

QUALITY PARAMETERS OF BUFFALO MEAT SAUSAGE CONTAINING ESSENTIAL OILS

Mohamed Abdallah Hussein^{1,*}, Rana Makram Moustafa Ali¹, Ahmed Elsayed Tharwat¹,
Waleed Rizk El-Ghareeb^{2,1} and Hesham Abdel-Moez Ismail^{2,3}

ABSTRACT

This study was performed to evaluate the utilization of buffalo meat in formulation of sausage side by side using of thyme essential oil (TEO), cinnamon essential oil (CEO) and garlic essential oil (GEO) at a concentration of 1%, in order to enhance the sensory and freshness parameters with the reduction of microbial load at chilling temperature 3°C for 8 days. Regarding sensory attributes, adding GEO resulted in insignificant lower scores of flavors and overall quality than control at the zero-time, 2nd, and 4th day, then sensory parameters were improved on the 6th and 8th day. Based on chemical findings, on the 8th day total volatile basic nitrogen (TVB-N) mean value in the control group increased to 26.17±0.21 mg/100 g exceeding the permissible limit of sausage acceptability in Egypt. Whereas TVB-N mean values were 18.21±0.23, 17.24±0.21, and 16.24±0.23 mg/100 for TEO 1%, CEO 1%, and GEO 1% treated groups, respectively. The final thiobarbituric acid (TBA) mean value for the control sample was 1.32±0.08 mg MDA/Kg, while the TBA mean values were 0.83±0.08, 0.79±0.08 and 0.76±0.08 mg MDA/Kg for TEO 1%, CEO 1%,

and GEO 1% treated groups, respectively. Results revealed that treated buffalo sausages had a slower rate of the increase in the microbial count than the control and buffalo sausage containing GEO 1% possessed the lowest microbial count at the end of the chilling period. The best reduction counts achieved were 0.92, 1.03, and 1.08 log₁₀CFU/g for aerobics plate count (APC), total mould count (TMC) and Enterobacteriaceae count on the 8th day under the effect of GEO 1%. It could be concluded that the examined essential oils (EOs) can be used as a natural preservative in buffalo meat sausage without any considerable unfavorable sensory effects.

Keywords: *Bubalus bubalis*, buffaloes, buffalo meat, sausage, thiobarbituric acid, total volatile basic nitrogen, essential oils, aerobic plate count

INTRODUCTION

Buffalo meat is considered as a healthy red meat since it has less fat and cholesterol than beef and pork. The meat from aged buffaloes above 5 years is not preferred because of its

¹Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt,
*E-mail: elged2010@yahoo.com

²Department of Public Health, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia

³Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

toughness. The utilization of tough buffalo meat in the manufacturing of comminuted meat products will enhance the meat industry (Modi *et al.*, 2004). Different types of sausages including fermented sausage, cooked sausage, and fresh sausage are produced and consumed all over the world, while the fresh sausage is common in Egypt (Saleh *et al.*, 2017). Microbial growth during the preservation of meat products is considered a major problem from the consumer health aspect in addition to deterioration of protein and oxidative rancidity that reduce the shelf life of meat and meat products. Bio preservatives can be a good tool in extending the shelf life of meat products, by reducing or eliminating survival of spoilage or pathogenic bacteria and increasing overall quality parameters of meat products through the reduction of oxidative rancidity (Naveena *et al.*, 2006). Essential oils are derived from a variety of aromatic plants that are often found in temperate or tropical climates, and they are frequently used in the traditional pharmacopeia. These plants are often employed in the preservation of foods due to their smell and antioxidant effects. Also, as analgesics, sedatives, anti-inflammatories, spasmolytics, and local anesthetics, antiseptic and therapeutic characteristics (Bakkali *et al.*, 2008). Essential oils are generally recognized as safe for human consumption as safe food additives in the European Union at concentrations <2 mg/kg body weight/day (Commission, 2012). Thyme essential oil obtained from (*Thymus vulgaris*), contains 20 to 54% thymol that acts on microbial cells and causes functional and structural damage to bacterial cell membranes that result in increased permeability. Also, contains a range of additional compounds, such as *P*-cymene, myrcene, borneol, and linalool (Borugă *et al.*, 2014). The antibacterial effects of TEO related to the presence of thymol,

