OPEN ACCESS
Journal of Animal Health and Production
Research Article

Effect of Cinnamon and Wheat Germ Essential Oils on the Chemical and Bacteriological Quality of Oriental Sausage

Rasha M. El Bayomi[1*], Aya Mohamed Elsobky[1], Dalia Fathy Khater[2], Adel I. El-Atabany[1]

[1]Food Control Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt; [2]Cheif Researcher, Animal Health Research Institute, Tanta Branch.

Abstract | Current study aimed to investigate the effects of cinnamon essential oil (CEO) and wheat germ essential oil (WGO) on the chemical and bacteriological quality of oriental sausage. Five batches of beef sausage first as control, second and third groups contained CEO at concentrations of 1% and 2%, while the fourth and fifth groups added with WGO at concentrations of 1% and 2%. The effect of different treatments on pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), aerobic plate count (APC), *Enterobacteriaceae* count, *Staphylococcus* count and *Enterococcus* count was studied at zero, 3rd, 6th, and 9th day of chilling at 3±1°C. The mean values of pH, TVB-N and TBA in Control group was 7.03 ± 0.05, 33.46± 0.46 mg/100g and 1.67± 0.03 mg MDA/kg however, it was reduced ($p<0.05$) in 2% CEO (6.29 ± 0.03, 19.74 ± 0.21mg/100g and 0.85 ± 0.03mg MDA/kg respectively) and 2% WGO (6.36 ± 0.04, 19.99 ± 0.13 mg/100g and 0.89 ±0.025 mg MDA/kg respectively) treated group on 9th day. The mean values of APC, *Enterobacteriaceae*, *Staphylococcus* and *Enterococcus* in control group were 7.23 ± 0.34, 5.43 ± 0.28, 5.12 ± 0.23 and 4.98 ± 0.25 $\log_{10}$CFU/g, while these were reduced ($p<0.05$) in 2% CEO (6.11 ± 0.31, 4.24 ± 0.26, 4.08 ± 0.24 and 3.98 ± 0.23 $\log_{10}$CFU/g respectively) and in 2% WGO (6.45 ± 0.31, 4.35 ± 0.24, 4.25± 0.23 and 4.08± 0.21 $\log_{10}$CFU/g set respectively) in treated group on 9th day. These results exhibited that CEO and/or WGO at concentration 2% could be added in oriental sausages to retard the chemical changes and decrease the bacterial growth during storage.

Keywords | Sausage, TBA, TVN, Cinnamon oil, Wheat germ oil.

Received | July 21, 2021; **Accepted** | August 08, 2021; **Published** | August 25, 2021
***Correspondence** | Rasha M El Bayomi, Food Control Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt; **Email:** rmazab_2010@yahoo.com
Citation | El Bayomi RM, Elsobky AM, Khater DF, El-Atabany AI (2021). Effect of cinnamon and wheat germ essential oils on the chemical and bacteriological quality of oriental sausage. J. Anim. Health Prod. 9(s1): 49-55.
DOI | http://dx.doi.org/10.17582/journal.jahp/2021/9.s1.49.55
ISSN | 2308-2801

INTRODUCTION

Oriental sausages are comminuted processed meat product made from beef meat, chicken meat, or a combination of these with water, binders, and seasoning. Sausage usually stuffed into a casing. However, in last years, due to increased consumer needs and competition in the meat processing, ingredients have been incorporated into meat and meat products to develop a healthier meat product. The demand for natural replacements of chemicals with natural additives, in foodstuffs to extend shelf life, decreasing lipid oxidation and deteriorative changes, so using of essential oils (EOs) is obtaining considerable attention (Hussein et al., 2018).

Cinnamomum zeylanicum (cinnamon essential oil) (CEO) contains cinnamaldehyde, thymol and carvacrol. The cinnamaldehyde is more efficient against Gram positive and Gram-negative bacteria than its other compound in cinnamon (Chang et al., 2001).

Triticum vulgare oil (Wheat germ oil) (WGO) contains es-

sential fatty acids as alpha linolenic and linoleic acid. These essential fatty acids are helpful in decreasing the cholesterol level, and improving endurance. In addition to high amount of vitamin E and essential fatty acids, WGO also have Vitamin B complex and is significant for chemoprevention. WGO protects mammalian cells counter to free radicals (Liu, 2007). Wheat germ extract have antibacterial activity and Gram-positive bacteria were highly sensitive than Gram-negative bacteria (Mahmoud et al., 2015). This study was conducted to evaluate the effect of cinnamon oil and wheat germ oil at concentration 1 and 2% during formulation of oriental sausage to improve chemical and microbiological quality under chilling condition.

