SUMMARY AND CONCLUSION

Bolti fish is considered one of the main type of fish widely used in Egypt especially, Aswan an its better with differential classes of people. Fresh fish are highly perishable and susceptible to spoilage. Effects of some antimicrobials and antioxidants on growth of *Escherichia coli* O157:H7 ATCC 69373 in (TSB) were studied to select the best concentration of antimicrobials and antioxidants which inhibit the growth of strain besides, the best concentration for combination of antimicrobials and antioxidants which inhibit the growth of *E coli* O157:H7 ATCC 69373 and *Pseudmonas aeruginasa.* Chemical composition, freshness tests, physical properties, microbiological assay and sensory evaluation were done for untreated and treated Bolti fish samples under cold and frozen storage. Four-culture plate methods as a new technique for detect and enumerate of some important bacteria were done. The obtained results were illustrated in three parts and could summarized as follows:

PART I:

1. Effect of antimicrobials and antioxidants against *E. coli* O157:H7 ATCC 69373 at 37°C:

Antimicrobial, sodium lactate (SL), sodium acetate (SA), sodium citrate (SC), tri-sodium phosphate (TSP), potassium sorbate (PS), ascorbic acid (AA), butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) against *E. coli* O157:H7 ATCC 69373 in laboratory media (Tryptic Soy Broth) at 37°C through 24 hrs was done.

None of the additives was bactericidal at different concentrations at zero time but, the inhibition percentage was increased with increasing concentrations.

- Sodium lactate was more effective than others antimicrobials at zero, 12 and 24 hrs. Wherever, 1% SL was ineffective on bacterial growth but, it was very effective at concentration ≥ 2%. A 3% SL had bactericidal effect af 24 hrs against test strain
- Sodium acetate and Sodium citrate had bacteriostatic effect at different concentrations from 1 to 4% against test strain. Sodium acetate was more effective than Sodium citrate on growth of strain.
- Tri-sodium phosphate was more effective than sodium acetate and sodium citrate. Tri-sodium phosphate was highly bacteriostatic effect at concentration from 1 to 3%. While, 4% TSP was bactericidal effect against strain.
- Potassium sorbate and ascorbic acid had bacteriostatic effect at different series levels concentrations against test strain. Potassium sorbate was more effective than ascorbic acid at zero, 12 and 24 hrs.
- BHA was more effective than BHT. BHT was bacteriostatic effect at 100 to 400 ppm. While, BHA was very effective at concentration >200 ppm then 300 ppm was bactericidal effect at 24 hrs.
- All additives (more concentration) well be tested had bacteriostatic effect at zero time. wherever, potassium sorbate and BHT were bactericidal effect at 6% and 500 ppm, respectively. Sodium lactate, tri-sodium phosphate,

potassium sorbate and BHA given highly antimicrobial effect against test strain at low concentrations.

2. **Effect of combined antimicrobials and antioxidant against *E. coli* O157:H7 ATCC 69373 at 37°C:**
   - Mixture (1) had bacteriostatic effect against test strain then the initial number was $4.10x10^6$ CFU ml^{-1} and become $1.40x10^6$, $7.85x10^6$ and $1.36x10^6$ CFU ml^{-1} at zero, 12 and 24 hrs, respectively. A slightly decreasing was occurred in number of cells. Bacteriostatic effect of mixtures 2, 3 and 4 were increased with increasing of mixtures concentrations. Inhibition percentage were 88.17, 98.78, 99.77 and 99.90% for mixtures 1, 2, 3 and 4 at 24 hrs, respectively. Either mixture (5) or mixture (6) had bactericidal effect on test strain at 24 hrs. However, mixture (7) was bactericidal at 12 hrs. While, mixture (8) was more bactericidal effect at zero time. The bactericidal effect for mixture (5) and mixture (8) was shown at 24 hrs and zero time, respectively. Thus, they were selected the mixture number (8) for using in part III from Thesis.

3. **Effect of combined antimicrobials and antioxidants against *P. aeruginosa* at 20°C:**
   - Mixtures 1, 2 and 3 had a slight bacteriostatic effect against test strain when compared to control. Bacteriostatic effect of mixture (4) was more high effective than mixtures 1, 2 and 3. Bactericidal effect was shown by mixtures 5, 6, 7 and 8 at 36 hrs. Most bactericidal effect was mixture (9) then killed all cells at zero time. Results obtained indicated that a high

bactericidal effect with low concentrations was for mixture (5) and mixture (9) at 36 hrs and zero time, respectively. Thus, they were selected the mixture (9) for using in part II from Thesis.