act on the cytoplasmic membrane of the bacterial cell is likely to suffer functional and structural damage. The basic mechanism of thymol's antibacterial impact is unknown; however, it is thought to entail disruption of the outer and inner membranes, as well as interactions with membrane proteins and intracellular targets (Sikkema *et al.*, 1995). Cinnamon (*Cinnamomum zeylanicum*) contains vital oils and other derivatives, such as cinnamic acid, cinnamate, and cinnamaldehyde, in addition, to being an antioxidant, antidiabetic, anti-inflammatory (Helander *et al.*, 1998). The antimicrobial action of CEO due to the electro-negativity of the compound trans-cinnamaldehyde, that is abundant in essential oil. Such electro-negative complex interferes in biological processes involving electron transmission and interacts with nitrogen-containing components, such as nucleic acids and proteins therefore, inhibits the growth and activity of the bacteria (Gupta *et al.*, 2008). Furthermore, cinnamon essential oil contains eugenol, linalool, and other phenolic substances that have either bactericidal or bacteriostatic agents. These components have the ability to attack the phospholipid in cell membrane, causing higher permeability and the leakage of cytoplasm, or interact with enzymes located on the cell wall of microorganism (Stefanini *et al.*, 2004). Garlic (*Allium sativum*) has antimicrobial activity and antioxidant activity. The active principle known as allicin (diallyl-thiosulfinate) is one of the main biologically active organosulfur compounds in garlic (Rahman, 2007). The current study was conducted to evaluate the use of buffalo meat in sausage manufacturing. Furthermore, it evaluated the effect of essential oils on sensory, chemical, and microbial parameters of chilled sausage.

MATERIALS AND METHODS

Preparation of sausage

Buffalo meat composed of boneless chuck, neck, and rounds with accompanying fats were obtained from butchers' shops at Zagazig, Egypt. Buffalo meat was obtained within 24 h of slaughter and were used for preparing buffalo sausage. Buffalo meat sausage was prepared according to Saleh *et al.* (2017). The control group contains 70%, 12%, 2.3%, 9.3%, 1%, 1.2% and 1.2%. lean meat, fat, sodium chloride, water, garlic, onion, and spices mixture. The treated groups TEO 1%, CEO 1%, and GEO 1% were obtained by the addition of 1% of the corresponding oil obtained from the agricultural research center, Dokki, Giza, Egypt, during mixing before stuffing. Finally, each group was packaged, identified clearly, and then stored at $3\pm 1^\circ\text{C}$. The prepared groups were examined directly after preparation at zero-time, 2nd, 4th, 6th, and 8th day.

Sensory evaluation

Fresh buffalo sausage was cooked on an electric grill to 71°C core temperature before being evaluated by a 9-member trained panel under similar location, light, and dish conditions (rectangular pieces approximately 2 cm 1.5 cm with 1 cm thickness were cut from the center of sausage samples). According to Lim, (2011) the hedonic method assesses the liking of the product being tested by using hedonic scales (9-point hedonic). The panelists evaluated the following characteristics: flavour, tenderness, and overall acceptability (1 - disliked strongly; 2 - disliked; 3 - moderately disliked; 4 - slightly disliked; 5 - indifferent; 6 - liked slightly; 7 - liked moderately; 8 - liked; and 9 - liked very much).

Chemical parameters

TVB-N was estimated according to FAO (1986) briefly; 10 g of buffalo sausage were homogenized and blended for 2 minutes with 100 mL distilled water then sample washed in distillation flask with 200 mL of water followed by addition of two g of magnesium oxide and two drops of antifoaming agent. The mixture was left to boil for 10 minutes and distilled into 25 mL of 2% boric acid solution with a few drops of screened methyl red indicator in a 500 mL flask for around 25 minutes using the same heating rate. Heating stopped, followed by washing down the condenser with distilled water. Using 0.1 N H_2SO_4 (titer), the flask contents and the blank solution (25 mL of 2% boric acid) were titrated and then TVB-N (mg N/100 g flesh) was determined using the formula: $\text{TVB-N} = 14$ (titer-blank).

TBARS was calculated according to Schmedes and Hølmer (1989) by combining ten grams of buffalo sausage with 25 mL of 20% trichloroacetic acid (w/v) and then homogenized for 30s in a blender. Two mL of the prepared solution were added to 2 mL of 0.02 mL of aqueous TBA after filtration and then incubated in the dark for 20 h at room temperature. The UV-vis spectrophotometer measured the absorbance at 532 nm (model UV-1200, Shimadzu, Japan). TBARS estimates were expressed in the sample of camel minced meat as mg malonaldehyde (MDA)/kg.

Microbiological parameters

In a vertical lamina-flow cabinet, a buffalo sausage sample (25 g) was collected aseptically and then transferred to a stomacher bag; 225 mL of 0.1% peptone water mixture was homogenized and total aerobic plate count were counted per gram on standard plate count agar medium (Oxiod CM325). For 48 h, the plates were

inoculated and incubated at 30°C according to ISO 4833-1: (2013). Enumeration of total mould count according to ISO 21527-1: (2008) briefly, 100 µl each of the prepared serial dilutions was inoculated into surface of two duplicate plates of sabouraud's dextrose agar medium supplemented with 0.05 mg chloramphenicol /ml, then incubated at 25°C for 5 days for enumeration of total mould. Enterobacteriaceae counts were enumerated on Violet Red Bile Glucose Agar by the pour plate method (VRBGA; Difco, Detroit, Michigan, USA). At 37°C for 24 h, the plates were overlaid with a virgin layer of the same growth medium prior to incubation (ISO 21528-2, 2004).