MATERIALS AND METHODS

Samples Preparation

Beef sausage samples were prepared according to the method described by Saleh et al. (2017). In brief, meat and fat tissues were cut into pieces of about egg-size, then it was ground to particles of about a rice size, then the ingredients were blended to prepare sausage mixture emulsion, which was then stuffed by sausage filling machine previously washed by hot water and cased in mutton casings.

The CEOand WGO were purchased from National Research Center, Dokki, Giza to determine the effect of these oils on the chemical and bacteriological quality of meat sausage. The experimental trails were repeated in triplicates, the samples were aseptically divided into five groups; Control group: lean meat 70%, fat 12%, sodium chloride 2.3%, water 9.3%, garlic1%, onion 1.2% and spices mixture 1.2%. Group 1(1% CEO): after mixing 1% removed and replaced with 1% cinnamon oil then mixed again. Group 2(2% CEO): after mixing 2% removed and replaced with 2% cinnamon oil then mixed again. Group 3 (1% WGO): after mixing 1% removed and replaced with 1% wheat germ oil then mixed again. Group 4 (2%WGO): after mixing 2% removed and replaced with 2% wheat germ oil then mixed again. For evaluation the effect of essential oils as natural preservatives the prepared beef sausage groups was kept under chilling condition at 3±1 ^{O}C and examined after formulation at zero time and after 3rd, 6th and 9th day.

Chemical Exminations

The pH value was determined by using an electrical pH meter according to Pearson (2006). The total volatile basic nitrogen (TVB-N) content was estimated according to EOS 63-9 (2006). The thiobarbituric acid (TBA) content was calculated by the method recommended by EOS 63-10 (2006).

Bacteriological Examinations

The preparation of the sausage samples and serial dilutions were performed according to ISO 6887-2 (2003). Briefly, twenty-five grams of each sample were homogenized aseptically in 225 ml of 0.1 % Buffered Peptone Water (BPW, Himedia, M614-500G) in a stomacher (Colworth, 400) for 2.5 min and then allowed to stand for 5 min to provide a homogenate which represents the dilution of 10^{-1}. One ml of the homogenate was transferred into a sterile test tube containing 9 ml of 0.1% BPW, then ten folds serial dilutions were prepared up to the required dilution (10^{-9}). Enumeration of aerobic plate count (APC) was done according to ISO 4833-1 (2013) using pour plate method of 1ml onto plate count agar (Oxoid, CM325) then incubated at 30 ºC. *Staphylococcus* count carried out on Baired-Parker agar medium plates (Oxoid, CM275) according to ISO, 6888-1 (1999). *Enterobacteriaceae* enumeration was carried out on violet red bile glucose ager (VRBG, Himedia) according to ISO 21528-2 (2004). Enumeration of *Enterococcus* was carried out on Bile Esculin Azide ager (BEA, Himedia, M340) as recommended by ISO 7899-2 (2000).

Statistical Analysis

All values of chemical and bacteriological analysis were presented as means ± standard error (SE). All microbial counts were converted to $\log_{10}$ CFU/g values.

Kruskal-wallis H One-way analysis of variance (ANOVA) post hoc Bonferroni correction was carried out to estimate the differences in chemical and bacterial counts. The difference between the treatments groups in the decontamination trial were analyzed by One-way ANOVA. P-values less than 0.05 were considered statistically significant.

RESULTS

Chemical Parameters

The mean values of pH were slightly decreased in the treated groups than the control group at zero time. On the 9th day the pH values increased in control group and in all the treated groups but the treated groups exhibited less pH values than the control group (Table 1). Regarding to TVB-N, there were no significant effects of essential oil at zero time within various concentration. Significant differences ($p<0.05$) appeared from the 3rd day between all treated group and the control group. The TVB-N values gradually increased with increasing chilling time from the 3rd day. Finally, on the 9th day the TVB-N values were increased in all groups but the control group had the highest values (Table 1).

Thiobarbituric acid at zero time has no significant differences ($p>0.05$) but significant differences ($p<0.05$) appeared from the 3rd day of chilling in all treated groups. The lowest TBA value was detected by 2% CEOtreated groups at zero-time, 3rd ,6th and 9th day of chilling (Table 1).

