Effect of Some Essential Oils on the Bacteriological Quality of Some Chicken Meat Products

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A B S T R A C T

A total of 1500g of the fresh minced chicken fillet was purchased from butcher shops in El Menofiya Governorate and directly transferred to the laboratory under complete aseptic condition. Experimental trials were pointed toward the ability to control the outgrowth of *Staph aureus* in chicken fillet using *Nigella sativa* oil (0.1% and 0.5%) and *rosemary oil* (0.1% and 0.5%). The sample was divided into 5 groups. All groups were injected with *Staph. aureus* reference strain (ATCC®25923) and the initial load was $2.6 \times 10^7 \pm 1.04 \times 10^7$ cfu/g. Control group was inoculated with the tested culture and was stored without treatment with oils. Both oils reduced *Staph. aureus* levels significantly from zero day till 6th day in the following order: *Nigella sativa 0.5% > Nigella sativa 0.1% > Rosemary 0.5% > Rosemary 0.1%*. In contrast in the control group, the count increased rapidly till the 4th day of the treatment. Moreover, both oils improved the overall acceptability and prolonged the shelf life of treated samples as it remains without putrefactive changes till 6th day in comparison with control samples which remain without putrefactive changes till 4th day only. Finally, it can be concluded that essential oils possess significant antibacterial activity against the *Staph. aureus* and increases with increasing their concentration.

Key words: Preservatives, Nigella sativa, Rosemary.

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1. INTRODUCTION

Meat preservation methods became a necessary to transport meat for long distances without spoiling and loss of nutritional value (Nychas *et al*., 2008).

Traditional chemical or physical preservation processes are very often; but they have various disadvantages such as deterioration of product quality, high degree of reactivity, potential health risks, and consumer product safety concerns, high cost or adverse sensory changes (Lyon *et al*., 2007).This explains why manufacturers have turned now to use the natural extracts after the consumer refuse to chemical additives because of their serious health problems (Amin *et al*., 2015).

Because of the awareness among the consumers, they prefer the meat without any chemical preservatives. Nowadays it has been proven that the best alternative for the chemical preservatives are the essential oils of spices, which can stabilize the meat from

microbial deterioration (Jagadeesh *et al*., 2012).

Essential oils and their extracts can be used as natural additives to reduce the use of chemical preservatives and to reduce their risks. Such materials can extend the shelf life of meat and their products and control/inhibit the microbial growth (Stiles and Hastings, 1991**)**. Many of the essential oils have potential benefits in food production since they showed antibacterial, antifungal and antioxidant effects (Politeo *et al*., 2007).

Different essential oils of different plants showed an effective antimicrobial effects on bacterial count when they are applied in meat products during storage (Angioni et al., 2004).

Nigella sativa (black cumin), belonging to family *Ranunculaceae*, is famous for its medicinal properties. Seeds of *Nigella sativa* contain alkaloids, volatile as well as fixed oils and a variety of pharmacologically active substances like thymoquinone, dithymoquinone, carvacrol, thymol, nigellicine-N-oxide, nigellidine and α-hedrin. Black cumin is also enriched with the fat content of 35.5%; the seeds of *Nigella sativa* contain volatile oil (0.5-1.6%), fixed oil (35.6-41.6%), protein and amino acids (22.7%) (Azeem *et al*., 2014).

Rosemary (Rosmarinus officinalis) is a small ever-green bush, belonging to Labiatae family. It grows principally in the basin of the Mediterranean Sea. Active substances which were contained in Rosmarinus yield have a series of properties, desirable from the point of view of the food industry and medicinal phytology (Djeddi *et al*., 2007).

2. MATERIALS AND METHODS

2.1. Strain used:

Staphylococcus aureus reference strain (ATCC®25923), used in this study, was obtained from Bacteriology Unit, Reference Laboratory For Veterinary Quality Control of Poultry Production, Animal Health Research Institute, Dokki, Giza, Egypt.

2.2. Essential oils:

Rosemary and *Nigella sativa* oils were purchased from Cap Pharma for Extraction Natural Oils, Plants and Cosmotics. License of Ministry of Health No.33/2006. The concentrations were adjusted to 0.1% and 0.5% for each.

