

Synergism between *Saccharomyces cerevisiae* probiotic and rosemary nano-emulsion: Effect on broiler chicken meat quality and shelf life

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ABSTRACT

Although several studies have investigated the effect of either probiotic feed additives or postmortem meat treatment on the quality of obtained chicken meat, the impact of combined treatment with probiotic feed additives along with meat dipping in essential oil nano-emulsion on meat shelf-life is barely examined. Therefore, this study investigated the effect of combined treatment with *Saccharomyces cerevisiae* yeast (SCY) and rosemary oil nano-emulsion (RNE) on the quality and shelf-life of chilled broiler meat. The experimental part consisted of adding SCY as a feed additive to broiler ration and/or dipping the resulting chicken meat in RNE 10% for 24 hours. Afterward, chicken meat from different treated groups, as well as the control one was refrigerated ($4\pm 1^\circ\text{C}$) and periodically examined on 0, 3rd, 7th, and 9th days of storage. The obtained results revealed significant reductions in total colony, Total *Enterobacteriaceae*, total *Staphylococci*, and total fungal counts of the SCY+RNE-treated group were reduced by about 79.4%, 34%, 72.8% and 32.5% as compared to control, respectively ($p<0.001$). While of RNE-treated group, they were decreased by about 71.6%, 16.5%, 14.4% and 26% as compared to control, respectively ($p<0.001$). Whereas of the SCY-treated group, they were reduced about 57.2%, 59%, 28.5% and 24.7% as compared to control, respectively ($p<0.001$). Additionally, meat spoilage indicators (pH, TBA-RS, TVBN) came in harmony with the microbiological results. As control group samples had the highest values of pH, TBA-RS, and TVBN, followed by the RNE-treated group and the SCY-treated group. On the other hand, the SCY+RNE-treated group showed the lowest pH, TBA-RS, and TVBN levels ($p<0.001$). These results confirm that treatment with SCY alone, RNE alone, and SCY+RNE prolonged the shelf-life of broiler chicken meat. To conclude, the addition of SCY as a probiotic additive to chicken feed in combination with meat dipping in RNE has a potential synergistic favorable effect on chicken meat quality and shelf-life.

Introduction

Improving the shelf-life of meat is considered significant from the economic and nutritional points of view. The antioxidant capacity of meat including chicken meat depends mainly on the activities of endogenous reducing enzymes at early postmortem time (Serpen *et al.*, 2012). This capacity decreases when muscular cells lose their homeostasis, consequently, the formation of free radicals is increased leading to meat spoilage and deterioration. Moreover, meat storage stability depends on several internal factors, such as metal catalysts, fatty acid profile, pH, and the presence of other inhibitors (Xiong, 2000; Min and Ahn, 2005).

Lipid and protein deterioration is considered the priority issue affecting meat quality characteristics (Ahn *et al.*, 2009; Lund *et al.*, 2011). In the meat industry, they have extensively used synthetic antioxidants, yet, because of the negative impression regarding the use of synthetic antioxidants; natural antioxidants (NA) are considered a suitable replacement (Velasco and Williams, 2011). Moreover, several studies reported possible carcinogenic and toxicological effects of the synthetic antioxidants that are used in human foods (Kumar *et al.*, 2015). Therefore, recently consumers have preferred food that is free of any chemical additives.

Essential oils (EOs) are aromatic and volatile lipophilic plant extracts with antimicrobial and antioxidant properties which could be a suitable alternative to synthetic chemical preservatives (Prakash *et al.*, 2015). The direct incorporation of EOs in the polymeric matrix has disadvantages, including volatilization of EOs during water evaporation due to the coarseness of its droplets and the creation of porous structures in the coating (Tastan *et al.*, 2016). Therefore, the application of EOs in the form of nano-emulsions with tiny droplet sizes has many advantages including, high stability, enhancement of physicochemical properties, and improved biological properties by increasing the specific surface area and hence

lowering the required amounts of the active component (Mushtaq *et al.*, 2023). Nano-emulsions are emulsions with particle sizes of 2–200 nm produced by high-energy or low-energy emulsification methods (McClements and Rao, 2011; Meneses *et al.*, 2019). The application of essential oil nano-emulsions is an emerging technique that is used in the food industry to prolong the shelf-life, manage food safety concerns, and replace synthetic preservatives (Amin, 2013).