Statistical analysis

The microbiological data was translated to a value of \log_{10} . The variations among groups were tested using the General Linear Model of SAS statistical system Package (SAS, 2008). Kolmogorov-Smirnov test was applied to guarantee the homogeneity and normality of variances among the different study groups. The statistical model was as the following:

$$Y_{ijk} = \mu + G_i + B_j + e_{ijk}$$

where Y_{ijk} showed the studied traits, μ is the overall mean for each trait, G_i is the fixed effect of i th groups with 4 levels where (i =control group, 2= TEO 1%, 3= CEO 1% and 4= GEO 1%), B_j is the fixed effect of replicates ($j = 1, 2, 3$ and 4) and e_{ijk} is the random residual effect. The difference between means was detected with Tukey's test. Log geometric mean was calculated for microbiological data. The significant was estimated at $P < 0.05$.

RESULTS AND DISCUSSIONS

Quality

Sensory evaluation has been used to accept or reject meat products since the beginning of humanity and very important tool for developing new products. Aside from technological and safety analysis, foods are distinguished by their organoleptic properties (taste, smell, texture, etc.), which must be considered when innovating, as these are the characteristics that will determine whether the consumer will purchase the product again or not (Ruiz-Capillas *et al.*, 2021).

Flavor, texture, and overall quality of buffalo meat sausage are presented in Table 1. Primarily the flavor scores at zero time were 8.22 ± 0.65 , 8.50 ± 0.64 , 8.65 ± 0.64 , and 6.19 ± 0.63 and on the 8th day of chilling were 4.14 ± 0.62 , 6.55 ± 0.65 , 6.79 ± 0.56 , and 7.22 ± 0.51 in the control, TEO 1%, CEO 1%, and GEO 1% groups, respectively. The texture score was 8.66 ± 0.51 and 3.29 ± 0.61 , 8.71 ± 0.52 and 6.32 ± 0.51 , 8.72 ± 0.52 and 6.57 ± 0.46 , 8.69 ± 0.55 and 6.95 ± 0.48 at zero time and 8th day of chilling in the control, TEO 1%, CEO 1%, and GEO 1% groups, respectively. The overall quality was lowered in the control group 4.19 ± 0.54 than 6.95 ± 0.47 , 7.11 ± 0.49 , and 7.43 ± 0.46 in TEO 1%, CEO 1%, and GEO 1% treated groups, respectively. Comparing the control and the three treatments, a significant decrease on flavor score and overall quality score in garlic 1% ($P < 0.05$) at zero time which attributed to presence of allicin, a sulfur-containing molecule resulting in, pungent garlic smell (Rahman, 2007). Araújo *et al.*, (2018) found that GEO slightly reduced flavor and overall acceptability of sausage directly after addition. On the other hand, significant reduction in the flavor, texture, and overall quality of the control group ($P < 0.05$) on 6th and 8th day of chilling that

attributed to the effect of essential oils on treated groups. This result agrees with that obtained by Sasse *et al.* (2009), who declared that many EO contain antioxidant components that enhance both color and flavor stability in meat products. Also, addition of TEO or CEO with different concentrations of 0.5, 1 and 2% to minced meat improved sensory properties where, free radicals eliminated and oxidation process delayed (Shaltout *et al.*, 2017).

Chemical parameters

TVB-N has been usually used as a biomarker of protein and amine degradation in meat and meat products. The effect of essential oils on the progression of TVB-N of fresh buffalo sausage during the 8-day storage $3 \pm 1^\circ\text{C}$ is shown in Figure 1. The initial TVB-N in the control and essential oil treated sample ranged from 4.18 to 4.22 mg/100 g. Higher initial TVB-N value reported 9.22 mg/100 g in Italian pork sausage (Zhang *et al.*, 2019) and 10.21 mg/100 g in Balkan-style beef sausage (Ajourloo *et al.*, 2021). The difference could be attributed to the length of time meat is processed after slaughter and the quality of the meat used. As it is demonstrated in Figure 1, TVB-N values were increased in both control and essential oil treated samples during storage time; meanwhile, the mean values were significantly lower ($P < 0.05$) in the treated samples in comparison with the control group at 4th, 6th, and 8th days of storage. The obtained results were with the same line with other published data on the effect of EO in decrease of TVB-N in pork sausage (Zhang *et al.*, 2019) and fresh beef sausage (Ajourloo *et al.*, 2021). Regarding comparing the obtained results with the Egyptian standard (1972-2005) of sausage, we found that all essential oil treated groups located within the limit 20 mg/100

g. The low concentration of TVB-N in essential oil treated groups may attributed to the antibacterial effects of EO in delaying microbial growth then reduction in the amount of nonprotein nitrogen compounds such as, ammonia, primary, secondary, and tertiary amines (Hussein *et al.*, 2017). Lipid oxidation (oxidative rancidity) considered as second spoilage parameter of fresh meat products is. Also, it is known that presence of light, oxygen, high storage temperature, enhance lipid oxidation (Hugo and Hugo, 2015).

