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THE EFFECT OF LIQUID SMOKE ON FOODBORNE PATHOGENS IN READY-TO-EAT MEAT PRODUCTS

Krste Popovski, Mirko Prodanov[2], Marija Ratkova Manovska[2], Dean Jankuloski[2]*

[1] Lecker ltd, Tetovo, R.N. Macedonia
[2] Ss. "Cyril and Methodius" University in Skopje, Faculty of Veterinary Medicine – Skopje, Skopje, R.N. Macedonia

Abstract

Liquid smoke is increasingly used in ready-to-eat meat products as a natural preservative with antimicrobial and antioxidant properties. This study investigated the efficacy of a commercial liquid smoke preparation (Cloud S9, Kerry, USA) against *Listeria monocytogenes* ATCC 13932, *Escherichia coli* ATCC 25922, and *Salmonella Enteritidis* ATCC 13076 in chicken and beef ham. Meat products were inoculated with each bacterial strain and treated with liquid smoke at concentrations of 1%, 2.5%, and 5%, and untreated samples were used as controls. Bacterial counts were assessed after 2 hours, 7 days, and 14 days of refrigerated storage (4 °C). Liquid smoke significantly inhibited bacterial growth in a concentration-dependent manner. For *Listeria monocytogenes*, 5% liquid smoke achieved > 2 log cfu/g reductions after 14 days, while lower concentrations suppressed growth without elimination. *Escherichia coli* and *Salmonella Enteritidis* exhibited comparatively lower sensitivity, with most treatments producing bacteriostatic rather than bactericidal effects; however, 5% liquid smoke achieved a measurable reduction of *Escherichia coli* in chicken ham. These findings confirm the antimicrobial potential of liquid smoke as a complementary hurdle to enhance the microbial safety of ready-to-eat meat products, while also underscoring the ongoing necessity for stringent hygiene practices.

Key words: Liquid smoke, smoked meat, *Listeria monocytogenes, Escherichia coli, Salmonella* Enteritidis

* Corresponding Author: djankuloski@fvm.ukim.edu.mk

UTICAJ TEČNOG DIMA NA PATOGENE U PROIZVODIMA OD MESA SPREMNIM ZA KONZUMIRANJE

Krste Popovski, Mirko Prodanov[2], Marija Ratkova Manovska[2], Dean Jankuloski[21]

[1] Lecker ltd, Tetovo, Republika Severna Makedonija
[2] Ss. "Kirilo i Metodije" Univerzitet u Skoplju, Fakultet veterinarske medicine – Skoplje, Skoplje, Republika Severna Makedonija

Kratak sadržaj

Tečni dim se sve više koristi u proizvodima od mesa spremnim za konzumiranje kao prirodni konzervans koji ima antimikrobna i antioksidativna svojstva. Predmet ovog rada je efikasnost komercijalnog tečnog dima (Cloud S9, Kerry, USA) protiv bakterija *Listeria monocytogenes* ATCC 13932, *Escherichia coli* ATCC 25922, i *Salmonella* Enteritidis ATCC 13076 u pilećoj i goveđoj grubousitnjenom barenom kobasici. Proizvodima od mesa su dodati bakterijski sojevi i tretirani su tečnim dimom u koncentracijama od 1%, 2,5% i 5%, dok su netretirani uzorci korišćeni kao kontrolna grupa. Broj bakterija je beležen nakon 2 sata, 7 dana i 14 dana čuvanja u frižideru (4 °C). Tečni dim je značajno inhibirao rast bakterija u zavisnosti od koncentracije. Kada je u pitanju *Listeria monocytogenes*, tečni dim u koncentraciji od 5% doveo je smanjenja broja bakterija za više od 2 log cfu/g nakon 14 dana, dok su niže koncentracije inhibirale rast, ali bez potpune eliminacije. *Escherichia coli* i *Salmonella* Enteritidis pokazali su relativno manju osetljivost, pri čemu je većina tretmana imala bakteriostatski, a ne baktericidni efekat. Međutim, tečni dim u koncentraciji od 5% doveo je do znatnog smanjenja broja *Escherichia coli* u pilećoj grubousitnjenoj barenoj kobasici. Ovi rezultati potvrđuju antimikrobni potencijal tečnog dima kao komplementarnog faktora za poboljšanje mikrobiološke bezbednostiproizvoda od mesa spremnih za konzumiranje, ali istovremeno ukazuje na to da je neophodno strogo sprovođenje higijenskih mera.