Rosemary is a plant source of bioactive substances containing phenolic compounds with antioxidant properties such as diterpenes, carnosol, and rosmarinic acid (Chao *et al.*, 2020). Nano encapsulation form of these phenolic compounds avoid exposure to oxygen, heat, humidity and light to protect them to achieve a higher antioxidant activity (Duarte and Larroza, 2019; Rashidaie *et al.*, 2019). Achieving increased stability, protection, controlled release and reducing the possible adverse impact on the organoleptic properties in meat and meat products (Duarte and Larroza, 2019). Rosemary (*Rosmarinus officinalis* L.) has a wide antimicrobial range (El Bayomi *et al.*, 2021). It was added to food because of its palatability, safety for consumers, and delaying effect on fat rancidity in meat (Yu *et al.*, 2002; Jongberg *et al.*, 2013). There is no limitation on using rosemary essential oil and its extract in meat products as it is generally recognized as safe (GRAS) (European Food Safety Authority, EFSA 2008). Moreover, rosemary extract and its essential oils were reported as potential antioxidant substances that are widely used in the food industry (Balentine *et al.*, 2006; Hussain *et al.*, 2010). Accordingly, the application of rosemary nano-emulsion (RNE) could be a promising approach to hinder the deterioration of chicken meat.

Additionally, probiotics are a significant feed additive that has the potential to increase antimicrobial resistance and reduce enteric diseases in poultry and subsequent contamination of poultry products (Patterson and Burkholder, 2003). Probiotics are microorganisms, such as fungi and

bacteria, living in nature and have beneficial effects on the host (Khan *et al.*, 2011). The most widely used type of yeast, *Saccharomyces cerevisiae* (SC), has been reported to improve feed quality and the performance of animals, as well as work as an immune stimulant to the host. The cell wall of SC contains chitin, mannan, and glucagon, working as immune stimulants and prebiotic sources (Li and Gatlin, 2003). Moreover, the yeast cell wall contains 1,3-1,6 D glucagon and mannan-oligosaccharides which act as natural growth promoters (van Leeuwen *et al.*, 2005; Ghosh *et al.*, 2007). In addition, the SC cell wall can minimize the toxic effect of aflatoxins in poultry through biodegradation (Parlat and Oguz 2001). Consequently, the addition of SC to broiler chicken ration could enhance the quality, safety, and stability of the resulting meat.

Despite there are several studies have examined the effect of probiotics as feed additives on the quality of meat products, to our knowledge, the investigation of probiotic application as ration additives along with meat treatment by essential oil nano-emulsion on the quality and shelf-life stability of poultry meat is very limited.

Therefore, the current study aimed to investigate the impact of the combined use of SC as a probiotic feed additive and RNE as a food additive on the microbiological and physicochemical status, as well as the shelf-life of obtained broiler meat, in comparison with individual use of each treatment and control (untreated) group.

Materials and methods

Probiotics

Saccharomyces cerevisiae is a probiotic that contains live yeast cells (20 billion CFU/g), active dry yeast (*Saccharomyces cerevisiae* Sc 47 (Allgau vet German. Allgau yeast, Germany). It is used in a dose of 2.5 billion CFU/Kg ration (Maksimović *et al.* 2022).

Rosemary nano-emulsion 10%

The nano-emulsion was prepared in the Nanomaterial's Research and Synthesis Unit (Animal Health Research Institute, Doki, Giza, Egypt) by mixing rosemary oil (10 ml) and Tween 80 (10 ml) for half an hour in a homogeneous blender (1500 watts), then 80 ml of distilled water was slowly added to the mixed oil phase with continuous mixing (Rao and McClements, 2011; Sorour *et al.* 2021). Characterizing the nano-emulsion and measuring electrical conductivity zeta potential (surface charge), and both size droplet and distribution (polydispersity indexes PDI) of micro-emulsion using Microtrac FLEX (12.0.1.0) Instrument was done.

Bird rearing and application of probiotics

Thirty-one-day-old Ross broiler chicks were obtained from Ommat Arab poultry breeder company, Giza, Egypt. They were incubated on Sacrolyte g/L water for 12 hours alternately with florfenicol 10% antibiotics ml/L water for the other 12 hours for three successive days. Chicks were reared under optimum conditions at a temperature range started at 35–37°C then decreased gradually till reached 25–23°C on the 42nd day, and relative humidity of about 50%. They received local standard ration composed of yellow corn, soybean meal, fish meal, corn oil, Glut, Di-calcium phosphate, limestone ground, and NaCl. Starter ration for 0–19-day old chick encompassed of crude protein 23%, crude fat 4.41%, crude fiber 2.42%, representative energy (Kcal/Kg diet) 3050, Calorie/Protein ratio 132.61, D-L-methionine 0.65%, L-lysine 1.49%, calcium 1.06%, total phosphorous 0.36%. Grower ration for 20- 35 day old chick composed of crude protein 21%, crude fat 5.42%, crude fiber 2.4%, RE (Kcal/Kg diet) 3115, calorie/Protein ratio 148.33, D-L-methionine 0.55%, L-lysine 1.19%, calcium 1.06%, total phosphorous 0.35% and finishing ration for the last week contained crude protein 17%, crude fat 6.43%, crude fiber 2.35%, RE (Kcal/Kg diet) 3265, Calorie/Protein ratio 186.57, D-L-methionine 0.42%,

