is categorized into seven forms, from A to G, according to the four toxins production (alpha, beta, epsilon, iota, CPE, and NetB). The most producing C. perfringens Enterotoxin (CPE) are belonging to type A [37] which is produced by the spores of C. perfringens in the small intestine. After ingestion, it causes food poisoning because the presence of large number of vegetative cells can withstand the acidic pH of the stomach, then sporulation and enterotoxin production occur in the small intestine [38]. The main signs of the illness are diarrhea, abdominal pain and nausea, in addition to wound infection and human gas gangrene may be induced by some lecithinase positive strains [39]. In the healthy individuals, the disease symptoms are generally minor and self-limiting, with symptoms clearance within 24 hours [32, 37]. Frey and Vileie [40] illustrated that C. perfringens has been incrimented in several anaerobic infections via the production of toxins which have the ability to damage the nervous system tissues in addition to inflammation and destruction of the large and small intestinal wall, resulting in necrotizing enteritis. C. perfringens type A is the main cause of human foodborne gastroenteritis, such as food poisoning, sporadic diarrhea, antibiotic-associated diarrhea, and nosocomial diarrhea outbreaks [41]. Deaths from C. perfringens food poisoning type A are occasional to occur but can happen in debilitated patients and the old people. In Egypt, the most common circulating C. perfringens strain in humans is type A [42]. C. perfringens was isolated from diarrheic and non-diarrheic human stool samples at a rate of 37.5% (15/40) and 15% (3/20), respectively. Two human enteritis strains of C. perfringens type A were positive for cpe and another two strains were cpb2 positive [42].

Virulence factors in C. perfringens

The virulence of C. perfringens strains is associated with the production of various toxins. The alpha-toxin is encoded by cpa gene and it is produced by all C. perfringens strains, it has the ability to hydrolyze the phospholipids of cell membrane, causing necrosis of the cells; the main gas gangrene characteristic [8]. The α-toxin can play three main roles in gas gangrene pathology; initially, with unknown mechanism it can affect the transfer of neutrophils, therefore reducing the clearance of the pathogen at the infected sites. Secondly, this toxin can lead to blood vessels constriction and reduces the tissues blood supply, creating micro-aerophilic environment that helps the overgrowth of C. perfringens. Finally, it stimulates the inflammation metabolism in the host cell, which may cause direct immune-mediated pathology of tissues [43]. Other main lethal toxins include beta (β), epsilon (ε) and iota (ι) and some strains (0 to 5 %) have the ability to produce enterotoxin (CPE) which is the principle common cause of C. perfringens food poisoning type A [44-46]. The CPE is mediated by the cpe gene and it has been reported that the probable food poisoning pathogenesis mechanisms may be induced from pore-formation or CPE-induced tight junction rearrangements [8]. Therefore, C. perfringens pathogenicity is mainly due to its capability to form various virulence factors such as hydrolytic enzymes and well-characterized pathogenic toxins [45].

Antibiotic resistance in C. perfringens

Emergence of resistant C. perfringens strains to diverse antimicrobial agents has become a public health alarm worldwide. Constant and improper uses of antibiotics in human and veterinary medicine, particularly in developing countries, can result in antibiotic resistance transfer among different bacteria; consequently,
it is vital to screen the susceptibilities of the identified pathogenic bacteria to the antimicrobial agents [47]. Large numbers of bacteria become resistant to antibiotics, while some are multidrug resistant (Figure 1) [48].

**Pumpkin seed oil**

There is an overstate concern of the distinctive formulation of vegetable oils; the oil of pumpkin seeds is a promising ambition in this regard. Oil can be extracted by either cold press or steam distillation yielding a dark greenish-colored pumpkin seed oil that can be used in cooking and salad dressing [16]. Moreover, oil can be consumed in cereal bars, chocolates, cakes, bread, muffins, soups, pasta, pesto and a garnish of stew [17], furthermore, the butter of pumpkin seeds are employed as an adorable alternative to peanut butter [18].