Ključne reči: Tečni dm, suhomesnati proizvodi, *Listeria monocytogenes, Escherichia coli, Salmonella* Enteritidis

INTRODUCTION

Traditional smoking has long served as an effective food-preservation method, valued not only for imparting characteristic flavor, color, and aroma but also for providing important antioxidant and antimicrobial benefits (Suñen et al., 2001; Lingbeck et al., 2014; Maga, 2018). Modern food processing has increasingly adopted liquid smoke, an all-natural condensate of wood smoke components, which offers practical advantages over traditional methods, such as ease and consistency of application, precise concentration control, reduced processing time, and the significant removal of carcinogenic polycyclic aromatic hydrocarbons (PAHs) (Sofos et al., 1988; Guillen et al., 2000; Šimko, 2005; Lingbeck *et al.*, 2014). This transition is particularly relevant given the increasing consumer demand for "all-natural" and "clean label" products, free from synthetic additives, aligning liquid smoke with a natural extract appeal (Morey et al., 2012; Lingbeck *et al.*, 2014).

The multifaceted preservative effects of liquid smoke are primarily attributed to its complex composition, notably phenolic compounds, carbonyls, and organic acids, which are generated during the pyrolysis of wood (Morey et al., 2012; Lingbeck et al., 2014). These compounds exert their antimicrobial activity by damaging microbial cell walls and membranes, inactivating essential enzymes, inhibiting cellular metabolism, and lowering intracellular pH, thereby inducing bacteriostatic or bactericidal effects (Morey et al., 2012; Dien et al., 2022). Numerous studies have demonstrated liquid smoke's efficacy against various foodborne pathogens, including *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus*, as well as spoilage microorganisms like *Aeromonas hydrophila* and various molds (Suñen et al., 2001; Van Loo et al., 2012; Morey et al., 2012; Lingbeck et al., 2014; Soares et al., 2016; Deliephan et al., 2023). Beyond direct pathogen inhibition, its potent antioxidant properties effectively retard lipid oxidation, playing a critical role in extending the shelf life of food products (Milly et al., 2005; Soares et al., 2016). For example, liquid smoke has been shown to suppress *L. monocytogenes* growth in frankfurters for up to 130 days and significantly reduce pathogen and histamine levels in tuna loin sashimi (Lingbeck et al., 2014; Dien et al., 2022). The effectiveness of liquid smoke is influenced by its concentration, the specific chemical composition of its components, and the pH of the food matrix (Van Loo et al., 2012; Morey et al., 2012; Lingbeck et al., 2014). Antimicrobial effectiveness can also be aaffected by the composition of the food matrix (Hao et al., 1998). Currently in literature most of the studies on the antimicrobial effect of liquid smoke are focused on listeriocidical effects on salmon and salmon products (Messina et al., 1988; Suñen,

1998; Suñen et al., 2001; Van Loo et al., 2012). There are limited studies of ready-to-eat (RTE) meat sausages, and other foodborne pathogens like *Salmonella* and *Escherichia coli* (Lingbeck et al., 2014).

The aim of this study was to determine the effectiveness of topically applied commercial liquid smoke preparation (Zesty Smoke Cloud S9, Kerry, USA) in inhibiting the growth of major foodborne pathogens (*Listeria monocytogenes* ATCC 13932, *Escherichia coli* ATCC 25922, and *Salmonella* Enteritidis ATCC 13076) in RTE meat products (chicken and beef hams).

MATERIAL AND METHODS

RTE meat products

Two types of RTE meat products were used in this study: Chicken Ham - Coarsely Ground Sausage (CH) and Beef Ham - Coarsely Ground Sausage (BH). The CH formulation consisted of the following ingredients: chicken breasts min. 50%, water, salt, soy isolate, celery spice, carrageenan (E407), disodium diphosphate (E450), sodium tripolyphosphate (E451), disodium inosinate (E316), potassium nitrate (E252), sodium nitrate (E250), monosodium glutamate (E621). The BH consisted of the following ingredients: beef meat category I min. 50%, chicken meat 5%, water, starch, soya protein, salt, spice, E450, E451, E407, E252, E250, E316, E621. Both products were prepared from coarsely chopped meat, marinated, and stuffed into permeable cellulose casings. They were then steam-cooked at 80 °C until the internal temperature reached 74 °C. The composition of RTE meat products is shown in Table 1.