L-lysine 1%, calcium 1.06%, total phosphorous 0.33%) and water for one week and then divided on the 8th day into two equal groups each of 15 chicks. Group A was used as a control which received water and a standard ration till the end of the experiment on the 42nd day. Group B was fed the same ration as the control group but mixed with SC probiotics at 2.5 billion CFU/Kg ration and free water till the end of the experiment. All birds were slaughtered, and thigh and breast muscles were collected. The ethical approval of this experimental design was obtained from the Institutional Animal Care and Use Committee (ARC- IACUC), Animal Health Research Institute, Agriculture Research Center, Egypt, with the number ARC, AHRI, 32, 24 before starting the experiment.

Poultry meat treatment with rosemary nano-emulsion and grouping

The collected breast and thigh muscles either from Group A or Group B were divided into two parts. The first part from each group was dipped in sterile distilled water for 24 hours before being chilled at 4±1°C for up to 9 days. While the second part was dipped in rosemary nano-emulsion 10% for 24 hours before being chilled as in part one. Accordingly, four groups of meat samples (breast and thigh muscles) were obtained, individually refrigerated, and then periodically collected at various time intervals on 0, 3rd, 7th, and 9th days of storage for microbiological and physicochemical analyses. Group I was considered as control which was not exposed to either SC probiotics or RNE. Group II was exposed to SC probiotics only, whereas Group III was exposed to RNE only, and Group IV was exposed to both SC probiotics and RNE treatments.

Poultry meat examination

Physicochemical analysis

The treated samples were analyzed for keeping quality indices by measuring pH, Total volatile basic nitrogen (TVBN) and thiobarbituric acid reactive substances (TBA-RS) according to the Egyptian Organization for Standardization and Quality control (EOS, 2006).

Microbiological examination

Samples were examined at 0, 3, 7, and 9 days of chilling at 4°C, where 25g of each sample was blended with 225 ml of 0.1% peptone water for 1-2 minutes in a sterile blender jar. Further decimal serial dilutions were prepared for testing. Determination of total colony count was done according to USDA (2011), total *Enterobacteriaceae* count (ISO, 2001), total fungal count according to FAO (1992). total *Staphylococci* count and *Staphylococcus aureus* count (USDA, 2011), where morphological examination of the suspected colonies was applied (Cruickshank *et al.*, 1975) then biochemical identification (MacFaddin, 2000) and examination for catalase activity test, oxidase test, growth at 10% NaCl, detection of Arginine decarboxylase (ADH), bile esculent test, mannitol test, detection of hemolysis, coagulase test, thermostable nuclease test "D-Nase activity" (Lachia *et al.*, 1971) and fermentation of sugars. Finally, isolation of *Salmonella* spp. (ICMSF, 1978) where smears of suspected colonies were stained with Gram stain and examined morphologically for staining characters. Presumptive *Salmonella* colonies were then subjected to initial screening tests using triple sugar iron agar (TSI), lysine iron agar (LIA), urea broth (Merck) and lysine decarboxylase. All biochemical tests were performed at 37°C for 18–24 hours including citrate utilization, indol production test, methyl red, urea hydrolysis, and Voges- Proskauer (Andrews and Hammack, 1998).

Statistical analysis

Preliminary statistical analysis was carried out using Graph Pad In-Stat software (version 3, ISS-Rome, Italy). Unless differentially specified,

groups of data were compared with one-way analysis of variant (ANOVA) followed by Tukey-Kramer (TK) Multiple Comparison post-test. The data as indicated are reported in tables and figures as mean±standard error (SEM). Values of P ≤ 0.05 were regarded as significant.

Results

SCY and RNE reduced the deterioration indicators of broiler chicken meat

The obtained results showed that pH in SCY+ RNE-treated group were 6.05±0.029, 5.9±0.026, 5.95 ±0.05 and 6.003 ±0.003, in RNE-treated group were 6.2 ±0.011, 5.89±0.018, 6.187±0.0088 and 6.26±0.011, in SCY-treated group were 5.74±0.063, 5.89±0.012, 6.177±0.0088 and 6.32±0.011 and in control group were 5.94±0.026, 5.68 ±0.017, 6.19±0.0088 and 6.34±0.0088 on 0, 3rd, 7th and 9th day respectively there is a highly significant decrease (p<0.001) in pH in SCY+ RNE-treated group, RNE-treated group and SCY-treated group in compare with control (Figure 1).