Table 1: Effect of CEOand WGO on pH, total volatile basic nitrogen (TVB-N) mg/100g and thiobarbituric acid (TBA) mg malonaldehyde/Kg of chilled sausage stored at 3±1 ^{O}C.

Parameters	Group	Zero day	3rd day	6th day	9th day
pH	Control	5.91 ± 0.02^{a}	6.31 ± 0.04^{a}	6.88 ± 0.08^{a}	7.03 ± 0.05^{a}
	1% CO	5.86 ± 0.03^{a}	6.01 ± 0.02^{b}	6.24 ± 0.04^{b}	6.44± 0.03^{b}
	2% CO	5.86 ± 0.06^{a}	5.92 ± 0.01^{b}	6.1 ± 0.03^{c}	6.29 ± 0.03^{c}
	1% WGO	5.89± 0.02^{a}	6.09 ± 0.04^{ab}	6.3 ± 0.02^{b}	6.53 ± 0.04^{ab}
	2% WGO	5.84 ± 0.02^{a}	5.98 ± 0.02^{b}	6.2 ± 0.05^{c}	6.36 ± 0.04^{b}
TVB-N	Control	3.6 ± 0.11^{a}	16.06 ± 0.34^{a}	25.5 ± 0.57^{a}	33.46± 0.46^{a}
	1% CO	3.49 ± 0.09^{a}	9.41 ± 0.26^{b}	18.81 ± 0.14^{b}	20.18± 0.52^{b}
	2% CO	3.42 ± 0.08^{a}	8.44 ± 0.14^{b}	16.8 ± 0.29^{b}	19.74 ± 0.21^{b}
	1% WGO	3.52 ± 0.10^{a}	9.93 ± 0.29^{b}	19.17 ± 0.19^{ab}	21.62 ± 0.47^{b}
	2% WGO	3.47 ± 0.08^{a}	8.99 ± 0.18^{b}	17.65 ± 0.19^{b}	19.99 ± 0.13^{b}
TBA	Control	0.08 ± 0.01^{a}	0.86 ± 0.06^{a}	1.08 ± 0.46^{a}	1.67± 0.03^{a}
	1% CO	0.075 ±0.005^{a}	0.525 ± 0.035^{b}	0.775 ± 0.049^{b}	0.94± 0.03^{b}
	2% CO	0.07 ± 0.01^{a}	0.43 ± 0.02^{c}	0.735 ± 0.015^{c}	0.85 ± 0.03^{c}
	1% WGO	0.08 ± 0.01^{a}	0.58 ± 0.04^{b}	0.853 ± 0.015^{b}	0.95 ± 0.06^{b}
	2% WGO	0.075 ± 0.015^{a}	0.49 ± 0.02^{b}	0.773 ± 0.025^{b}	0.89 ±0.025^{bc}

$^{(a,b,c)}$ different superscript letters in the same column (of each parameter) indicate significant differences ($p < 0.05$).
CO= Cinnamon oil.
WGO= Wheat germ oil.

Table 2: Effect of CEOand WGO on aerobic plate count (APC) log $_{10}$ CFU/g of chilled sausage stored at 3±1 ^{O}C.

Groups	Zero day	3rd day	6th day	9th day
Control	5.07 ± 0.18^{a}	5.47± 0.21^{a}	6.32 ± 0.29^{a}	7.23 ± 0.34^{a}
1% CO	4.79 ± 0.17^{a}	5.12 ± 0.18^{a}	5.90 ± 0.27^{b}	6.89 ± 0.32^{a}
Reduction count	0.28	0.35	0.42	0.34
2% CO	4.54 ± 0.2^{b}	4.95 ± 0.19^{b}	5.55 ± 0.31^{c}	6.11 ± 0.31^{c}
Reduction count	0.53	0.52	0.77	1.12
1% WGO	4.86 ± 0.19^{a}	5.22 ± 0.18^{a}	6.02 ± 0.24^{ab}	7.10 ± 0.36^{a}
Reduction count	0.21	0.25	0.30	0.13
2% WGO	4.63 ± 0.17^{ab}	5.02 ± 0.19^{b}	5.86 ± 0.28^{b}	6.45 ± 0.31^{b}
Reduction count	0.44	0.45	0.46	0.78

$^{(a,b,c)}$ different superscript letters in the same column indicate significant differences ($p < 0.05$).
Reduction count = control group – treated group

Table 3: Effect of CEOand WGO on *Enterobacteriaceae* count log $_{10}$ CFU/g of chilled sausage stored at 3±1 ^{O}C.