2.3. Minced chicken fillet:

A grand total of 1500g of the fresh minced chicken fillet used in this study was purchased from butcher shops in El Menofiya Governorate. The purchased minced fillet was divided into 5 groups (100gm).

The 5 groups were arranged as follows:

- 1st subgroup: was treated with s. *aureus* and 0.1% of *Nigella sativa* oil.
- 2nd subgroup: was treated with s. *aureus* and 0.5% of *Nigella sativa* oil.
- 3rd subgroup: was treated with s. *aureus* and 0.1% of *rosemary* oil.
- 4th subgroup: was treated with s. *aureus* and 0.5% of *rosemary* oil.
- Control: was inoculated with the tested culture *(S.aureus)* and was stored without treatment as (Control +ve).

2.4. Experimental application:

- All five groups were inoculated with *S. aureus* reference strain with infective dose 10^6 cfu/gm**,** then mixed thoroughly by gently squeezing the bags by hand and leaved for 30 minutes for complete attachment between microorganisms and minced chicken fillet.
- Initial load of *S. aureus* was detected before addition of essential oils. It was 2.6 x $10^7 \pm 1.0^4$ x 10^7 CFU/g.
- The essential oils were added with their certain concentrations to the samples then

leave them for a further 30 minutes to ensure even mixing.

- Each sample was packed in polyethylene bag, labeled and stored in refrigerator chamber at 4 ºC.
- The bacterial *S. aureus* count was estimated at zero day and every 48 hrs. to evaluate the effect of the essential oils treatments. Also, organoleptic examination (color, odor, texture and overall acceptability) were conducted after 3 hours and every day 24 hrs intervals during storage until spoilage of samples. (Penny *et al*., 1993).
- Tests were performed in triplicate.

2.5. Statistical analysis:

The data was statistically treated by one way ANOVA using SPSS program for windows (Version 16) (SPSS Inc. Chicago, IL and USA).

3. RESULTS

Results in Table (1) showed that the overall acceptability in case of using *Nigella Sativa* oil at the concentrations of 0.1% and 0.5%, the scores were 7, 6, 5, 5, 4, 4, 3 and 7, 7,7, 6, 6, 5, 4 at zero day,1st, 2nd, 3rd, 4th , 5th day and 6th day of the storage period respectively, while in case of using *Rosemary* oil at the concentrations of 0.1% and 0.5%, the scores were 6, 5, 4, 3, 3, 2, 1 and 7, 5, 5, 4, 4, 3, 2 at zero day,1st, 2nd, 3rd, 4th , 5th day and 6th day of the storage period, respectively, comparing to the scores of overall acceptability in the control samples which were 6, 4, 4, 3, 2, 1, 1 at zero day,1st, 2nd, 3rd, 4th , 5th day and 6th day of the storage period, respectively.

The results in Table (2) noted that the initial load of *Staph. aureus* after inoculating the reference strain in the sample is 2.6 x 10^7± 1.04x 10^7 CFU/g. In case of *Nigella sativa oil at* the concentrations of 0.1% and 0.5%, the mean *Staph. aureus* levels decreased to 1.8 x 10^7 ± 3.0 x 10^6 cfu/g and 1.2 x 10^7 ±1.5 x 10^6 cfu/g at zero day, and to 8.1 x 10^6 ±2.2 x 10^6 cfu/g and 5.2 x 10^6 ± 1.0 x 10^6 cfu/g in 2nd day, and to 9.2 x 10^5 ± 3.8 x 10^5 cfu/g and 8.6 x 10^4 ± 1.9 x 10^4 cfu/g in 4th day, and to 7.1 X10^5 ± 3.8 x 10^5cfu/g and 2.7 x 10^3± 1.0 X10^3 cfu/g in 6th day, respectively. In case of *Rosemary* oil at the concentrations of 0.1% and 0.5%, the mean *Staph. aureus* levels decreased to 2.4 x 10^7 ±7.2 x 10^6cfu/g and 1.9 x 10^7 ±1.5 x 10^6 cfu/g at zero day, and to 2.1 x 10^7 ± 5.5 x 10^6cfu/g and 8.3 x 10^6 ±0.4x 10^6cfu/g in 2nd day and to 6.2 x 10^6 ± 2.4 x 10^6cfu/g and 2.7 x 10^6 ±6.8 x 10^5cfu/g in 4th day, and to 2.9 x 10^6 ± 2 x 10^5/g and 9.7 x 10^5 ±1.1 x 10^5cfu/g in 6th day, respectively. In control, the count was 2.8x 10^7± 6.5 x 10^6, 1.6 x 10^8 ± 2.6 x 10^7 and 8.3 x 10^8 ± 3.4 x 10^8 at zero, 2nd and 4th day.