The changes in TBA values are demonstrated in Figure 2. The TBA of the control and essential oil treated groups at the beginning of the study ranged from 0.07 to 0.09 mg MDA/kg. In the control group, the TBA value reached to 0.98 and 1.32 mg MDA/kg on the 6th and 8th day of chilling exceeding the maximum permissible limit established in Egypt for sausage (Egyptian standard 1972-2005). Meanwhile, the values for the essential oil treated groups remained lower than the maximum permissible limit of 0.9 mg MDA/kg. The present results are in line with those of Zhang *et al.* (2019) who noticed that adding of cinnamon oil to fresh sausage with different concentrations led to a significant reduction in TBA values of treated pork sausage compared with control. Also, addition of thyme oil at concentration (0.125%) to chicken sausage significantly reduced TBA values (Sharma *et al.*, 2017). The activity of EO in producing of antioxidant effect could be recognized to their ability of free radical scavenging, retarding growth of mesophilic and psychrophilic bacteria that enhances metal ions chelation and saturated fatty acids oxidation (Bozin *et al.*, 2007; Sharma *et al.*, 2016).

Microbial parameters

Changes in total aerobic plate counts

(APC) of buffalo meat sausage during refrigerated storage are illustrated in Table 2. The initial APC of the control group was $4.38 \pm 0.17 \log_{10}$ CFU/g and gradually increased to $7.11 \pm 0.55 \log_{10}$ CFU/g at the end of storage. It was revealed that APC of fresh sausages in Canada ranged from 3.18 to $8.32 \log_{10}$ CFU/g with a mean of $5.65 \log_{10}$ CFU/g (Farber *et al.*, 1988). Also, it was ranged from $4.08 \log_{10}$ CFU/g at zero time to $8.32 \log_{10}$ CFU/g on 13th day of storage in Balkan beef sausage (Ajourloo *et al.*, 2021). Overall, APC significantly decreased in buffalo sausage samples containing EO in comparison with the control groups ($P < 0.05$), except for zero time and 2nd day. Addition of essential oils to buffalo sausage reduced APC below $7 \log_{10}$ CFU/g until the 8th day of chilling. Similar findings were obtained by Shaltout *et al.* (2017) who found APC of in chilled minced meat reduced significantly ($P < 0.05$) in the treated samples with thyme or cinnamon at concentration 1, 1.5 and 2%. With concern to the use of essential oils in fresh buffalo sausage, the results of this study in agreement with Zhang *et al.* (2019), who found cinnamon essential oil significantly reduced of APC in pork sausage. The reduction counts under the effect of TEO 1%, CEO 1%, and GEO 1% were 0.93 and 0.72, 0.98 and 0.84, 1.12 and 0.92 \log_{10} CFU on 6th and 8th day, respectively.

Mould contamination of meat products can occur during animal slaughter, transportation, or processing of meat products using contaminated equipment or contaminated additives and spices, which are the most common sources of mould contamination in meat products. (Jay *et al.*, 2005). The total mould count in buffalo meat sausage in control group was $2.23 \pm 0.13 \log_{10}$ CFU/g at zero time and gradually increased to $4.82 \pm 0.41 \log_{10}$ CFU/g on the 8th day of chilling, Table 3. In comparing the control and EO treated groups, a significant

reduction ($P < 0.05$) in total mould count in all treated groups on the 6th and 8th day of chilling was obtained. Moreover, significant reduction obtained only in GEO 1% on 2nd and 4th day which declared the more potency of GEO against mould in buffalo sausage. Nearly similar effect of EO on total mould count was obtained after treatment of minced meat with 0.5, 1% TEO or GEO (Habashy *et al.*, 2019). The effect of EO on *Aspergillus flavus* *in vitro* and *in vivo* was studied (Kumar *et al.*, 2020). Oliveira *et al.* (2020) used TEO *in vitro* for inhibition of *A. flavus*. The CEO possess great effect against food-contaminating moulds, *A. fumigatus*, *A. flavus*, *Aspergillus niger*, *A. chevalieri*, *A. versicolor*, *penicillioides*, and *Cladosporium herbarum* (Singh *et al.*, 2020). The addition of GEO to the stored foods have been correlated with the presence of several phenolic and terpenoids components which affect mould growth (Lasram *et al.*, 2019). CEO showed activity against *A. ocharceus*, *A. niger*, and *A. oryzae* isolated from wheat bread (Hu *et al.*, 2021).

The Enterobacteriaceae count is important for determining the sanitary conditions in which meat products are processed. Based on the findings, the zero-time *Enterobacteriaceae* count was $2.75 \log_{10}$ CFU/g and reached $5.92 \pm 0.54 \log_{10}$ CFU/g, of the control buffalo sausage, Table 4. A significant ($P < 0.05$) reduction in the count of *Enterobacteriaceae* in GEO treated group noticed on 2nd and 4th day of chilling. Meanwhile, on the 6th and 8th days significant reduction in all EO treated groups on comparing with the control group. The obtained results on *Enterobacteriaceae* growth, regarding using of EO, agree with Zhang *et al.* (2019) who found reduction in *Enterobacteriaceae* count during cold storage of pork sausage under effect of 0.1% CEO or 0.5% CEO. In the study held out by Shaltout *et al.* (2017), it was demonstrated

Table 1. Effect of different essential oils on sensory quality of buffalo meat sausage (Mean±SE) stored at 3±1°C (N=5).