Groups	Zero day	3rd day	6th day	9th day
Control	3.06 ± 0.17^{a}	3.95± 0.19^{a}	4.69 ± 0.23^{a}	5.43 ± 0.28^{a}
1% CO	2.78 ± 0.18^{a}	3.54 ± 0.17^{ab}	4.19 ± 0.21^{b}	5.01 ± 0.27^{b}
Reduction count	0.28	0.41	0.50	0.42
2% CO	2.56 ± 0.17^{b}	3.36 ± 0.14^{b}	3.95 ± 0.24^{b}	4.24 ± 0.26^{c}
Reduction count	0.50	0.59	0.74	1.19
1% WGO	2.84 ± 0.16^{a}	3.67 ± 0.16^{a}	4.28 ± 0.20^{ab}	5.12± 0.23^{a}
Reduction count	0.22	0.28	0.41	0.31
2% WGO	2.73 ± 0.15 ^{ab}	3.44 ± 0.15^{b}	4.04 ± 0.23^{b}	4.35 ± 0.24^{c}
Reduction count	0.33	0.51	0.65	1.08

$^{(a,b,c)}$ different superscript letters in the same column indicate significant differences ($p < 0.05$).
Reduction count = control group – treated group

Table 4: Effect of CEOand WGO on *Staphylococcus* count log $_{10}$ CFU/g of chilled sausage stored at 3±1 ^{O}C.

Groups	Zero day	3rd day	6th day	9th day
Control	3.56 ± 0.23^{a}	4.12± 0.21^{a}	4.85 ± 0.22^{a}	5.12 ± 0.23^{a}
1% CO	3.21 ± 0.24^{a}	3.75 ± 0.19^{a}	4.45 ± 0.19^{a}	4.68 ± 0.21^{b}
Reduction count	0.35	0.37	0.4	0.44
2% CO	2.94 ± 0.21^{b}	3.54 ± 0.21^{b}	3.86 ± 0.22^{b}	4.08 ± 0.24^{c}
Reduction count	0.62	0.58	0.99	1.04
1% WGO	3.26 ± 0.23^{a}	3.81 ± 0.18^{a}	4.49 ± 0.23^{a}	4.71± 0.25^{ab}
Reduction count	0.3	0.31	0.36	0.41
2% WGO	3.05 ± 0.19^{ab}	3.62 ± 0.18^{b}	3.91 ± 0.21^{b}	4.25± 0.23^{b}
Reduction count	0.51	0.50	0.86	0.87

$^{(a,b,c)}$ different superscript letters in the same column indicate significant differences ($p < 0.05$).

Reduction count = control group – treated group

Table 5: Effect of CEOand WGO on *Enterococcus* count log $_{10}$ CFU/g of chilled sausage stored at 3±1 ^{O}C.

Groups	Zero day	3rd day	6th day	9th day
Control	3.22 ± 0.18^{a}	3.55 ± 0.24^{a}	4.12 ± 0.24^{a}	4.98 ± 0.25^{a}
1% CO	2.95 ± 0.19^{a}	3.23 ± 0.18^{ab}	3.74 ± 0.21^{ab}	4.51 ± 0.23^{b}
Reduction count	0.27	0.32	0.38	0.47
2% CO	2.82 ± 0.22^{b}	2.98 ± 0.19^{b}	3.53 ± 0.21^{b}	3.98 ± 0.23^{c}
Reduction count	0.4	0.57	0.59	1.00
1% WGO	2.98 ± 0.18^{a}	3.26 ± 0.21^{a}	3.76 ± 0.22^{a}	4.59± 0.22^{a}
Reduction count	0.24	0.29	0.36	0.39
2% WGO	2.89 ± 0.20^{ab}	3.02 ± 0.19^{b}	3.58 ± 0.23^{b}	4.08± 0.21^{b}
Reduction count	0.33	0.53	0.54	0.90

$^{(a,b,c)}$ different superscript letters in the same column indicate significant differences ($p < 0.05$).