The results recorded in Table (3) and Figure (1) revealed that the reduction % of *Staph. aureus* with *Nigella sativa 0.1%, Nigella sativa 0.5%, Rosemary 0.1% and Rosemary 0.5%* treatment *is* (30.77%, 53.85%, 7.69%, 26.92%) at zero day, and (68.86%, 80.00%, 19.23%, 68.07%) at 2nd day, and (96.46%, 99.67%, 76.15%, 89.61%) at 4th day, and (97.27%, 99.99%, 85.00%, 96.27%) at 6th day.

Table (1): overall acceptability of minced chicken fillet inoculated with *Staph. aureus* and treated with different concentrations of *Nigella Sativa* and *Rosemary* during cold storage at 4ºC.

Groups	oil conc.	Zero day	1st day	2nd day	3rd day	4th day	5th day	6th day
Control	–	6	4	4	3	2	1	1
Nigella Sativa	0.1%	7	6	5	5	4	4	3
	0.5%	7	7	7	6	6	5	4
Rosemary	0.1%	6	5	4	3	3	2	1
	0.5%	7	5	5	4	4	3	2

Score System for Sensory Evaluation:

9: Excellent 6: Good 3: Poor

8: Very very good 5: Medium 2: Very poor

7: Very good 4: Fair 1: Very very poor

Table (2): Antimicrobial effect of different concentrations of *Nigella Sativa* and *Rosemary* essential oils against *Staph. aureus* count artificially inoculated in minced chicken fillet during cold storage at 4ºC.

Durations	Control	*Nigella sativa* oil 0.1%	*Nigella sativa* oil 0.5%	Rosemary oil 0.1%	Rosemary oil 0.5%
Zero day ^{NS}	$2.8x\ 10^{7a}$ $\pm 6.5\ X10^{6}$	1.8×10^{7a} $\pm 3.0 \times 10^{6}$	1.2×10^{7a} $\pm 1.5 \times 10^{6}$	2.4×10^{7a} $\pm 7.2 \times 10^{6}$	1.9×10^{7a} $\pm 1.5 \times 10^{6}$
2nd day $^{++}$	1.6×10^{8a} $\pm 2.6X10^{7}$	8.1×10^{6b} $\pm 2.2 \times 10^{6}$	5.2×10^{6b} $\pm 1.0 \times 10^{6}$	$2.1x\ 10^{7b}$ $\pm 5.5 \times 10^{6}$	8.3×10^{6b} $\pm 0.4x\ 10^{6}$
4th day $^{+}$	8.3×10^{8a} $\pm 3.4 \times 10^{8}$	9.2×10^{5b} $\pm 3.8 \times 10^{5}$	8.6×10^{4b} $\pm 1.9 \times 10^{4}$	6.2×10^{6b} $\pm 2.4 \times 10^{6}$	$2.7x\ 10^{6b}$ $\pm 6.8 \times 10^{5}$
6th day $^{++}$	-	7.1×10^{5a} $\pm 3.8 \times 10^{5}$	2.7×10^{3a} $\pm 1.0\ X10^{3}$	3.9×10^{6b} $\pm 2.0 \times 10^{5}$	9.7×10^{5a} $\pm 1.1 \times 10^{5}$

Initial load of *Staph. aureus* = $2.6 \times 10^{7} \pm 1.04 \times 10^{7}$ CFU/g

The values represent Mean counts ± S.E. (CFU/g) of three experiments.

Means within the same row not bearing the same superscripts are significantly different.

NS = Non significant differences.

+ = Significant differences ($P<0.05$)

++ = High significant differences ($P<0.01$)

Table (3): Reduction % of *Staph. aureus* count artificially inoculated into minced chicken fillet samples treated with different concentrations of *Nigella Sativa* and *Rosemary* essential oils.