PART II: Effect of cold storage alone or accompanied with dipping treatment on Bolti fish:

1. Chemical composition of untreated and treated chilled Bolti fish:

- Moisture content was ranged from 78.74 to 77.66% for all treatments. During subsequent cold storage, a significant decremental pattern was shown in moisture content. At 9 days, total loss in moisture content was 4.42, 1.46, 0.94, 1.90 and 1.51%, for RB, TWB, ETWB, ITWB and EITWB, respectively
- Bolti fish samples contained 83.43 to 84.29% protein (on dry weight basis), such content consequently dropped during cold storage period. The rate of protein loss was more highly in RB than other all treatments. Less loss in protein content was shown in ETWB and EITWB.
- Total lipid content was around 10% at the beginning of cold storage then significantly ($P<0.05$) increased up to the end of various storage periods. The total lipid seemed to be slightly increased during cold storage in all samples. RB samples showed higher lipid content during storage period.
- The ash content was around 6.0% in all of the investigated treatments at zero time of cold storage. During subsequent

cold storage a significant ($P<0.05$) increase was observed till the end of storage periods.

2. Chemical freshness indices of untreated and treated chilled Bolti fish:

- The mean values of T.V.N. were ranged between 13.07 to 14 mg/100 g (on wet weight basis). With the progression of cold storage, a significant increase ($p<0.05$) in TVN for all investigated treatments. The lowest rate of TVN rise was observed in ETWB and EITWB means along life storage (18 days).
- TMA values were ranged between 0.84 to 1.21 mg/100 g (on wet weight basis) at zero time. During cold storage, great differences between treatments were anticipated. ETWB and EITWB had a long life when compared to other treatments.
- The TBA values were ranged from 0.50 to 0.78 mg malonaldhyde/kg. No significant difference ($P>0.05$) among all treatments was shown at zero time of cold storage. ETWB and EITWB treatments showed the lowest incremental pattern during cold storage than TWB and ITWB treatments when compared to RB treatment.

3. Physical properties of untreated and treated chilled Bolti fish:

- The average initial pH value of Bolti fish was around 6.48 and it increased continuously during cold storage till the end of the corresponding storage periods for each treatment. ETWB and EITWB had a lowest increasing rate.
- Optical density of Bolti fish gills extract ranged from 0.77 to 0.90 (as O.D. at 542 nm). A slight decrease between

eviscerated treatment and others. A significant ($P<0.05$) decremental pattern was shown in O.D. of gills extract for all treatments.

- Optical density of Bolti fish muscles extract ranged from 0.19 to 0.23 (as O.D. at 420 nm). The incremental trend of the ETWB and EITWB treatment were lower than that of TWB and ITWB when compared to RB treatment at 18 days.
- Pigments concentration ranged from 0.75 to 0.84 (as O.D. at 540 nm) at zero time of cold storage. During subsequent cold storage the pigments concentration was generally decreased with increasing the time. The decremental trend of the ETWB and EITWB treatments were lower than that of TWB and ITWB treatments when compared to RB treatment (at samples were organoleptically rejected).
- Refractive index of eye fluid (RI) ranged between 1.3364-1.3381 before storage then continuously increased till the end of storage periods to ranged from 1.4248 to 1.3992 for all treatments. However, the highest value was shown with RB treatment at 9 days of cold storage.
- Water holding capacity (WHC) was ranged from 6.87 to 8.97 cm^2/0.3 g sample. During cold storage, the dipping treatment was improved the (WHC) of treated samples, whereas the WHC was progressively decreased in RB treatment means the highest rat decreased.
- Plasticity ranged from 3.57 to 3.07 cm^2/0.3 g at zero time of cold storage. A continuous decrease was recorded till the end of cold storage for all treatments. At 9 days of cold

storage, ETWB and EITWB treatments showed higher plasticity than TWB and ITWB treatments when compared to RB treatment.