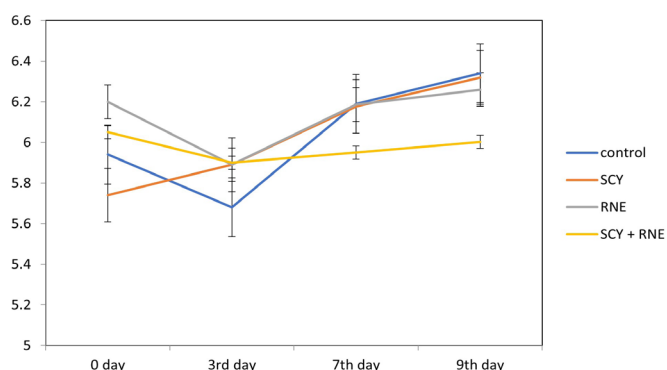


Figure 1. pH changes in broiler chicken meat exposed to *Saccharomyces cerevisiae* yeast (SCY) and rosemary nano-emulsion (RNE) 10% either individually or together at different time intervals during chilled storage at 4±1°C. Data are represented by means±standard errors of at least 3 replicates (n≥3).

Figure 2 shows the changes in TVBN of treated and untreated broiler chicken meat at different time intervals during refrigerated storage at 4±1°C. The obtained results showed that the changes in TVBN of SCY+ RNE-treated group were 8.77±0.082, 9.87±0.067, 12.62±0.35 and 18.73±0.088, in RNE-treated group were 9.67±0.234, 11.34±0.081, 13.46±0.087 and 18.8±0.04, in SCY-treated group were 8.77±0.1, 11.55±0.39, 15.25±0.15 and 18.76±0.06 and in Control group were 11.67±0.285, 12.97±0.23, 16.48±0.057 and 21±0.1 on 0, 3rd, 7th and 9th day respectively there is a highly significant decrease at (p<0.001) in the TVBN changes of SCY+ RNE-treated group, RNE-treated group and SCY-treated group in compare with control.

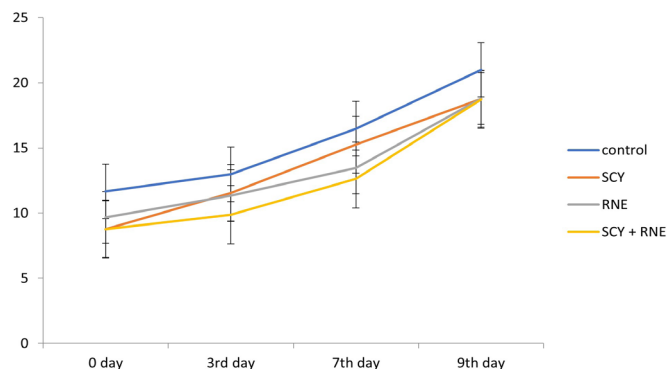


Figure 2. The changes in TVBN of broiler chicken meat exposed to *Saccharomyces cerevisiae* yeast (SCY) and rosemary nano-emulsion (RNE) 10% either individually or together at different time intervals during chilled storage at 4±1°C. Data are presented as means±standard errors of at least 3 replicates (n≥3).

chicken meat at different time intervals during refrigerated storage at 4±1°C. The changes in TBA of SCY+ RNE-treated group were 0.114±0.025, 0.126±0.007, 0.148±0.005 and 0.1716±0.005, of RNE-treated group were 0.218±0.018, 0.277±0.0021, 0.383±0.0035 and 0.5746±0.014, of SCY-treated group were 0.187±0.013, 0.226±0.018, 0.391±0.007 and 0.4756±0.0088 and of control group were 0.241±0.014, 0.475±0.028, 0.803±0.042 and 0.892 ±0.011 at 0, 3rd, 7th and 9th day respectively. There is a highly significant decrease at (p<0.001) in the TBA changes of SCY+ RNE-treated group, RNE-treated group and SCY-treated group in compare with control.