Durations	*Nigella sativa* oil (0.1%)	*Nigella sativa* oil (0.5%)	Rosemary oil (0.1%)	Rosemary oil (0.5%)
Zero day	30.77	53.85	7.69	26.92
2nd day	68.86	80.00	19.23	68.07
4th day	96.46	99.67	76.15	89.62
6th day	97.27	99.99	85.00	96.27

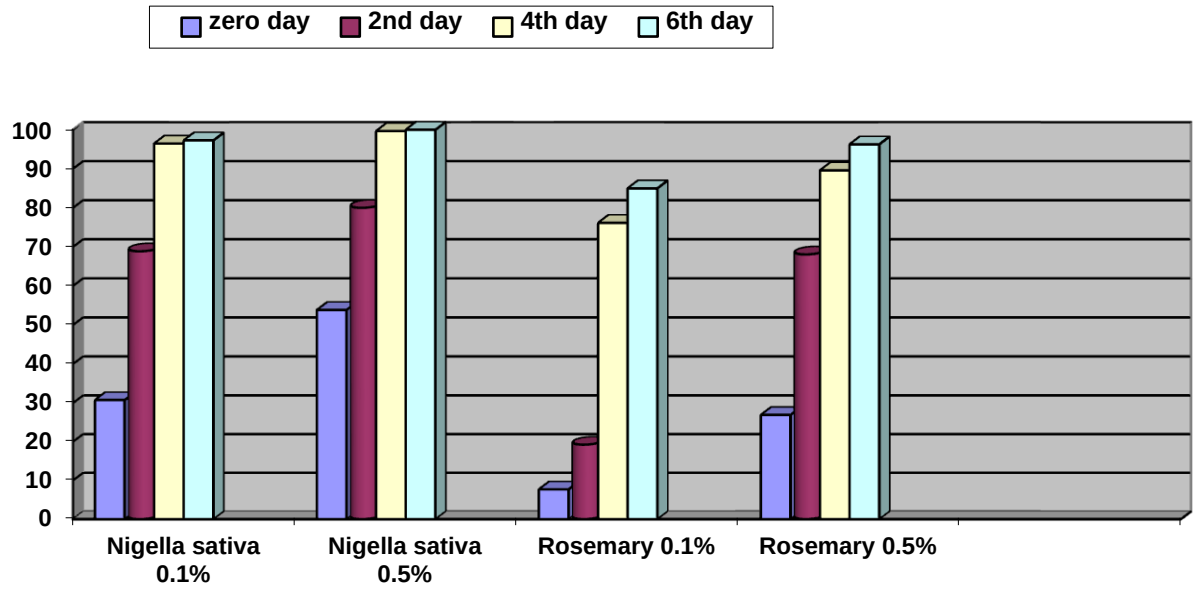

Figure (1): Reduction % of *Staph. aureus* count artificially inoculated into minced chicken fillet samples treated with different concentrations of *Nigella Sativa* and *Rosemary* essential oils.

4. DISCUSSION

There is an increase in using plant-origin food-preservative essential oils since the 1990s, with more utilization of spices and their essential oils as natural bio-preservatives, to increase shelf life and overall quality of food products (Simitzis *et al*., 2008).

Sensory evaluation is an easy, quick and efficient method for getting idea about the quality of the product and its overall acceptance; sensory methods were used to assess the degree of freshness based on organoleptic characteristics such as color, odor, texture and overall acceptability of the product (Haq *et al*., 2013).

From the obtained results in Table (1) there was a decline of sensory attributes begin after the first day of storage with marked reduction of overall acceptability values in the control samples at the 4th day of storage.

Generally, it is obvious that the sensory attributes and shelf life of different treated minced chicken fillet samples during cold storage (4ºC) were improved by using different concentrations of *Nigella sativa* and *Rosemary* oils, compared to the control samples during the storage period. Samples treated with *Nigella Sativa* oil revealed the higher improvement of sensory attributes than samples treated with *Rosemary* oil.

These results nearly agreed with Amin (2013) who illustrated that different essential oils improve the sensory attributes of treated meat samples. Also, she concluded that *Rosemary oil* give lowest effect on sensory attributes of treated samples.