Reduction count = control group – treated group

Bacteriological Parameters

The APC was increased with the extension of chilling time. On the 6th day of chilling, the counts were increased in control group than the other treated groups. On the 9th day, the counts were 7.23 ± 0.34, 6.89 ± 0.32, 6.11 ± 0.31, 7.10 ± 0.36 and 6.45 ± 0.31 log $_{10}$ CFU/g in control, 1% CO, 2% CO, 1% WGO and 2% WGO, respectively. The greater reduction count was obtained in 2% CEOtreated groups at all chilling periods (Table 2).

Enterobacteriaceae counts at zero day were decreased from 3.06 ± 0.17 in control group to 2.78 ± 0.18, 2.56 ± 0.17, 2.84 ± 0.16 and 2.73 ± 0.15 in 1% CEO, 2% CEO, 1% WGO respectively and 2% WGO with high reduction count of 0.50 log in 2% CEO. The counts were increased in all treated groups but the counts still lower than the control group on 9th day. The best reduction counts achieved for *Enterobacteriaceae* count was under the effect of 2% CEOby 0.5, 0.59, 0.74 and 1.19 log at zero-time, 3rd, 6th and 9th day of chilling, respectively (Table 3).

Staphylococcus count decreased from 3.56 ± 0.23 in control groups to 3.21 ± 0.24, 2.94 ± 0.21, 3.26 ± 0.23 and 3.05 ± 0.19 log$_{10}$CFU/g in the treated group at zero-time. The counts increased gradually by the time, but the count in all treated groups was lower than in control group (Table 4). The counts of *Enterococcus* have the same pattern of increasing in control group more than treated group. The reduction in *Enterococcus* population increased by using of cinnamon oil 2% all over the chilling days (Table 5).

DISCUSSION

The pH value is an important physicochemical characteristic to decide the quality and shelf life of sausage. In the current study, the pH of sausage at zero time in all examined groups were in agreement with Ferrari and Torres (2002) who reported that pH value in sausage samples collected from São Paulo (Brazil) ranged from 5.08 to 6.48. From the 3rd day, the pH in control group significantly increased ($p< 0.05$) than treated groups. These results were coincided with Shaltout et al. (2017) who found that addition of CEOin concentrations of 0.5, 1 and 1.5% decreased the pH of stored minced meat. The increase in pH values in control groups over the treatment groups may be attributed to the activity of essential oils in treated group against

bacteria acting on protein with formation of ammonia leading to increase in pH (Valencia et al., 2008). Protein and non-protein nitrogenous materials in sausage are degraded by several enzymatic action and microbial activity, which lead to the production of volatile nitrogen.

The amount of TVB-N is good chemical indicator to assess the quality and freshness of sausage. The TVB-N values at zero time in control group were nearly similar to values obtained in oriental sausage samples collected from Egypt (Girgis et al., 2015; Saleh et al., 2017). On the 9th day the TVB-N values were coincide with 18 mg VBN/100 g in stored Chinese-style sausage reported by Lin and Lin (2002). Significant increase ($p< 0.05$) in TVB-N content of control group comparing with 1% CO, 2% CEOand 2% WGO treated groups appeared from the 3rd day of chilling. These findings may be attributed to the rapid growth and multiplication of bacteria acting on protein in control groups which led to degradation of protein and the formation of free amines, trimethylamine, dimethylamine and ammonia (Saleh et al., 2017). The TBA is an important measure that indicates the degree of lipid oxidation state and rancidity. In the current study the TBA at zero time nearly similar to the result obtained by Khan and Ahmad (2015) who found that TBA in buffalo meat sausage was in the range of 0.093 mg MDA / kg. Using of essential oil CEOand WGO in both concentration 1 and 2% reduced TBA values in treated groups ($P< 0.05$) compared with the control group from the 3rd day of chilling. Nearly similar finding in minced meat treated with cinnamon oil was reported by Shaltout et al. (2017). The effect of wheat germ oil as antioxidant in chicken nuggets were reported by Arshad et al. (2017). The antioxidant effect of essential oils is due to high percentage of phenolic compounds which can efficiently retard oxidative reactions (Sharma et al., 2017).