Table 1. Composition of RTE meat products

	Chicken Ham (CH) (per 100 g)	**Beef Ham (BH) (per 100 g)**
Total protein	14 g	14 g
Meat protein	12 g	12 g
Collagen	0.6 g	0.6 g
Carbohydrates	1.6 g	2.65 g
sugars	0.25 g	0.1 g
Total fat	0.8 g	1.5 g
saturated	0.24 g	0.6 g
Salt	2.9 g	2.9 g
Water	up to 80 g	up to 80 g

The sausages were sliced into 100 g portions, individually vacuum-packed, stored and transported to the laboratory at the temperature of 4 °C. For the purposes of this study, a total of 122 CH and 122 BH packages were used.

Bacterial strains

One strain of *Listeria monocytogenes* ATCC 13932, *Escherichia coli* ATCC 25922, and *Salmonella* Enteritidis ATCC 13076 were used in this study. All strains were obtained commercially (Microbiologics, USA).

The pellets were resuscitated according to the manufacturer's instructions. Each strain was inoculated in Brain Heart Infusion (BHI, Oxoid Ltd, UK) and incubated at 37 °C for 24 hours. After incubation each strain was stroked on a plate of Nutrient Agar (NA, Oxoid Ltd, UK) in a way to produce individual colonies and incubated at 37 °C for 24 hours. After incubation, individual colonies were suspended in maximum recovery diluent (MRD, Oxoid Ltd., UK) and adjusted to a turbidity up to 0.5 McF. Serial dilutions were made for every strain in order to obtain the required number of bacteria present in the suspensions. Through serial dilutions the number of every strain in the suspension was determined. 6.84 log cfu/mL for *L. monocytogenes*, 6.80 log cfu/mL for *E. coli* and 6.09 log cfu/mL for *S*. Enteritidis.

This suspension was used to contaminate the meat products.

Liquid smoke

Commercially available liquid smoke Zesty smoke Cloud S9 (Kerry, USA was used for this study. The specification of the product is that it has neutral taste and color, pH 5.5. liquid intended for spaying and dosing on meat products. The smoke was stored at room temperature until use. It was sprayed on the meat products using a handheld spray bottle. One spray shot contained 1.5 mL smoke suspension. Three different concentrations were used for this study: 1%, 2.5% and 5% concentration of the liquid smoke, diluted with sterile deionized water.

Bacteriological examination

Detection of *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* in RTE meat samples without contamination and without liquid smoke applicatio*n* was carried out according to ISO 6579-1, ISO 11290-1, and ISO 16649-2 respectively.

ISO 11290-2 was used for the enumeration of *Listeria monocytogenes*, ISO 16649-2 for the enumeration of *E. coli* and for the enumeration of *Salmonella* spp. The same protocol used for Listeria monocytogenes was followed, with the exception of the agar employed for inoculation. According to the above-mentioned standards, the initial inoculation for all the samples was made in BPW (Biolife italiana S.r.l., Italy) and then from the initial suspension decade serial dilution was made in Maximum recovery diluent MRD (Biolife italiana S.r.l., Italy). From each of the serial dilutions, 1 ml was transferred on three plates selective agar and spread out with hockey stick. For Salmonella Enteritidis, XLD agar (Biolife Italiana S.r.l., Italy) was used; for Listeria monocytogenes, Ottaviani–Agosti agar (Biolife Italiana S.r.l., Italy) was employed, while XLD agar (Biolife Italiana S.r.l., Italy) was used for *E. coli.*

Experimental design

All manipulations were carried out in a BLU Space Class II Bio-safety Cabinet (PBI Internacional, Italy).

Before the contamination with *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* Enteritidis, as well as the application of liquid smoke, five samples of each type of ham were tested for the absence of *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* Enteritidis.