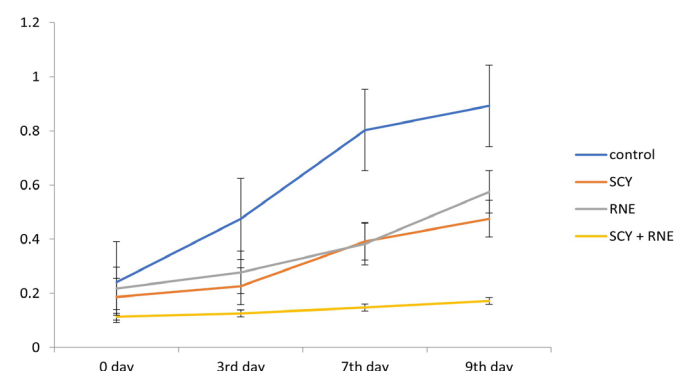


Figure 3. The changes in TBA of broiler chicken meat exposed to *Saccharomyces cerevisiae* yeast (SCY) and rosemary nano-emulsion (RNE) 10% either individually or together at different time intervals during chilled storage at 4±1°C. Data are presented as means±standard errors of at least 3 replicates (n≥3).

SCY and RNE improved the microbiological quality of broiler chicken meat

Table 1 summarizes the TCC of treated and untreated broiler chicken meat at different time intervals during refrigerated storage at 4±1°C. The obtained results showed that TCC in SCY+ RNE-treated group were 4.3x10³±44, 0.9x10⁴±57.74, 6.3x10⁴±120.2 and 8.3x10⁴±136.4, while in RNE-treated group were 5x10³±57.7, 1.2x10⁴±88.2, 7.6x10⁴±66.67 and 1.26x 10⁵±120.2, and in SCY-treated group were 5.3x10³±16.7, 1.26x10⁴±115.5, 8.3x10⁴±66.67 and 2.3x10⁵±233.3 on 0, 3rd, 7th and 9th day, respectively. On the other hand, they were 7x10³±76.4, 3.6x10⁴±333.33, 1.373.5 in the control group on 0, 3rd, 7th and 9th day, respectively. There was a significant decrease (p<0.001) in TCC in SCY+ RNE-treated group, RNE-treated group, and SCY-treated group in comparison with the control group.

The total *Enterobacteriaceae* counts of treated and untreated broiler chicken meat at different time intervals during refrigerated storage at 4±1°C were shown in Table 2. The obtained results showed that Total *Enterobacteriaceae* counts in SCY+ RNE-treated group were 2.3x10³±16.67, 6x10³±57.7, 8.3x10³±33.33 and 5x10⁴±577.35, in RNE-treated group were 3.3x10³±60.1, 6.6x 10³ ±88.2, 8.3x10³ ±33.33 and 6.6x10⁴ ±60.2, in SCY-treated group were 3.3x10³±44.1, 7.3x10³ ±44.1, 9.3x10³±72.65 and 2.1x10⁴±88.2 and in Control group were 4.3x10³±16.67, 7.6x10³±120.2, 1.3x10⁴ ±76.4 and 7.6x10⁴±88.2 on 0, 3rd, 7th and 9th day, respectively. There is a highly significant decrease (p<0.001) in Total *Enterobacteriaceae* counts in SCY+ RNE-treated group, RNE-treated group, and SCY-treated group in comparison with control.

Table 3 shows the Total fungal counts of treated and untreated broiler chicken meat at different time intervals during refrigerated storage at 4±1°C. Total fungal counts in SCY+ RNE-treated group were 2x10³±28.9, 4.6x10³±33.33, 9x10³±57.35 and 5.9x10⁴±440.1, in RNE-treated group were 3.3x10³±60.1, 6x10³±50, 9.3x10³±16.67 and 6.3x10⁴±333.3, in SCY-treated group were 3.6x10³±44.1, 5x10³±57.7, 1.6x10³ ±33.33 and 7.3x10⁴±333.3 and in Control group were 4.3x10³±44.1, 7.3x10³±33.33, 2.6x10⁴±72.65 and 7.3x10⁴±166.67 on 0, 3rd, 7th and 9th day respectively there is a highly significant decrease at (p<0.001) in Total fungal counts in SCY+ RNE-treated group, RNE-treated group and SCY-treated group in compare with control.

Figure 3 shows the changes in TBA of treated and untreated broiler

Staphylococci counts of treated and untreated broiler chicken meat at different time intervals during refrigerated storage at 4±1°C were presented in Table 4. The obtained results showed that in Total *Staphylococci* counts SCY+ RNE-treated group were 1x10³±5.8, 2.6x10³±44.1, 5.3x10³±16.67 and 1.3x10³±132.3, in RNE-treated group were 1.6x10³±33.3, 4x10³±57.7, 6.6x10³±44.1 and 2x10⁴±86.6, in SCY-treated group were 1.6x10³±44.1, 3.3x10³±44.1, 6x10³±57.7 and 1.6x10⁴±33.33 and in Control group were 2.3x10³±16.67, 4.3x10³±33.3, 8x10³±76.4 and 2.3x10⁴±16.67 on 0, 3rd, 7th and 9th day respectively, there is a highly significant decrease at (p<0.001) in Total *Staphylococci* counts in SCY+ RNE-treated group, RNE-treated group and SCY-treated group in compare with control.