Cinnamon EO as an antimicrobial on meat has been studied in treatment of mutton patties using 0.25% CEO (Luo et al., 2007) and chicken fillet using 1% CEO (Babuskin et al., 2014). Total bacterial count is a commonly used microbiological method for estimating shelf life of food. In the current study APC at zero time in control group was similar to 5.18 $\log_{10}$ CFU/g in sliced dry sausages in Addis Ababa, Ethiopia (Assaye and Ashenafi, 2014). However, the count was slightly lower than 6.3 to 6.4 $\log_{10}$CFU /g in sausages sold in Nigeria (Oluwafemi and Simisaye, 2006). These counts of control group increased on 3rd day till 9th day of the experiment and there was a significant decrease in 2% CEO at zero day, on the 3rd day significant decreases in 2% CEO and 2% WGO, meanwhile on 6th day significant decreases in 1% CEO, 2% CEO and 2% WGO when compared with control group. Nearly similar effect of cinnamon on APC obtained during chilling of minced meat (Shaltout et al., 2017). The effect of cinnamon oil is due to presence of cinnamaldehyde which poses antibacterial activity (Quattara et al., 2000). The effect of wheat germ oil due to dimethoxy benzoquinone (Kim et al., 2010).

Presence of *Enterobacteriaceae* indicates enteric contamination and reflect the bad hygienic quality of raw food and may cause a public health hazard to consumers as well as deteriorative changes of meat, the presence of a considerable count of *Enterobacteriaceae* indicates inadequate processing and post processing recontamination (ICMSF, 1980). The *Enterobacteriaceae* counts in the present study were comparable to 3.4 and 3.9 $\log_{10}$ CFU/g (Guillier et al., 2013) in France and 3.77$\log_{10}$CFU/g (Sofy et al., 2017) in ready-to-eat foods in Egypt.

The significant decreases in *Enterobacteriaceae* count at zero time ($p< 0.05$) occurred in 2% CO, on 3rd day occurred in 2% CEOand 2% WGO, meanwhile on 6th and 9th day occurred in 1% CO, 2%CEOand 2%WGO when compared with control group of the same chilling day. Nearly similar effect of cinnamon on *Enterobacteriaceae* count were obtained during chilling of minced meat by Shaltout et al. (2017). In addition, Quattara et al. (2000) reported the effect of cinnamon oil to inhibit *Enterobacteriaceae* on cooked meat products. The activity of wheat germ described on members of *Enterobacteriaceae* like *Escherichia coli* KCTC2593, *Salmonella typhimurium* KCTC2054 (Kim et al., 2010).

Presence of high number of *Staphylococcus*/g of sausage may indicate a tendency towards presence of *S.aureus* and predication of health hazard. At zero-time significant reduction ($p<0.05$) in *Staphylococcus* count in 2% CEOtreated group by 0.62 than control group. Significance difference was reported on 3rd day extend to 2% CEOand 2% WGO treated groups. On the 6th and 9th day of chilling significant reductions ($p<0.05$) in 2% CEOand 2%WGO treated groups when compared with control group. In vitro studies proved that wheat germ essential oil able to be potent inhibitor against *Staphylococcus aureus* KCTC1927 and *Staphylococcus aureus* (NCINB 50080) (Kim et al., 2010; El-Moez et al., 2013).

Enterococcus can be used as an indicator of both fecal contamination of foods and the dissemination of antimicrobial resistance related to the use of antimicrobials in farming (Van den Bogaard et al., 2002). The results achieved in the current study declared that the *Enterococcus* counts at zero time were progressively increased on the 6th and 9th day of chilling. Nearly similar count of 3.68± 0.19 log CFU/ g was reported in beef sausage (Selvan et al., 2007) in Chennai city, India.

At zero-time significant reduction ($p< 0.05$) occurred in

2%CEOtreated group. On the 3rd day of chilling significant reduction in *Enterococcus* count (p<0.05) occurred in 2% CEOand 2% WGO, respectively. Using of 2% CEOand 2% WGO significantly reduced the *Enterococcus* counts (P<0.05) on comparing with the other groups on the 6th day of chilling. On the 9th day of chilling significant decreases (p<0.05) achieved in 1% CO, 2% CEOand 2% WGO. The effect of essential oils on *Enterococcus* is due leakage of cellular components after damaging of cell membrane protein, interfering with integrated enzymes in membrane, leading to coagulation of cytoplasm (Raccach, 1984). The depleting of the proton motive force, changing phospholipid constituents and fatty acid, impairing enzymatic mechanisms used for energy production and metabolism, altering electron transport and nutrient uptake (Taniguchi et al., 1988).

CONCLUSION

Essential oils could inhibit the growth and multiplication of studied bacteria in chilled sausage. Cinnamon and wheat germ oil at concentration 2% have the ability to minimize chemical changes of chilled sausage. Therefore, using of these essential oils in sausage formulation could be considered as a promising solution to be used instead of chemical preservative.