The vacuumed packages were cut with sterile scalpers and 3 mL liquid smoke was sprayed in each package. After that, 1 mL bacterial suspension was added. The packages were then vacuumed, re-sealed and stored at 4 °C until the analysis.

For each meat preparation / bacterial strain combination, 3 different concentrations of liquid smoke (1%, 2.5% and 5%) were applied. For every meat preparation / bacterial strain combination nine samples were made- three samples for each time interval of testing.

For positive control, only the bacterial strains were applied to each product, whereas for the negative control, three different concentrations of liquid smoke were tested on the meat products without the addition of any bacterial strain. The controls were also done in triplicate for each testing interval.

Bacterial counts were done in three intervals, at two hours, seven days and fourteen days after the meat product inoculation. Three samples from each combination of meat preparation/pathogen/Liquid smoke concentration were tested. In addition, three samples of each positive and negative controls were tested.

Enumeration of *L. monocytogenes* was performed according to ISO 11290-

2:2017, *E. coli* according to ISO 16649-2:2021, and for *Salmonella* spp., the same protocol as for *Listeria* was applied, differing only in the inoculation agar used. The meat product was diluted 1:10 in Buffered peptone water, with successive decimal dilutions in MRD. After that, 1 mL was transferred from each decimal dilution into three 90 diameter Petri dishes with selective agar. Ottaviani–Agosti agar (Biolife Italiana S.r.l., Italy) was employed for *L. monocytogenes*, Xylose Lysine Deoxycholate (XLD) agar (Biolife Italiana S.r.l., Italy) for *S. Enteritidis*, and Tryptone-Bile-X-Glucuronide (TBX) agar (Biolife Italiana S.r.l., Italy) for *Escherichia coli*. *L. monocytogenes* and *S.* Enteritidis agars were incubated at 37 °C for 24 hours and then counted, while E. coli was incubated at 44 °C for 24 hours and then counted. The results were expressed as log cfu/g, and from the three tests of each combination the mean value was calculated.

Statistical analysis

The experiments were conducted in triplicate and data was analyzed using SPSS Statistics (IBM, USA). Following ANOVA, significant differences in the treatments were determined with Tukey's LSD test at $p \leq 0.05$.

RESULTS AND DISCUSSION

For the purposes of this study a total of 244 samples of RTE sausages were tested. 122 of them were CH, and same number were BH. No bacterial growth was observed in any of the negative controls.

Table 2. Chicken ham – *L. monocytogenes* (CH-LM)

LM (log cfu/g)											
Without liquid smoke			**1%**			**2.5%**			**5%**		
2 hours	7 days	14 days	2 hours	7 days	14 days	2 hours	7 days	14 days	2 hours	7 days	14 days
4.72 ± 0.3	5.11 ± 0.26	6.94 ± 0.31	4.65 ± 0.3	4.96 ± 0.25	4.88 ± 0.3	4.59 ± 0.16	4.72 ± 0.26	3.61 ± 0.37	4.04 ± 0.19	4.48 ± 0.16	2.32 ± 0.28

Listeria monocytogenes inoculated in the CH which were not infused with liquid smoke entered log phase and increased from 4.72 ± 0.3 to 5.11 ± 0.26 log cfu/g in 7 days, to 6.94 ± 0.31 log cfu/g in 14 days. In CH treatment with 1% liq-

uid smoke resulted in modest suppression, with day 14 counts of 4.88 ± 0.3 log cfu/g, while 2.5% liquid smoke showed greater efficacy, reducing counts to 3.61 ± 0.37 log cfu/g by day 14. The 5% liquid smoke treatment produced the greatest reduction, lowering the Listeria count to 2.32 ± 0.28 log cfu/g (Table 2.).