Both type of essential oils prolonged the shelf life of the treated samples (as samples remain without putrefactive changes till 6th day while in control it remain without putrefactive changes till 4th day).

Regarding the results recorded in Table (2), the mean *Staph. aureus* levels in the examined samples in the four groups treated by the essential oils (each of *Nigella sativa* and *Rosemary* 0.1% and 0.5%) were significantly reduced from zero day after treatment till 6th day. The mean bacterial count at 6th day recorded the lowest level among all the examined days. In the control group, the bacterial count increased significantly and rapidly from the beginning of the experiment till the 4th day from the treatment. The organoleptic examinations exhibited putrefactive changes in the control group samples at the 5th day; subsequently, these samples were unfit for the microbiological examinations.

This result agreed with those reported by Amina (2016) who estimated the *Staph. aureus* count in the broiler fillet of the control group increased rapidly from the beginning of the experiment till the 4th day without putrefactive changes and putrefied at 6th day. While this result disagreed with those obtained by Abd El-Dayem and Marzouk (2010), who estimated the *Staph. aureus* count in the broiler fillet of the control group till the 10th day without putrefactive changes, this variation may be explained by the lower initial bacterial count in their study comparing with those in the current investigation.

The lowest *Staph. aureus* levels in the examined samples in the four groups was achieved by *Nigella sativa* 0.5% which gave the highest protection, followed by *Nigella sativa* 0.1%, followed by *Rosemary 0.5%* and finally *Rosemary 0.1%*) which gave the lowest effect on *Staph. aureus.* The results partially agreed with those reported by Abd El-Dayem and Marzouk (2010), who detected slight decrease of *Staph. aureus* count in chicken fillet treated with 0.5% *Nigella sativa* oil at the 2nd, 4th and 7th days after treatment comparing with those in the control group. Moreover, Bessedik and Allem (2013) concluded that 0.4% of *Nigella sativa* oil had an inhibitory effect against *Staph. aureus.*

The antibacterial effect of *Nigella sativa* oil may be due to its content of alkaloids, volatile as well as fixed oils and a variety of pharmacologically active substances like thymoquinone, dithymoquinone, carvacrol, thymol, nigellicine-N-oxide, nigellidine and α-hedrin. Black cumin is also enriched with the fat content of 35.5%; the seeds of *Nigella sativa* contain volatile oil (0.5-1.6%), fixed oil (35.6-41.6%), protein and amino acids (22.7%) (Azeem *et al*.2014).

The antibacterial effect of *Rosemary* oil may be due to α-pinene which is reported as the major component of *Rosemary* essential oil, followed by 1,8 – cineole, camphene, β-myrcene, camphor and borneole. It was determined that *Rosemary* essential oil exhibits antimicrobial activity by passing through the cell wall and cytoplasm membranes and disrupting their structure as a

typical lipophilic substance (Stojanović-Radić *et al*., 2010). Another potent compound is ßcaryophyllene, which may affect accumulation of some substances by increasing permeability of the plasma membrane. In that way, it affects and increases cytotoxic effect of compounds with which it interacts (Legaule, and Pichette, 2007).

The results recorded in Table (3) and Figure (1) revealed that *Nigella sativa oil 0.5%* treatment gave the highest reduction % of *Staph. aureus.*

Natural preservatives and /or essential oils in certain concentrations have a great role in inhibiting and reduction microbial growth of some microorganisms that have public health hazards.

5. CONCLUSION

From this study, it could be concluded that the studied essential oils possess significant antibacterial activity against *Staph. aureus*, in the following order: *Nigella sativa 0.5% > Nigella sativa 0.1% > Rosemary 0.5% > Rosemary 0.1%*. Their antibacterial activity increases, when increasing their concentration. Therefore, these essential oils may be selected for use as potential food biopreservatives and anti- *Staph. aureus* agents in minced chicken meat and other foods, depending upon the desired flavor of the products.

6. REFERENCES

Abd-El-Daym, R. and Marzouk, N.M. (2010): Effect of *Nigella sativa* oil on the microbial quality of chilled chicken fillets. Alex. J. Vet. Sci., 29(1): 51-59.