Pumpkin seed oil possesses a comparatively modest composition of fatty acids, predominantly the essential fatty acids: linoleic, stearic, oleic and palmitic acids [12], those four fatty acids estimate almost (98 ± 0.13%) of the total amount of fatty acids [19], as presented in Table (3). Besides, phytoestrogens and phytosterols such as β-sitosterol, secoisol ariciresinol, genistein, daidzein are included [13]. Pumpkin seed oil also includes elevated amounts of numerous non-triacylglycerol constituents such as tocopherols that play a vital role in suppression of free radical formation in biological systems [9]. Tocopherols are highly affected by the processing methods utilized in oil extraction from the pumpkin seeds [20]. Moreover, pumpkin seed oil contains specific amounts of polyphenols; Andjelkovic et al. [21] evaluated the total concentration of polyphenols in pumpkin seed oil with ≈25-51 mg/kg as gallic acid equivalents. The HPLC analysis of individual phenolic ingredients valued the polyphenolics of pumpkin seed oil including vanillic acid, tyrosol, o-coumaric acid, caffeic acid [22] and trans-cinnamic acid [23]. Conventionally, the pumpkin seed oil is attained by pressing either roasted pumpkin seeds or the unroasted pumpkin seeds to gain the cold-pressed pumpkin seed oil, where the difference in the two oils is in the sensory appearances such as taste, color and odor [24]. Research on vegetable oils got from roasted seeds disclose that pumpkin roasted seed oil are enriched with mono- and poly-unsaturated fatty acids, minerals, vitamins, pigments, phytosterols, phenolic compounds, and pyrazine derivatives [25]. For all that, the pumpkin seed oil is considered as desirable edible oil as well as it attracts interest as potential nutraceutical oil.

It was found that pumpkin seed oil attains numerous critical medicinal impacts, including suppressing growth and decrease size of the prostate, delay of the hypertension progression, alleviation severity of arthritis and hypercholesterolemia and diabetes, suppression of urethral and bladder pressure, and depressing the levels of breast, gastric, colorectal and lung cancer cells [20].

![Figure 1: How antibiotic resistance happens](image-url)
The development of resistance might be attributed to the misuse of antibiotics for sub-therapeutic and therapeutic treatments of humans and food animals. In addition, the resistance of bacteria to antibiotics can be transferred from a strain to another one through the transfer of resistance genes [49]. *C. perfringens* high resistance rates may be as a result of the antimicrobials widespread use and these resistant strains can act as a reservoir of resistance genes that can be transferred among bacteria in various environments [50]. The antibiogram of the isolated *C. perfringens* from buffalo meat samples in Bareilly city, India was tested against 16 antibiotics by disc diffusion method. The results revealed that *C. perfringens* isolates displayed resistance to gentamicin (57.1%), ampicillin (60%), cephalothin (65.7%), colistin sulfate (88.5%), ceftazidime (100%) and streptomycin (100%). The strains were 100% sensitive to ciprofloxacin, ofloxacin and nitrofurantoin, but sulfatriad (68.5%) and tetracycline (71.4%) revealed moderate sensitivity [47]. In the same time, Tansuphasiri *et al.* [51] studied the antibiotic resistance pattern of 89 *C. perfringens* isolates based on the minimal inhibitory concentrations by the agar dilution method and the results declared that *C. perfringens* was resistant (56.2%) to tetracycline, 24.9% to imipenem, 9.5% to metronidazole, 9% to penicillin G, 4.5% to vancomycin, 3% to chloramphenicol and 1% to ceftriaxone. Additionally, Mohammed [52] evaluated the antimicrobial susceptibility of 30 *C. perfringens* isolates by disc diffusion technique and the results revealed that all the isolates were highly susceptible to both ampicillin and furazolidone (100%), penicillin (90%) and cloxacillin (80%). However, the susceptibilities for bacitracin, erythromycin and lincomycin were 73%, 70% and 50%, respectively. All of the isolates were resistant to both kanamycin and gentamycin (100%, each).

All *C. perfringens* strains revealed sensitivity to enrofloxacin, tylosin, erythromycin, bacitracin and florfenicol, however, 24 (61%) and 26 (66%) of the 39 tested isolates revealed resistance to lincomycin and tetracycline, respectively [53]. There was a higher resistance of *C. perfringens* to neomycin sulfate (87.5%), lincomycin and tetracycline (80%, each), while, all the isolates were sensitive to chloramphenicol reported by Shojaadoost and Peighambari [54]. Meanwhile, Mwangi *et al.* [55] investigated the antimicrobial susceptibility of *C. perfringens* isolates by the disk diffusion method. The results revealed that *C. perfringens* had resistance to multiple antibiotics. The resistance rates were 98%, 73%, 67%, 65% and 53% to streptomycin, gentamicin, erythromycin, bacitracin, and tetracycline. While over 80% of *C. perfringens* isolates were susceptible to norfloxacin, amoxicillin, cefepime and ampicillin.