Characteristics	Storage days	Control	TEO 1%	CEO 1%	GEO 1%
Flavor	Zero time	8.22±0.65	8.50±0.64	8.65±0.64	6.19±0.63
	2 nd day	8.12±0.64	8.44±0.65	8.55±0.64	6.87±0.64
	4 th day	7.06±0.56	7.92±0.55	7.96±0.52	6.77±0.56
	6 th day	6.12±0.53 ^b	7.13±0.57 ^a	7.35±0.54 ^a	7.83±0.56 ^a
	8 th day	4.14±0.62 ^b	6.55±0.65 ^a	6.79±0.56 ^a	7.22±0.51 ^a
Texture	Zero time	8.66±0.51	8.71±0.52	8.72±0.52	8.69±0.55
	2 nd day	7.06±0.52	7.96±0.49	7.94±0.51	8.11±0.53
	4 th day	6.85±0.53	7.83±0.52	7.86±0.49	7.96±0.49
	6 th day	4.26±0.48 ^b	7.09±0.43 ^a	7.12±0.47 ^a	7.89±0.45 ^a
	8 th day	3.29±0.61 ^b	6.32±0.51 ^a	6.57±0.46 ^a	6.95±0.48 ^a
Overall quality	Zero time	8.24±0.49	8.34±0.51	8.45±0.52	7.55±0.48 ^b
	2 nd day	7.49±0.46	8.12 ±0.47	8.39±0.49	8.44±0.51
	4 th day	6.57±0.52	7.98±0.53	8.12±0.51	8.38±0.47
	6 th day	5.47±0.42 ^b	7.21±0.43 ^a	7.54±0.48 ^a	7.98±0.45 ^a
	8 th day	4.19±0.54 ^b	6.95±0.47 ^a	7.11±0.49 ^a	7.43±0.46 ^a

Values with different letters (a, b, c) in the same row are significantly different (P<0.05).

Each value is a mean ± SE. of five replicates.

Table 2. Effect of essential oils on total aerobic plate count (Mean ± SE) log₁₀ CFU/g in buffalo meat sausage stored at 3±1°C (N=5).

Storage days	Control	TEO 1%	RC	CEO 1%	RC	GEO 1%	RC
Zero time	4.38±0.17	4.28±0.15	0.10	4.24±0.15	0.14	4.19±0.14	0.19
2 nd day	4.98±0.16	4.66±0.15	0.32	4.54±0.15	0.44	4.47±0.17	0.51
4 th day	5.45±0.29 ^a	4.92±0.23 ^a	0.53	4.85±0.24 ^{ab}	0.6	4.74±0.26 ^b	0.71
6 th day	6.45±0.48 ^a	5.52±0.41 ^b	0.93	5.47±0.42 ^{bc}	0.98	5.33±0.45 ^c	1.12
8 th day	7.11±0.55 ^a	6.39±0.54 ^b	0.72	6.27±0.49 ^b	0.84	6.19±0.48 ^b	0.92

Values with different letters (a, b, c) in the same row are significantly different (P<0.05).

Each value is a mean ± SE. of five replicates.

Reduction Count (RC) = Control - Treated.

Table 3. Effect of essential oils on total mould count (Mean \pm SE) \log_{10} CFU/g in buffalo meat sausage stored at $3\pm 1^\circ\text{C}$ (N=5).

Storage days	Control	TEO 1%	RC	CEO 1%	RC	GEO 1%	RC
Zero time	2.23 \pm 0.13	2.22 \pm 0.13	0.01	2.19 \pm 0.04	0.14	2.05 \pm 0.13	0.18
2 nd day	3.14 \pm 0.18 ^a	2.89 \pm 0.17 ^a	0.25	2.78 \pm 0.17 ^a	0.36	2.26 \pm 0.15 ^b	0.88
4 th day	3.97 \pm 0.22 ^a	3.25 \pm 0.19 ^{4a}	0.72	3.15 \pm 0.18 ^{ab}	0.82	2.95 \pm 0.19 ^b	1.02
6 th day	4.23 \pm 0.38 ^a	3.47 \pm 0.32 ^b	0.76	3.41 \pm 0.30 ^b	0.82	3.22 \pm 0.29 ^c	1.01
8 th day	4.82 \pm 0.41 ^a	4.11 \pm 0.37 ^b	0.71	4.05 \pm 0.34 ^b	0.77	3.79 \pm 0.32 ^c	1.03

Values with different letters (a, b, c) in the same row are significantly different ($P < 0.05$).

Each value is a mean \pm SE. of five replicates.

Reduction Count (RC) = Control - Treated

Table 4. Effect of essential oils on Enterobacteriaceae count (Mean \pm SE) \log_{10} CFU/g in buffalo meat sausage stored at $3\pm 1^\circ\text{C}$ (N=5).