ACKNOWLEDGEMENTS

The authors are grateful to staff members at Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Egypt for their kind support.

CONFLICT OF INTEREST

None of the authors has any conflict of interest to declare.

AUTHORS CONTRIBUTION

All authors contributed equally in conceptualization; data analysis; writing original draft & editing. All authors have read and agreed to the published version of the manuscript.

REFERENCES

- Arshad MS, Imran A, Nadeem MT, Sohaib M, Saeed F, Anjum FM, Ho Kwon J, Hussain S (2017). Enhancing the quality and lipid stability of chicken nuggets using natural antioxidants. Lipids. Health. Dis. 16:108-119. https://doi.org/10.1186/s12944-017-0496-4
- Assaye H, Ashkenazi M (2014). Microbiological profile of retail sliced dry sausages in Ethiopia. Int. Food. Res. J. 21 (6):2473- 2479.
- Babuskin S, Babu PAS, Sasikala M, Sabina K, Archana G, Sivarajan M, Sukumar M (2014). Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. Int. J. Food. Microbiol. 171: 32-40. https://doi.org/10.1016/j.ijfoodmicro.2013.11.011
- Chang ST, Chen PF, Chang SC (2001). Antibacterial activity of leaf essential oils and their constituents from Cinnamomum. osmophloeum. J. Ethnopharmacol.77 (1): 123-127. https://doi.org/10.1016/S0378-8741(01)00273-2
- (EOS) Egyptian Organization for Standardization (2006a). Methods of analysis and testing for meat. Part 9: determination of total volatile nitrogen (TVN).ES:63-9/2006.
- (EOS) Egyptian Organization for Standardization (2006b). Methods of analysis and testing for meat. Part 10: determination of thiobarbituric acid (TBA). ES: 63-10/2006.
- El-Moez SIA, Abdelmonem MA, Gomaa AM, Aziz MFA (2013). In vitro antibacterial activities of dietary medicinal ethanolic extracts against pathogenic reference strains of animal origin. Afr. J. Microbiol. Res.7 (46):5261-5270. https://doi.org/10.5897/AJMR2013.5477
- Ferrari CKB, Torres EAFS (2002). Lipid oxidation and quality parameters of sausages marketed locally in the town of S ăo Paulo (Brazil). Czech. J. Food. Sci. 20 (4):144-150. https://doi.org/10.17221/3525-CJFS
- Girgis AY, Hussein SA, Shahin MFSA (2015). Improving the properties of beef sausage. Life. Sci. J. 12(1):38-43.
- Guillier L, Danan C, Bergis H, Delignette-Muller M, Granier S, Rudelle S, Beaufort A, Brisabois A (2013). Use of quantitative microbial risk assessment when investigating foodborne illness outbreaks: The example of a monophasic Salmonella Typhimurium 4,5, 12: i: – outbreak implicating beef burgers. Int. J. Food. Microbiol. 166 (3):471-478. https://doi.org/10.1016/j.ijfoodmicro.2013.08.006
- Hussein MA, El-Ghareeb WR, Nasr MA (2018). The effect of rosemary extract and lactic acid on the quality of refrigerated broiler fillets. J. Food Sci. Technol. 55 (12):5025-5034. https://doi.org/10.1007/s13197-018-3441-2
- ICMSF (International Commission on Microbiological Specifications for Foods) (1980). Microbial Ecology of Foods. Academic Press, New York.
- ISO 21528-2 (2004). Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *Enterobacteriaceae*, part 2: colony count method. Enumeration ISO, Geneva.
- ISO 4833-1 (2013). Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30° C by the pour plate technique.
- ISO 6887-2 (2003). Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1-3: Specific rules f or the preparation of meat and meat products.
- ISO 6888-1 (1999) Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive Staphylococci (*Staphylococcus* aureus and other species) - Part 1: Technique using Baird-Parker agar medium.
- ISO 7899-2 (2000). Water quality - Detection and enumeration of intestinal enterococci - Part 2: Membrane filtration method. Geneva, Switzerland.
- Khan I, Ahmad S (2015). Studies on physicochemical properties of cooked buffalo meat sausage as influenced by incorporation