Table 3. Beef ham – *L. monocytogenes* (BH-LM)

LM (log cfu/g)											
Without liquid smoke			**1%**			**2.5%**			**5%**		
2 hours	7 days	14 days	2 hours	7 days	14 days	2 hours	7 days	14 days	2 hours	7 days	14 days
4.62 ± 0.3	4.95 ± 0.31	6.86 ± 0.35	4.99 ± 0.26	5.35 ± 0.31	5.51 ± 0.3	4.93 ± 0.29	5.03 ± 0.29	4.99 ± 0.3	4.00 ± 0.26	4.48 ± 0.26	2.51 ± 0.3

Similarly to the CH, BH inoculated with *L. monocytogenes* entered log phase and the number increased from 4.62 ± 0.3 to 4.95 ± 0.31 log cfu/g in 7 days, to 6.86 ± 0.35 log cfu/g in 14 days, while the BH treated with 1% liquid smoke showed modest suppression of 5.51 ± 0.3 log cfu/g in 14 days. The 2.5% smoke treated BH had 4.99 ± 0.3 log cfu/g by day 14 showing greater efficacy, and the highest efficacy was observed in 5% smoke treated BH with 2.51 ± 0.3 log cfu/g (Table 3.).

The ability of *L. monocytogenes* to adapt and grow rapidly under psychrotrophic conditions is well documented (Chan and Wiedmann, 2009). Some authors (Buchanan and Klawitter, 1991) observed that at 5 °C L. monocytogenes can easily adapt and exhibit exponential growth rates. The growth rate of *L. monocytogenes* was suppressed ($p < 0.05\%$) with the addition of the liquid smoke over the period of 14 days. All infused concentrations had this effect. The 2.5 % liquid smoke infusion showed decrease in the cfu/g in CH, but for BH the results were similar as with the 1% infusion in 14 days. Both types of products that were infused with 5% liquid smoke exhibited bactericidal effect on *L. monocytogenes* over the 14 days.

The findings of this study are consistent with those reported by (Morey et al., 2012), who investigated the incorporation of liquid smoke into meat products. Liquid smoke has been widely applied in RTE products as a strategy to mitigate the risk of *L. monocytogenes*. Its antimicrobial activity is primarily attributed to compounds such as phenols, carbonyls, and organic acids, which are typically condensed in water or other carriers and subsequently applied to

products, such as frankfurters, by spraying or dipping (Vitt et al., 2001; Holley and Patel, 2005). The efficacy of commercial liquid smoke formulations against *L. monocytogenes* has been well documented. For example, (Murphy et al., 2005) found that treatment of frankfurters with Select 23P reduced inoculated *L. monocytogenes* populations by 3.2 log cfu/cm² within 4 h, and to below 1 log cfu/cm² after 12 days of storage at 4 °C. This effect was largely attributed to the low pH (2.4) and the presence of phenolic constituents in the condensate. Similarly, (Gedela et al., 2007) reported that topical application of the commercial preparation Zesti-B reduced *L. monocytogenes* on frankfurters and fully cooked turkey chubs by approximately 2 log cfu/g ($p < 0.05$) within one week.

Table 4. Chicken ham – *Salmonella* Enteritidis (CH-SE)

SE (log cfu/g)											
Without liquid smoke			**1%**			**2.5%**			**5%**		
2 hours	7 days	14 days	2 hours	7 days	14 days	2 hours	7 days	14 days	2 hours	7 days	14 days
4.90 ± 0.14	5.40 ± 0.28	6.30 ± 0.14	4.81 ± 0.45	4.24 ± 0.46	4.88 ± 0.10	4.74 ± 0.38	4.15 ± 0.49	3.76 ± 0.31	3.90 ± 0.33	4.06 ± 0.11	3.49 ± 0.27

The CH samples inoculated with *S.* Enteritidis not treated with liquid smoke entered log phase and increased from 4.90 ± 0.14 log cfu/g on day 0 to 5.40 ± 0.28 log cfu/g on day 7 and 6.30 ± 0.14 log cfu/g on day 14. Significant reduction in bacterial count was observed in CH treated with 2.5% and 5% liquid smoke from 4.74 ± 0.38 log cfu/g and 3.9 ± 0.33 log cfu/g on day 0 to 3.76 ± 0.31 log cfu/g and 3.49 ± 0.27 log cfu/g on day 14, respectively (Table 4.).