Regarding the incidence of *Salmonella* and *Staphylococcus aureus* in broiler chicken meat exposed to *Saccharomyces cerevisiae* yeast (SCY) and/or rosemary nano-emulsion (RNE) 10% at different time intervals during chilled storage at 4±1°C, it was failed to detect them throughout

the storage duration (data not shown).

Discussion

Lipid oxidation of meat is one of the most important factors affecting shelf life and one of the decisive factors in the consumer’s purchase decision of a product. So, the use of natural products that have an antioxidant capacity such as SMY and RNE can overcome and delay the oxidation process. Accordingly, they prolong shelf life and improve the quality of meat.

Our results in Figures 1, 2 and 3, confirm the antioxidant effect of SCY and RNE, where there was amelioration to the deteriorative characters (pH, TVBN and TBARS) all over the 9 days of storage in in SCY+ RNE-treated, RNE-treated and SCY-treated groups. Rosemary has a potential effect to inhibit the lipid oxidation of food, by eliminating free radicals and the chain reaction of metal ions such as Fe2 is terminated,

Table 1. Total colony counts (CFU/g) of broiler chicken meat exposed to *Saccharomyces cerevisiae* yeast (SCY) and rosemary nano-emulsion (RNE) 10% either individually or together at different time intervals during chilled storage at 4±1°C.

Treatment groups	Day of chilled storage			
	0 day	3 rd day	7 th day	9 th day
Group I (Control)	7×10 ³ ±76.4 ^a	3.6×10 ⁴ ±333.33 ^a	1.3×10 ⁵ ±881.9 ^a	6×10 ⁵ ±5773.5 ^a
Group II (SCY-treated)	5.3x10 ³ ±16.7 ^c	1.26×10 ⁴ ±115.5 ^b	8.3×10 ⁴ ±66.67 ^c	2.3×10 ⁵ ±233.3 ^c
Group III (RNE-treated)	5×10 ³ ±57.7 ^b	1.2×10 ⁴ ±88.2 ^b	7.6×10 ⁴ ±66.67 ^b	1.26×10 ⁵ ±120.2 ^b
Group IV (SCY+ RNE-treated)	4.3×10 ³ ±44 ^d	0.9×10 ⁴ ±57.74 ^c	6.3×10 ⁴ ±120.2 ^d	8.3×10 ⁴ ±136.4 ^d

Data are presented as means±standard errors of at least 3 replicates (n≥3). Different small letters superscripts (^{a,b,c,d}) within the same column indicate high significant differences between means at (P < 0.001).

Table 2. Total *Enterobacteriaceae* counts (CFU/g) of broiler chicken meat exposed to *Saccharomyces cerevisiae* yeast (SCY) and rosemary nano-emulsion (RNE) 10% either individually or together at different time intervals during chilled storage at 4±1°C.

Treatment groups	Day of chilled storage			
	0 day	3 rd day	7 th day	9 th day
Group I (Control)	4.3x10 ³ ±16.67 ^a	7.6x10 ³ ±120.2 ^a	1.3x10 ⁴ ±76.4 ^a	7.6x10 ⁴ ±88.2 ^a
Group II (SCY-treated)	3.3x10 ³ ±44.1 ^b	7.3x10 ³ ±44.1 ^b	9.3x10 ³ ±72.65 ^c	2.1x10 ⁴ ±88.2 ^c
Group III (RNE-treated)	3.3x10 ³ ±60.1 ^b	6.6x10 ³ ±88.2 ^a	8.3x10 ³ ±33.33 ^b	6.6x10 ⁴ ±60.2 ^b
Group IV (SCY+ RNE-treated)	2.3x10 ³ ±16.67 ^c	6x10 ³ ±57.7 ^c	8.3x10 ³ ±33.33 ^b	5x10 ⁴ ±577.35 ^d

Data are presented as means±standard errors of at least 3 replicates (n≥3). Different small letters superscripts (^{a,b,c,d}) within the same column indicate high significant differences between means at (P < 0.001).

Table 3. Total fungal counts of broiler chicken meat exposed to *Saccharomyces cerevisiae* yeast (SCY) and rosemary nano-emulsion (RNE) 10% either individually or together at different time intervals during chilled storage at 4±1°C.