of carrot powder during refrigerated storage. J. Food. Proc. Technol. 6 (436):2.
- Kim MH, Jo SH, Ha KS, Song JH, Jang HD, Kwon YI (2010). Antimicrobial activities of 1, 4-benzoquinones and wheat germ extract. J. Microbiol. Biotechnol. 20 (8): 1204-1209. https://doi.org/10.4014/jmb.1004.04037
- Lin KW, Lin SN (2002). Effects of sodium lactate and trisodium phosphate on the physicochemical properties and shelf life of low-fat Chinese-style sausage. Meat. Science. 60 (2):147-154. https://doi.org/10.1016/S0309-1740(01)00116-4
- Liu RH (2007). Whole grain phytochemicals and health. J. Cereal. Sci.46 (3): 207-219. https://doi.org/10.1002/9780470277607.ch15
- Luo H, Lin S, Ren F, Wu L, Chen L, Sun Y (2007). Antioxidant and antimicrobial capacity of Chinese medicinal herb extracts in raw sheep Meat. J. Food. Prot.70 (6): 1440-1445. https://doi.org/10.4315/0362-028X-70.6.1440
- Mahmoud AA, Mohdaly AA, Elneairy NA (2015). Wheat germ: an overview on nutritional value, antioxidant potential and antibacterial characteristics. Food. Nutri. Sci. 6 (2): 265-277. https://doi.org/10.4236/fns.2015.62027
- Oluwafemi F, Simisaye MT (2006). Extent of microbial contamination of sausages sold in two Nigerian cities. AJBR. 9 (2):133-136. https://doi.org/10.4314/ajbr.v9i2.48895
- Pearson D (2006). Chemical Analysis of Foods. 11th Ed, Publishing Co., Churchill Livingstone, Edinburgh, London, United Kingdom.
- Quattara B, Simard RE, Piette JPG, Begin A, Holley RA (2000). Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. Int. J. Food. Microbiol. 62(1-2):139-148. https://doi.org/10.1016/S0168-1605(00)00407-4
- Raccach M (1984). The antimicrobial activity of phenolic antioxidants in food: A review 1. J. Food. Safety. 6 (3) :141-170. https://doi.org/10.1111/j.1745-4565.1984.tb00479.x
- Saleh EA, Morshdy AME, Hafez AE, Hussein MA, Elewa ES, Mahmoud AF (2017). Effect of pomegranate peel powder on the hygienic quality of beef sausage. J. Microbiol. Biotech. Food. Sci. 6 (6): 1300-1304. https://doi.org/10.15414/jmbfs.2017.6.6.1300-1304
- Selvan P, Babu R N,Sureshkumar S, Venkataramanujam V (2007). Microbial quality of retail meat products available in chennai city. Am. J. Food. Technol. 2 (1):55-59. https://doi.org/10.3923/ajft.2007.55.59
- Shaltout FA, Thabet M G, Koura H A (2017). Impact of Some Essential Oils on the Quality Aspect and Shelf Life of Meat. BVM J. 33(2): 351-364. https://doi.org/10.21608/bvmj.2017.30503
- Sharma H, Mendiratta SK, Agarwal RK, Kumar S, Soni A (2017). Evaluation of anti-oxidant and anti-microbial activity of various essential oils in fresh chicken sausages.J. Food. Sci. Technol. 54 (2):279-292. https://doi.org/10.1007/s13197-016-2461-z
- Sofy AR, Sharaf AEMM, Al Karim AG, Hmed AA, Moharam KM (2017). Prevalence of the harmful Gram-negative bacteria in ready-to-eat foods in Egypt. Food. Public Health. J.7(3): 59-68.
- Taniguchi M, Yano Y, Tada E, Ikenishi KOS, Haraguchi H, Hashimoto K, Kubo I (1988). Mode of action of polygodial, an antifungal sesquiterpene dialdehyde. Agric. Biol. Chem. 52(6):1409-1414. https://doi.org/10.1080/00021369.1988.10868863
- Valencia I, O'Grady MN, Ansorena D, Astiasarán I, Kerry JP (2008). Enhancement of the nutritional status and quality of fresh pork sausages following the addition of linseed oil, fish oil and natural antioxidants. Meat Science. 80 (4): 1046-1054. https://doi.org/10.1016/j.meatsci.2008.04.024
- Van den Bogaard AE, Willems R, London N, Top J, Stobberingh EE (2002). Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. J. Antimicrobial. Chemoth. 49 (3):497-505. https://doi.org/10.1093/jac/49.3.497