Table 5. Beef ham – *Salmonella* Enteritidis (BH-SE)

SE (log cfu/g)											
Without liquid smoke			**1%**			**2.5%**			**5%**		
2 hours	7 days	14 days	2 hours	7 days	14 days	2 hours	7 days	14 days	2 hours	7 days	14 days
3.96 ± 0.35	4.76 ± 0.29	6.69 ± 0.07	5.09 ± 0.10	4.68 ± 0.61	4.26 ± 0.32	4.79 ± 0.41	4.24 ± 0.02	3.79 ± 0.39	3.91 ± 0.36	4.00 ± 0.92	2.78 ± 0.09

Similarly to CH, BH inoculated SE without smoke entered log phase and from 3.96 ± 0.35 log cfu/g on day 0 the bacterial count increased to 4.76 ± 0.29 log cfu/g on day 7 and 6.69 ± 0.07 log cfu/g on day 14. BH treated with 1% and 2.5% liquid smoke showed significant reduction in bacterial count from 4.09 ± 0.10 log cfu/g and 4.79 ± 0.41 log cfu/g on day 0 to 4.26 ± 0.32 log cfu/g and 3.79 ± 0.39 log cfu/g on day 14, respectively. Higher suppression was observed for BH treated with 5% liquid smoke with counts of 3.91 ± 0.36 log cfu/g on day 0 to 2.78 ± 0.09 log cfu/g on day 14 (Table 5.).

In the absence of liquid smoke infusion, meat products inoculated with S. Enteritidis showed a notable increase in bacterial growth over the 14-day storage period. These results indicate active bacterial proliferation and entry into the logarithmic growth phase in untreated products, highlighting the risk associated with unmitigated *Salmonella* contamination in RTE meat products during storage.

Conversely, the inclusion of liquid smoke significantly suppressed the growth of *S. Enteritidis* ($p < 0.05$). In CH infused with 1% liquid smoke, bacterial counts increased only slightly over the 14-day period, suggesting a strong bacteriostatic effect. In BH, the same concentration resulted in a moderate reduction. These findings show that liquid smoke, even at low levels, is capable of inhibiting Salmonella proliferation, particularly in beef.

A study by Soares et al. (2016) mentions that liquid smoke had an inhibitory effect on *S. Choleraesuis* with a minimal bactericidal concentration ranging from 7.5 to 15%. This is line with our study where we predominantly observed only bacteriostatic effect of the smoke on *Salmonella*. Another study screened MICs of eight commercial liquid smoke samples against *S.* Enteritidis and found ranges from 0.5 to 8.0% (Van Loo et al., 2012) The same study concluded that the effect of liquid smoke in *Salmo*nella and *E. coli* is similar.

However, it is important to note that while reductions were evident, complete elimination of pathogens was not achieved, reinforcing the notion that liquid smoke may function primarily as a bacteriostatic agent at lower concentrations, rather than exhibiting full bactericidal activity.

Table 6. Chicken ham – *E. coli* (CH-EC)

EC (log cfu/g)											
Without liquid smoke			**1%**			**2.5%**			**5%**		
2 hours	7 days	14 days	2 hours	7 days	14 days	2 hours	7 days	14 days	2 hours	7 days	14 days
4.60 ± 0.33	5.37 ± 0.35	6.70 ± 0.3	4.32 ± 0.3	4.40 ± 0.3	4.46 ± 0.35	4.26 ± 0.28	4.30 ± 0.3	4.28 ± 0.26	3.96 ± 0.25	4.18 ± 0.26	2.72 ± 0.3

E. coli inoculated CH samples which were not treated with liquid smoke entered log phase and increased from 4.60 ± 0.33 log cfu/g on day 0 to 5.37 ± 0.35 log cfu/g on day 7 and 6.70 ± 0.30 log cfu/g on day 14. In CH samples, treatment with 1% and 2.5% liquid smoke produced only modest suppression, yielding day-14 counts of 4.46 ± 0.35 log CFU/g and 4.28 ± 0.26 log CFU/g, respectively. Significant suppression was observed with 5% liquid smoke treated CH with count of 2.72 ± 0.30 log cfu/g (Table 6.).