Treatment groups	Day of chilled storage			
	0 day	3 rd day	7 th day	9 th day
Group I (Control)	4.3x10 ³ ±44.1 ^a	7.3x10 ³ ±33.33 ^a	2.6x10 ⁴ ±72.65 ^a	7.3x10 ⁴ ±166.67 ^a
Group II (SCY-treated)	3.6x10 ³ ±44.1 ^c	5x10 ³ ±57.7 ^c	1.6x10 ³ ±33.33 ^c	7.3x10 ⁴ ±333.3 ^a
Group III (RNE-treated)	3.3x10 ³ ±60.1 ^b	6x10 ³ ±50 ^b	9.3x10 ³ ±16.67 ^b	6.3x10 ⁴ ±333.3 ^b
Group IV (SCY+ RNE-treated)	2x10 ³ ±28.9 ^d	4.6x10 ³ ±33.33 ^d	9x10 ³ ±57.35 ^d	5.9x10 ⁴ ±440.1 ^c

Data are represented by means±standard errors of at least 3 replicates (n≥3). Different small letters superscripts (a, b, c, d) within the same column indicate high significant differences between means at (P < 0.001).

Table 4. Total *Staphylococci* count in broiler chicken meat exposed to *Saccharomyces cerevisiae* yeast (SCY) and rosemary nano-emulsion (RNE) 10% either individually or together at different time intervals during chilled storage at 4±1°C.

Treatment groups	Day of chilled storage			
	0 day	3 rd day	7 th day	9 th day
Group I (Control)	2.3x10 ³ ±16.67 ^a	4.3x10 ³ ±33.3 ^a	8x10 ³ ±76.4 ^a	2.3x10 ⁴ ±16.67 ^a
Group II (SCY-treated)	1.6x10 ³ ±44.1 ^b	3.3x10 ³ ±44.1 ^c	6x10 ³ ±57.7 ^c	1.6x10 ⁴ ±33.33 ^c
Group III (RNE-treated)	1.6x10 ³ ±33.3 ^b	4x10 ³ ±57.7 ^b	6.6x10 ³ ±44.1 ^b	2x10 ⁴ ±86.6 ^b
Group IV (SCY+ RNE-treated)	1x10 ³ ±5.8 ^c	2.6x10 ³ ±44.1 ^c	5.3x10 ³ ±16.67 ^d	1.3x10 ³ ±132.3 ^d

Data are represented by means±standard errors of at least 3 replicates (n≥3). Different small letters superscripts (a, b, c, d) within the same column indicate significant differences between means at (P < 0.001).

reducing the activated oxygen molecules rate of formation (Afonso *et al.*, 2013; Rašković *et al.*, 2014; Aminzare *et al.*, 2019; Rashidaie *et al.*, 2019).

Rosemary is a natural antioxidant widely used for food conservation (Feng *et al.*, 2016). Yeast having manno-oligosaccharides or mannose which confirmed by Lyons (2002) and Oyfo *et al.* (1989). mannanoligosaccharides and β -glucans content of *Saccharomyces cerevisiae* yeast make them used as a growth promoter and immune stimulant (Celik *et al.*, 2000; Çelyk *et al.*, 2003). Also, it has antimicrobial properties and other health-related benefits through the maintenance of the intestinal biostructure (Wang and Gu, 2010; Zhang, L. *et al.*, 2016). Also, it prevents some specific intestinal pathogens, produce various nutrients, improve the chicken intestinal metabolism (Zarei *et al.*, 2018), and enhances the general performance as well as improve the local and systemic immunity (Korver, 2012; Wang *et al.*, 2017).

Additionally, data in Tables 1, 2, 3 and 4 showed the beneficial effects of adding probiotics (SCY) to chicken ration (SCY), dipping chicken meat in rosemary nano-emulsion (RNE) and a combination of both treatments (SCY+RNE) on chicken meat quality and shelf life, where the great reduction in microbial counts was achieved SCY+RNE, followed by RNE and finally SCY.

The antioxidant and antimicrobial activities of rosemary extract (RE) is linked with their contents of phenolic diterpenes such as carnosic acid, carnosol, rosmanol, rosmaridiphenol and rosmariquinone, ursolic acid, and caffeic acid (Aruoma *et al.*, 1992). Moreover, several research studies have reported RE as an antimicrobial food additive because of the phenolic constituents and their ferrous ion-chelating effect (Zhang, H. *et al.*, 2016). Moreover, adding RE or their essential oils has an impact retarding microbial growth of both Gram-positive and Gram-negative bacteria in different meat systems (Liu *et al.*, 2009; Jiang *et al.*, 2011). The antimicrobial mechanism of these plant extracts and their essential oils was explained by their effect on the bacterial cell membrane integrity and permeability (Ojeda-Sana *et al.*, 2013).