Amin, Reham, A. (2013): Screening of antibacterial activity of *Cinnamon*, *Clove* and *Rosemary* essential oils against common food borne pathogens in minced beef. J. Benha Vet. Med., 25 (2):151-164.

Amin, Reham A.; Nesrin, Z.E. and Naglaa, A.E. (2015): Effect of thyme oil and clove oil on Staphylococcus aureus in chicken minced meat. J. Agriculture and Food Safety Sciences, 2:45-55.

Amina, H.H. (2016): Control of microbial contamination of chicken meat using some natural additives and surfactants. M.V.Sc. Thesis (Meat Hygiene), Fac. Vet. Med., Zagazig Univ,Egypt.

Angioni, A.; Barra, E.; Cereti, D.; Barile, J.; Coisson, D. and Arlorio, M. (2004): Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil (*Rosmarinusofficinalis L.*). J. Agric. Food Chem., 52(11): 3530-3535.

Azeem, T.; Zaib-Ur-Rehman; Umar, S.; Asif, M.; Arif, M. and Rahman, A. (2014): Effect of *Nigella Sativa* on poultry health and production: a review. Sci. Lett., 2 (2):76-82.

Bessedik, A. and Allem, R. (2013): Molecular composition and antibacterial effect of essential oil of *Nigella sativa*. Afr. J. Biotechnol., 12 (20): 3006- 3012.

Djeddi, S.; Bouchenah, N.; Settar, I. and Skaltsa, H.D. (2007): Composition and antimicrobial activity of the essential oil of *Rosmarinus officinalis* L. from Algeria. Chem. Natural Comp., 43 (4): 487- 490.

Haq, M.; Dutta, P.L.; Sultana, N. and Rahman, A. (2013): Production and quality assessment of fish burger from the grass carp, Ctenopharyngodon idella (Cuvier and Valenciennes, 1844), J. Fisheries, 1(1): 42-47.

Jagadeesh, A.; Rupasundari, A.; Sankar, B. and Sravanthi, M. (2012): Studies on the antimicrobial effectiveness of essential oils of garlic, clove and

cinnamon on *staphylococcus aureus* in chicken meat patties. Tamilnadu J. Veterinary & Animal Sciences 8 (1):45-49.

Legaule, J. and Pichette, A. (2007): Potentiating effect of ß-caryophyllene on anticancer activity of α- humulene, isocaryophyllene and paclitaxel. J. Pharmacy and Pharmacol., 59 (12):1643-1647.

Lyon, S.A.; Fletcher, D.L. and Berrang, M.E. (2007): Germicidal ultraviolet light to lower numbers of *Listeria monocytogenes* on broiler breast fillets. Poult. Sci., 86(5): 964-967.

Nychas, G.; Skandamis, C.; Tassou, A. and Koutsoumanis, K. (2008): Meat spoilage during distribution. J. Meat Sci., 78: 77-89.

Politeo, O.; Jukic, M. and Milos, M. (2007): Chemical composition and antioxidant capacity of free volatile aglycones from basil (Ocimumbasilicum.) compared with its essential oil. J. Food Chem., 101: 379-385.

Penny, N.; Hagyard, C.A. and Bell, R.G. (1993): Extension of shelf life of chilled sliced roast beef in carbon dioxide packing. Int. J. Sci. Tech., 28(2): 181-191.

Simitzis, P.E.; Deligeorgis, S.G.; Bizelis, J.A.; Dardamani, A.; Theodosiou, I. and Fegeros, K. (2008): Effect of dietary oregano oil supplementation on lamb meat characteristics. Meat Science, 79 (2): 217-223.

Steel, R.G.D. and Torrie, J.H. (1980): Principles and Procedures of Statistics, 2nd Edition, New York: McGraw-Hill.

Stiles, M.E. and Hastings, J.W. (1991): Bacteriocins production by lactic acid bacteria: potential for use in meat preservation. Trends Food Sci. Technol., 2: 247-251.

Stojanović-Radić, Z.; Nešić1, M.; LjiljanaČomić, L. and Radulović, N. (2010): Antimicrobial activity and cytotoxicity of commercial rosemary essential oil (*Rosmarinus officinalis L.*). Biologica. Nyssana., 1 (1-2): 83-88.