**Biofilm formation ability of C. perfringens**

Biofilm matrix acts as a protective barrier within which the bacterial cells become resistant to antibiotics because a concentration gradient is performed through the biofilm matrix reducing the actual antibiotics concentration reaching the pathogen [56]. It has been linked with persistent tissue infections and it is associated with chronic diseases and acute bacterial infections [57] and could protect the bacterial cells from cellular immune responses performed from antibiotics and by toxin production [58]. Low doses of antibacterial agents induce bacterial biofilm formation [59].

Biofilm is composed of a thick multi-component biofilm matrix; this matrix is able to protect the bacterial cell by saving the environment around it. Matrices of biofilm consist of an extracellular polymeric material, which is formed from polysaccharide, DNA and proteins. Flagellae play a significant role in the formation of biofilm by motile bacteria as they may be included in the maturation of Clostridial biofilms where flagellae play an important role in the architecture of mature biofilms [60]. The process of biofilm formation involves different stages including attachment, proliferation, maturation,
dispersion and planktonic cell formation (Figure 2) [61].

Figure 2: The biofilm formation Process [61].

Bacterial Planktonic forms are attached reversibly to the solid rock and produce a quorum sensing molecule which permits planktonic cells to irreversibly aggregate. Endogenously the sessile bacteria perform a medium round the cells, which maintain a position for multiplication and the cell division. A few numbers of the bacterial cells undergo inactive due to the stress presence and become non-replicating bacterial cells. The cells join in the mature biofilm. When the stress is released, the inactive bacteria are converted to active and they are found in planktonic forms to begin the subsequent cycle of biofilm formation (Figure 2) [61].

The characteristic features of cells in biofilms include: aggregation in suspension or on solid surfaces, protection from phagocytic and immune cells, resistance to environmental and physical stresses and increased antibiotic resistance [62]. Biofilm formation by C. perfringens can help the bacterial cell to adhere to the surfaces facilitating colonization and infection as well as biofilm could protect from oxygen stress [14].

Resistance of Clostridial biofilms to many antibiotics and different environmental stresses could be attributed to the thick biofilm matrix as well as the bacterial cells physiological state within biofilms [15, 63, 64]. The development of antibiotics resistance in biofilm can rise from 10 to 1,000-fold more as compared by the planktonic bacteria [65]. Römling and Balsalobre [66] reported that recurrent bacterial infections have been related to the ability of bacterial cells to form biofilms.

Antibacterial effect of oils on C. perfringens:

Essential oils (EOs) have been used for medicinal purposes as food preservatives, and for aroma and flavor since thousands of years. Several EOs have antimicrobial effectiveness versus a wide variety of bacteria, such as C. perfringens [67]. Numerous researchers have studied the effect of naturally occurring antimicrobial agents and their inhibition and inactivation properties on disease-causing microorganisms and spoilage. Several studies determined a strong bactericidal effect of EOs and some of them have an inhibitory against spore forming bacteria in food; therefore, there is a favorable potential of using these natural antimicrobial agents as food preservatives [68].

The mechanism of action of EOs due to the presence of phenolic components, includes disrupting the proton motive force, the cytoplasmic membrane, active transport, flow of electron, and coagulation of cell contents. All these actions are important for coagulation of the cytoplasmic inner cellular components and destroying the lipid bonds among the protein layers [66]. Also, EOs have the ability to increase the cell membrane permeability causing outflow of the necessary intracellular components, in addition to the impairment of cellular respiration and bacterial enzyme system [69]. The EOs antimicrobial activities are due to the chemical characteristics
including their hydrophobicity that allows them to react with bacterial cell membrane lipids, therefore the bacterial metabolism is affected and the permeability of cell membrane and wall is increased, resulting in sever escape of serious ions and molecules from bacterial cells [70]. In addition, the phenolic groups present in EOs compounds have the ability to change the function and structure of the bacterial cell membrane [71]. Many EOs revealed the antimicrobial activities against a broad range of bacteria, including C. perfringens [67]. Several studies prove that application of little concentrations of some essential oils may produce bores on the sensitive bacterial cell wall including C. perfringens, causing lysing of the vegetative forms [72].

Thyme (Thymus vulgaris), an aromatic plant of the Labiatae family, is widely used in foods field for different purposes. The phenols thymol in a concentration ranged from 44 to 60% and carvacrol in a concentration of 2.2-4.2% constitute the most active and major constituents besides the γ-terpinene (16.1-18.9%) and monoterpenic hydrocarbons ρ-cymene (18.5-23.5%) in thyme EO composition [17]. Thymol and carvacrol only exhibited adequate bactericidal and bacteriostatic ability. These compounds are responsible for the main phenolic compounds, which are responsible for the high antioxidant property in addition to the antimicrobial activity of thyme [73]. It has antibacterial actions against Gram-positive and Gram-negative bacteria [74]. The antibacterial effect of thyme is due to perturbation of the bacterial plasma membranes lipid fractions. This can affect the membrane permeability and induce leakage of the intracellular materials. Also, it causes cell membrane enlargement, which results in ions passive diffusion among the expanded phospholipids [75, 76].