Probiotic supplementation improved birds' immunity, which is proved by the results in samples of the SMY-treated group revealing significantly lower microbial counts as compared to control group, such findings agree with the results reported by Sarwar *et al.* (2023) and Ayanwale *et al.* (2006). Moreover, Cuenca *et al.* (2022) explained the mode of action of SCY as probiotics, where it has shown the capacity to cross the gastric barrier, multiplying and colonizing the intestine. When added to the diet of monogastric animals, it favors the development of the gastro-intestinal microbial flora and stimulates immunity and microvilli; it inhibits the action of microbial toxins and increases an antagonistic effect against pathogenic microorganism.

Samples of the SMY+RNE group showed the lowest microbial counts due to the double effect of using SC probiotics as feed additives and RE nano-emulsion as food additives, leading to prolonging the shelf life, where TCC did not reach 10^5 CFU/g as shown in Table 1.

Regarding the results of the effect of probiotic (*Saccharomyces cerevisiae*) on microbial properties of chicken meat, a significant reduction of microbial counts was observed in the RNE-treated group (Tables 1, 2, 3, and 4), as compared to the control (untreated) group.

Additionally, probiotics have been shown to inhibit pathogenic bacteria through different mechanisms including maintaining normal intestinal microflora by competitive exclusion and antagonism, altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production, inhibiting the action of microbial toxins and exerting an antagonistic effect against pathogenic micro-organisms, improving feed intake and digestion and finally stimulating the immune system (Kabir *et al.* 2005; Condoy *et al.* 2021; Castro and Rodríguez 2005).

Both total colony count (TCA) and *Enterobacteriaceae* count (EC) are used to evaluate food contamination, manufacturing system hygiene, quality control, and food safety (American Public Health Association, 1984). It is recommended that the flesh total colony count TCC should

not exceed 106/g wet weight (ICMSF, 1998). Whereas the Egyptian Organization for Standardization (EOS, 2005) recommended that TCC and *Enterobacteriaceae* count should not be more than 10^5 /g, and 10^3 /g, respectively.

Higher TCCs were reported by Bailey *et al.* (2000) (4.62 and 6.87 \log_{10} /g on 0 and 7th day of chilling at 4°C, respectively) and Mahmoud *et al.* (2020) (7.6×10^5 and 4.9×10^6 CFU/g for breast and thigh samples, respectively). Moreover, higher counts of *Enterobacteriaceae* were recorded by Mahmoud *et al.* (2020) who reported 1.6×10^5 CFU/g and 3.6×10^5 CFU/g for breast and thigh samples, respectively, while lower *Staphylococci* counts (6.3×10^2 CFU/g in breast and 2.5×10^3 CFU/g in thigh samples) and total fungal counts (1.1×10^3 CFU/g in breast and 4.7×10^3 CFU/g in thigh samples).

Samples treated with RNE showed lower total colony counts than those reported by Elzamzamy (2014). On the other hand, Al-Hijazeen, (2018) stated higher counts (4.15, 4.15, 7.11, 7.60 and 8.10 log CFU/g at 40C on 0, 2, 4, 6, and 8, respectively). Total *Enterobacteriaceae* counts in the current study revealed higher counts than those reported by Elzamzamy (2014). On the other hand, Al-Hijazeen 2018 reported higher counts in the treated samples during chilling at 4°C.

Table 3 showed similar counts of *Staphylococcus* to those reported by Elzamzamy (2014) in the case of the RNE-treated group. On the contrary, total fungal counts displayed in Table 4 revealed higher values than the results reported by Elzamzamy (2014).

In the current study, *Salmonella* was not detected in treated and untreated samples, on the contrary, Bailey *et al.* (2000) determined counts of 1.54 and 1.51 \log_{10} /g on 0 and 7th day of chilling at 4°C. While Mahmoud *et al.* (2020) reported *Salmonella* in 9% of the examined breast and thigh samples.

Conclusion

Adding *Saccharomyces cerevisiae* as probiotic additives to poultry feeds and then dipping meat in rosemary nano-emulsion improved meat quality and prolonged the shelf life of poultry meat. Further investigation on the cytotoxicity of rosemary nano-emulsion and sensory attributes of treated poultry samples is recommended.

Conflict of interest

The authors declare that they have no conflict of interest.

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