Storage days	Control	TEO 1%	RC	CEO 1%	RC	GEO 1%	RC
Zero time	3.11 \pm 0.17	2.98 \pm 0.15	0.13	2.91 \pm 0.16	0.20	2.75 \pm 0.14	0.36
2 nd day	3.96 \pm 0.19 ^a	3.41 \pm 0.16 ^a	0.55	3.38 \pm 0.15 ^a	0.58	2.97 \pm 0.13 ^b	0.99
4 th day	4.47 \pm 0.34 ^a	3.87 \pm 0.28 ^a	0.6	3.76 \pm 0.25 ^{ab}	0.71	3.49 \pm 0.23 ^b	0.98
6 th day	5.08 \pm 0.46 ^a	4.43 \pm 0.36 ^b	0.65	4.38 \pm 0.36 ^b	0.7	4.09 \pm 0.32 ^b	0.99
8 th day	5.92 \pm 0.54 ^a	5.07 \pm 0.49 ^b	0.85	4.95 \pm 0.47 ^{bc}	0.97	4.84 \pm 0.43 ^c	1.08

Values with different letters (a, b, c) in the same row are significantly different ($P < 0.05$).

Each value is a mean \pm SE. of five replicates.

Reduction Count (RC) = Control - Treated.

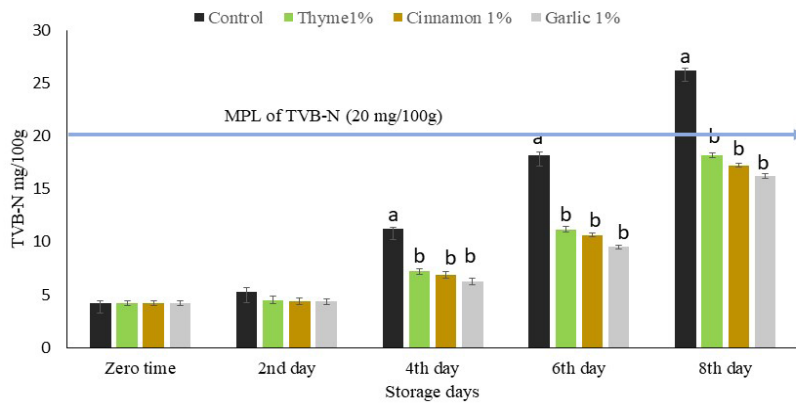


Figure 1. Effect of essential oils on Total volatile basic nitrogen mg/ 100 g in buffalo meat sausage stored at 3 ± 1 °C (N=5). Each value is a mean \pm SE. of five replicates. Values with different letters in the same day are significantly different (P<0.05).

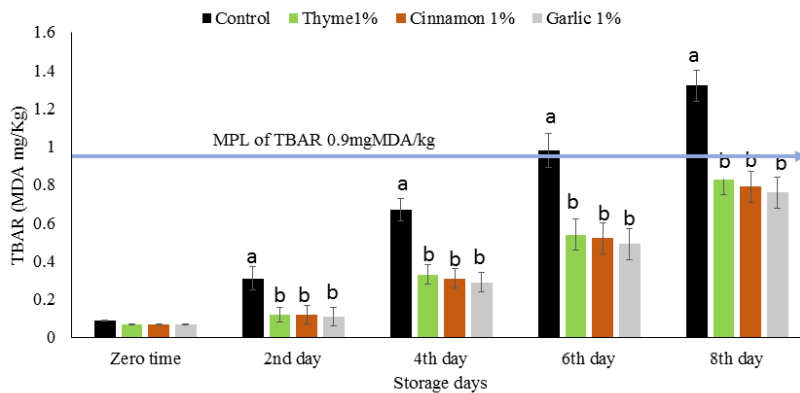


Figure 2. Effect of essential oils on Thiobarbituric acid mg malondialdehyde/kg in buffalo meat sausage stored at 3 ± 1 °C (N=5). Each value is a mean \pm SE. of five replicates. Values with different letters in the same day are significantly different (P<0.05).

that TEO and CEO reduce *Enterobacteriaceae* count in chilled minced on the 9th and 12th day. In addition, *E. coli* counts reduced under the effect of GEO in fresh mixed sausage during cold storage (Araújo *et al.*, 2018). Addition of TEO 1%, CEO 1%, GEO1% during processing of buffalo meat sausage led to reduction counts 0.65, 0.7, 0.99 log₁₀CFU/g on the 6th day which increased to 0.85, 0.97 and 1.08 log₁₀CFU/g on the 8th day of chilling, respectively.

CONCLUSION

Based on the findings of the current study, it can be concluded that buffalo meat could be used successfully in formulation of sausage. Furthermore, sensory and freshness parameters enhanced, and microbial load decreased with the addition of the studied essential oils during chilling period.