Table 7. Beef ham – *E. coli* (BH-EC)

EC (log cfu/g)											
Without liquid smoke			**1%**			**2.5%**			**5%**		
2 hours	7 days	14 days	2 hours	7 days	14 days	2 hours	7 days	14 days	2 hours	7 days	14 days
4.30 ± 0.42	4.57 ± 0.04	6.58 ± 0.56	4.28 ± 0.36	4.81 ± 0.18	4.86 ± 0.18	4.20 ± 0.38	4.70 ± 0.72	4.71 ± 0.29	3.93 ± 0.26	4.68 ± 0.30	4.36 ± 0.26

Similarly to CH, modest suppression was observed on BH treated with 1% and 2.5 % liquid smoke with counts of 4.86 ± 0.34 log cfu/g and 4.71 ± 0.29 log cfu/g in 14 days, respectively. Greater suppression was observed in BH treated with 5% liquid smoke with counts of 4.36 ± 0.26 log cfu/g (Table 7).

The results of this study clearly demonstrate that the application of liquid smoke has a significant inhibitory effect on the growth of *E. coli* in both CH and BH. In the control samples (without liquid smoke infusion), there was a rapid and consistent increase in *E. coli* counts over the 14-day period. These results confirm that under favourable conditions, *E. coli* can multiply rapidly

in meat products, posing a serious food safety concern. In contrast, the addition of liquid smoke, even at low concentrations (1%), significantly suppressed the growth of *E. coli*. One percent infusion reduced the growth rate in CH to a near-stagnant level for 14 days, while the BH saw a slower but more pronounced increase. Higher concentrations of liquid smoke (2.5% and 5%) had an even stronger inhibitory effect, especially in chicken meat products. Notably, the 5% concentration not only suppressed growth but reduced the *E. coli* count from 3.96 to 2.72 log CFU/g, suggesting potential bactericidal activity of the smoke components.

These findings are consistent with previous studies that have shown smoke extracts to be effective in controlling microbial growth in food products. Estrada-Muñoz et al., (1998) demonstrated similar suppression in beef products, while Dien et al. (2022) found comparable results in tuna and sashimi, suggesting that the antimicrobial effects of smoke are not limited to red meat but may extend to seafood as well.

These results not only confirm the antimicrobial efficacy of liquid smoke but also suggest a dose-dependent response, particularly against *E. coli*. The reduction in *E. coli* was more pronounced in beef products compared to chicken products, which could be due to matrix-specific interactions between the meat composition and the antimicrobial components of liquid smoke.

Interestingly, the data also supports the observation by Asita and Campbell (1990) that Gram-negative bacteria such as *E. coli* tend to be less sensitive to smoke extracts compared to Gram-positive organisms. Despite this general resistance, the fact that high concentrations of liquid smoke were able to reduce bacterial counts shows its potential as a natural preservative, especially in RTE and processed meat products.

CONCLUSION

This study demonstrated that liquid smoke has effects on *L. monocytogenes*, *E. coli* and *Salmonella* Enteritidis. Additionally, although the number of pathogenic bacteria was reduced, they were not eliminated. These findings emphasize that liquid smoke can help improve food safety, but proper hygiene practices are still crucial.

The present study demonstrates the potential of liquid smoke as a natural antimicrobial additive for improving the microbiological safety of meat products during refrigerated storage. Its use may act as an additional hurdle within a multi-intervention food safety strategy for RTE meats.

However, this study is not without limitations. The observed antimicrobial

effects were primarily bacteriostatic at the concentrations used, and further research is required in order to determine the sensory impact, optimal dosing, and potential synergies with other preservation techniques. Moreover, evaluating its effects under different packaging conditions (e.g., vacuum vs. modified atmosphere) and storage temperatures could further clarify the practical utility of liquid smoke in industrial applications.

Author's Contribution

KP and MP made contributions to conception and design of the article; KP, MP and MRM conducted the experiments; KP, MP and MRM wrote the manuscript; MP and DJ coordinated the work and revised the manuscript. KP and MRM prepared the final draft; MP and MRM contributed to the analysis and presentation of the results.

Competing interest

The authors declare that they have no competing interests.

Authors' ORCID iDs

Krste Popovski: https://orcid.org/0009-0004-3258-3009
Mirko Prodanov: https://orcid.org/0000-0003-0439-5401
Marija Ratkova Manovska: https://orcid.org/0000-0002-6680-1375
Dean Jankuloski: https://orcid.org/0000-0001-7448-6021

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