Marjoram (Origanum majorana) essential oil belongs to the family Lamiaceae that possesses a broad inhibitory spectrum against a variety of Gram-positive, Gram-negative, bacteria and yeasts [77,78]. Chemical analysis of marjoram oil revealed the presence of 21 components demonstrate 96.36%. The major components were β-caryophyllene (2.09%), linalyl acetate (2.53%), α-terpinolene (2.90%), p-cymene (3.25%), limonene (3.38%), α-terpineol (3.61%) sabinene (5.47%), sabinene hydrate (5.95%), α-terpinene (7.03%), γ-terpinene (12.38%), cis-sabinene hydrate (14.95%) and trans terpinen-4-ol (26.10%). The authors concluded that beef burger products contained marjoram oil (2.5% and 5% concentration) show little psychrotrophic and APC counts during the storage period, and these results revealed that marjoram has a significant antimicrobial property [79].

Marjoram EO has a greatest potential for uses in the industrial applications [80]. It is commercially used as a spice, as well as traditionally used for the treatment of asthma, indigestion, headache, rheumatism, dizziness, migraine and gastrointestinal disorders [81]. Marjoram EO is a natural product, classified as generally recognized as safe (GRAS) and it has antibacterial, antifungal, antiviral, insecticidal and antioxidant activities [82].

The antimicrobial mode of action of marjoram EO is due to its hydrophobic potential to introduce into the cell membrane of bacterial cells [83]. In addition, the application of marjoram EO dipping treatments possessed potential in the color enhancement, stability of lipid and microbial status during refrigerated storage as well as preservation of minced meat samples for longer periods without the need to use hazardous chemical preservatives [84]. Radaelli et al. [85] investigated the antibacterial effect of thyme and marjoram EO against C. perfringens. The results revealed that the minimal inhibitory and minimal bactericidal concentrations of thyme and marjoram essential oils against C. perfringens were 1.25 and 5 mg ml⁻¹, respectively. They concluded that marjoram and thyme oils showed bactericidal activity at their minimum inhibitory concentration on C. perfringens and using of essential oils might help as an alternative to the use of chemical preservatives for the inactivation and control of pathogens in food industry. Another study by Nuno et al. [86] evaluated the antibacterial effect of marjoram EO on C. perfringens. The results revealed that the mean diameter of the inhibition zone was 31 mm. Also, the value of MIC by the disc-diffusion method was 0.78%. The inhibitory effect of carvacrol, thymol, and oregano oils EOs against germination and
growth of *C. perfringens* spores has been evaluated after contaminating cooked ground meats with *C. perfringens* spores before cooling in a process from the Department of Agriculture Food Safety and Inspection Service in U.S. stabilization guidelines for cooling meat products [87,88]. Carvacrol and thymol (1 to 2%, each) and oregano oil (2%) effectively restricted the growth of *C. perfringens* throughout 12 to 21 h of cooling from 54.4 to 7.2°C, with an increase of less than 1 log CFU/g in the bacterial count in cooked minced beef [87]. Another investigation by Juneja and Friedman, showed that higher concentrations of 2%, wt/wt of carvacrol, thymol, and oregano oils were required to completely control the growth of *C. perfringens* spores following over 15 h of cooling in cooked ground turkey [88]. This obviously shows the inhibitory effects of these EOs on *C. perfringens* in meat.

**Conclusion**

*C. perfringens* is the largest toxin producer microbiota of humans and animals and in the soil among Clostridium species. *C. perfringens* is one of the most reported agents for food poisoning occurrences and has the most alarm in public health all over the world. The severity of *C. perfringens* illness depends on the toxin consumed, amount of contaminated food, age and health conditions. *C. perfringens* food poisoning take place through consumption of under cooked and improperly stored foods. Therefore, foods should be properly cooked and stored with proper sanitation by food handlers. In addition of the necessity of using thyme oil and marjoram oil to improve the wellbeing condition of meat and their products. Health procedures from slaughter to consumption must be followed.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

**References**


carvacrol, cinnamaldehyde, thymol, or oregano oil during chilling. J Food Prot. 69 (7):1546–1551.


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