REFERENCES

- Ajourloo, M., A. Khanjari, A. Misaghi, A.A. Basti, A. Kamkar, F. Yadegar and F. Fallah. 2021. Combined effects of Ziziphora clinopodioides essential oil and lysozyme to extend shelf life and control *Listeria monocytogenes* in Balkan-style fresh sausage. *Food Science and Nutrition*, **9**(3): 1665-1675. DOI: 10.1002/fsn3.2141
- Araújo, M.K., A.M. Gumiela, K. Bordin, F.B. Luciano and R.E.F. de Macedo. 2018. Combination of garlic essential oil, allyl isothiocyanate, and nisin Z as bio-preservatives in fresh sausage. *Meat Sci.*, **143**: 177-183. DOI: 10.1016/j.meatsci.2018.05.002
- Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar. 2008. Biological effects of essential oils - A review. *Food Chem. Toxicol.*, **46**(2): 446-475. DOI: 10.1016/j.fct.2007.09.106
- Borugă, O., C. Jianu, C. Mișcă, I. Goleț, A. Gruia and F. Horhat. 2014. Thymus vulgaris essential oil: Chemical composition and antimicrobial activity. *Journal of Medicine and Life*, **7**(Special Issue 3): 56-60.
- Bozin, B., N. Mimica-Dukic, I. Samojlik and E. Jovin. 2007. Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis L.* and *Salvia officinalis L.*, Lamiaceae) essential oils. *J. Agric. Food Chem.*, **55**(19): 7879-7885. DOI: 10.1021/jf0715323
- Egyptian Organization for Standardization and Quality Control. 2005. *Frozen Sausage. Egyptian Organization for Standardization and Quality Control*, Cairo, Egypt
- FAO. 1986. *Food and Nutrition Paper: Manual of Food Quality Control 14/8 Food Analysis*. Food and Agriculture Organization of the United Nations, Rome, Italy.
- FAO. 2012. Commission Regulation (EU) No 432/2012 Establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children development and health. *Official Journal of the European Union*, **L136**: 1-40. Available on: <http://extwprlegsl.fao.org/docs/pdf/eur112829.pdf>
- Gupta, C., A.P. Garg, R.C. Uniyal and A. Kumari. 2008. Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on some food-borne microbes. *Afr. J. Microbiol. Res.*, **2**(9): 247-251. Available on: <https://academicjournals.com>

org/article/article1380109444_Gupta%20et%20al.pdf

- Habashy, A.H.A., W.S. Darwish, M.A. Hussein and W.M.S. El-Dien. 2019. Prevalence of different mould genera in meat and meat products with some reduction trials using essential oils. *Advances in Animal and Veterinary Sciences*, **7**(S2), 79-85. DOI: 10.17582/journal.aavs/2019/7.s2.79.85
- Helander, I.M., H.L. Alakomi, K. Latva-Kala, T. Mattila-Sandholm, I. Pol, E.J. Smid and A. von Wright. 1998. Characterization of the action of selected essential oil components on Gram-negative bacteria. *J. Agric. Food Chem.*, **46**(9): 3590-3595. DOI: 10.1021/jf980154m
- Hu, Z., K. Yuan, Q. Zhou, C. Lu, L. Du and F. Liu. 2021. Mechanism of antifungal activity of *Perilla frutescens* essential oil against *Aspergillus flavus* by transcriptomic analysis. *Food Control*, **123**: 107703. DOI: 10.1016/j.foodcont.2020.107703
- Hugo, C.J. and A. Hugo. 2015. Current trends in natural preservatives for fresh sausage products. *Trends Food Sci. Tech.*, **45**(1): 12-23. DOI: 10.1016/j.tifs.2015.05.003
- Hussein, M.A., W.R. El-Ghareeb and M.A. Nasr. 2018. The effect of rosemary extract and lactic acid on the quality of refrigerated broiler fillets. *J. Food Sci. Tech.*, **55**(1): 5025-5034. DOI: 10.1007/s13197-018-3441-2
- ISO. 2008. *Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Enumeration of Yeasts and Moulds - Part 1: Colony Count Technique in Products with Water Activity Greater Than 0,95*. International Organization for Standardization, Geneva, Switzerland.
- ISO. 2004. *Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Detection and Enumeration of Enterobacteriaceae - Part 2: Colony Count Method*. International Organization for Standardization, Geneva, Switzerland.
- ISO. 2013. *Microbiology of the Food Chain - Horizontal Method for the Enumeration of Microorganisms - Part 1: Colony Count at 30oC by the Pour Plate Technique*. International Organization for Standardization, Geneva, Switzerland.
- Jay, J.M., M.J. Loessner and D.A. Golden. 2005. Taxonomy, role, and significance of microorganisms in foods. p. 13-37. *In Modern food Microbiology, Food Science Text Series*, Springer, Boston, Massachusetts, USA. DOI: 10.1007/0-387-23413-6_2
- Kumar, M., A.K. Dwivedy, P. Sarma, M.S. Dkhar, H. Kayang, R. Raghuwanshi and N.K. Dubey. 2020. Chemically characterized *Artemisia nilagirica*(Clarke)Pamp.essential oil as a safe plant-based preservative and shelf-life enhancer of millets against fungal and aflatoxin contamination and lipid peroxidation. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, **154**(3): 269-276. DOI: 10.1080/11263504.2019.1587539
- Lasram, S., H. Zemni, Z. Hamdi, S. Chenenaoui, H. Houissa, M.S. Tounsi and A. Ghorbel. 2019. Antifungal and antiaflatoxinogenic activities of *Carum carvi* L., *Coriandrum sativum* L. seed essential oils and their major terpene component against *Aspergillus flavus*. *Ind. Crop. Prod.*, **134**: 11-18. DOI: 10.1016/j.indcrop.2019.03.037
- Lim, J. 2011. Hedonic scaling: A review of methods and theory. *Food Qual. Prefer.*, **22**(8): 733-

747. DOI: 10.1016/j.foodqual.2011.05.008
- Modi, V.K., N.S. Mahendrakar, D.N. Rao and N.M. Sachindra. 2004. Quality of buffalo meat burger containing legume flours as binders. *Meat Sci.*, **66**(1): 143-149. DOI: 10.1016/S0309-1740(03)00078-0
- Naveena, B.M., M. Muthukumar, A.R. Sen, Y. Babji and T.R.K. Murthy. 2006. Improvement of shelf-life of buffalo meat using lactic acid, clove oil and vitamin C during retail display. *Meat Sci.*, **74**(2): 409-415. DOI: 10.1016/j.meatsci.2006.04.020
- Oliveira, R.C., M. Carvajal-Moreno, B. Correa and F. Rojo-Callejas. 2020. Cellular, physiological and molecular approaches to investigate the antifungal and anti-aflatoxigenic effects of thyme essential oil on *Aspergillus flavus*. *Food chemistry*, **315**(15): 126096. DOI: 10.1016/j.foodchem.2019.126096
- Rahman, M.S. 2007. Allicin and other functional active components in garlic: Health benefits and bioavailability. *Int. J. Food Prop.*, **10**(2): 245-268. DOI: 10.1080/10942910601113327
- Ruiz-Capillas, C., A.M. Herrero, T. Pintado and G. Delgado-Pando. 2021. Sensory analysis and consumer research in new meat products development. *Foods*, **10**(2): 429. DOI: 10.3390/foods10020429
- Sikkema, J., J.A.M. De Bont and B. Poolman. 1995. Mechanisms of membrane toxicity of hydrocarbons. *Microbiology Reviews*, **59**(2): 201-222. DOI: 10.1128/mr.59.2.201-222.1995
- Saleh, E.A., A.E.M. Morshdy, A.E.S.E. Hafez, M.A. Hussein, E.S. Elewa and A.F.A. Mahmoud. 2017. Effect of pomegranate peel powder on the hygienic quality of beef sausage. *Journal of Microbiology, Biotechnology and Food Sciences*, **6**(6): 1300-1304. DOI: 10.15414/jmbfs.2017.6.6.1300-1304
- SAS. 2008. *SAS Statistical System Package-Jmp 8 User's Guides*, 2nded. SAS Institute Inc, Cary, North Carolina, USA.
- Sasse, A., P. Colindres and M.S. Brewer. 2009. Effect of natural and synthetic antioxidants on the oxidative stability of cooked, frozen pork patties. *J. Food Sci.*, **74**(1): S30-S35. DOI: 10.1111/j.1750-3841.2008.00979.x
- Schmedes, A. and G. Hølmer. 1989. A new thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. *J. Am. Oil Chem. Soc.*, **66**(6): 813-817. DOI: 10.1007/BF02653674
- Shaltout, F.A., M.G. Thabet and H.A. Koura. 2017. Impact of some essential oils on the quality aspect and shelf life of meat. *Journal of Nutrition and Food Sciences*, **7**(6): 1-7. DOI: 10.4172/2155-9600.1000647
- Sharma, H., S. Mendiratta, R.K. Agarwal, S. Kumar and A. Soni. 2017. Evaluation of anti-oxidant and anti-microbial activity of various essential oils in fresh chicken sausages. *J. Food Sci. Tech. Mys.*, **54**(2): 279-292. DOI: 10.1007/s13197-016-2461-z
- Sharma, H., A.K. Sharma and A.K. Pandey. 2016. Medicinal attributes of major phenylpropanoids present in cinnamon. *BMC Complem. Altern. M.*, **16**(1): 1-11. DOI: 10.1186/s12906-016-1147-4
- Singh, A., Deepika, A.K. Chaudhari, S. Das, J. Prasad, A.K. Dwivedy and N.K. Dubey. 2021. Efficacy of *Cinnamomum cassia* essential oil against food-borne molds and aflatoxin B1 contamination. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, **155**(4): 899-907.

- Stefanini, M.B., R.O. Figueiredo, L.C. Ming and A.F. Junior. 2004. Antimicrobial activity of the essential oils of some spice herbs. *Acta Hortic.*, **597**: 215-216. DOI: 10.17660/ActaHortic.2003.597.30
- Zhang, X., H. Wang, X. Li, Y. Sun, D. Pan, Y. Wang and J. Cao. 2019. Effect of cinnamon essential oil on the microbiological and physiochemical characters of fresh Italian style sausage during storage. *Anim. Sci. J.*, **90**(3): 435-444. DOI: 10.1111/asj.13171