- Cooking loss was ranged from 9.50 to 11.24% in all treatments at zero time of cold storage. Cooking loss of RB treatment was sharply increased as cold storage period was extended. Such results were positively correlated with that of the WHC. The cooking loss was lower of both ETWB and EITWB than that of TWB and ITWB when compared to RB treatment. Moisture/protein percentage for ETWB and EITWB were 98.94 and 99.17% then the cooking loss were 7.88 and 8.22% at 18 days.

4. Microbiological quality attributes:

- Total mesophilic bacterial count (TMBC) of different treatments were 3.98, 3.25, 3.45, 3.78 and 3.62 log CFU/g enumerated by conventional plating method and 4.01, 3.11, 3.41, 3.71 and 3.71 CFU/g enumerated by four-culture method for RB, TWB, ETWB, ITWB and EITWB treatments, respectively at zero time. A considerable decrease of TMBC that noticed in treated sample when compared to RB samples at zero time of cold storage. A incremental trend in TMBC during subsequent cold storage was shown. ETWB and EITWB had a lowest increasing in the total mesophilic microbial load.
- Gram negative bacterial (GNB) of different treated Bolti fish had a incremental rate in all treatments. However, RB treatment was more highly than other treatments. ETWB and EITWB treatments had a lower increasing in GNB from

TWB and ITWB treatments when compared to RB treatment at end of cold storage periods on both methods.

- During cold storage, rapidly increasing in coliform group (CG) of RB treatments, but slowly increasing in dipped treatments was shown at 9 days. At end of storage periods, the CG load of dipped treatments samples not reached to the CG load of RB samples.
- *E. coli* count had a high different between RB samples and other dipped treatments then the dipping solution was more direct effect on *E. coli*. A incremental trend in all treatments at the end of storage periods was shown. However, the ETWB and EITWB treatments showed the lowest incremental pattern during cold storage from TWB and ITWB treatments when compared to RB treatment.
- The response of scatter plots exhibited a high degree of association between the four-culture methods and the conventional plating methods for the enumeration of total mesophilic microbial loads (r^2 = 0.94), gram-negative bacteria (r^2 = 0.89), coliform group (r^2 = 0.98) and *E. coli* (r^2 = 0.90). The association of the four-culture values with the results of the conventional plating methods suggested that the four-culture methods can be used to effectively enumerate the four critical bacterial group in fish over conventional plating methods. This method will facilitate sanitation monitoring at fish-processing plants by simplifying procedures.
- Psychrophilic count (PsC) were progressively increased with the prolonged cold storage. Regarding to the high effect of

dipping treatments on reducing (PsC) when compared to RB treatment was shown at zero time.

- Proteolytic bacteria counts were 3.41, 2.85, 2.40, 2.88 and 2.48 log CFU g^{-1} for RB, TWB, ETWB, ITWB and EITWB, respectively at zero time. With the progression of cold storage, a incremental trend in all treatments till the end of storage periods was observed.
- During subsequent cold storage, incremental trend in Lipolytic bacteria (LB) counts was recorded. RB treatment had a highest load when compared to all treatments at the end of storage periods.
- Moulds and yeasts (M&Y)was 2.35 log CFU g^{-1} for RB treatment, but not detected in other dipped treatment at zero time of storage. During subsequent cold storage, a slowly incremental trend was shown in M& Y counts till reached to 2.72 log CFU g^{-1} for RB treatment at 9 days.

5. Sensory evaluation of untreated and treated chilled Bolti fish:

5.1. Sensory evaluation of raw Bolti fish:

A significant differences ($P<0.05$) in appearance, color of gills, texture, odor, eye's lustrous, scales consistency, sliminess and overall acceptability between either different treatments or different storage periods was shown. During subsequent cold storage a decremental trend was shown in all characteristics. A significant differences ($P<0.05$) in sensory attributes between ETWB and RB treatments. Also, a same trend was shown between TWB and RB treatment. While, the ETWB was better than TWB treatments because, it obtained a highest values

indicate the best quality. A significant differences ($P<0.05$) in sensory characteristics with increasing storage periods was shown.

5.2. Sensory evaluation of cooked Bolti fish:

A significant difference ($p<0.05$) in appearance, taste, texture, juiciness, odor and Overall acceptability scores of different treated bolti fish after cooked between either different treatments or different storage periods was shown. Multiple comparisons for different treatments showed significant differences ($P<0.05$) between ETWB and RB treatments. Also, significant differences ($P<0.05$) between TWB and RB treatments. No significant differences ($P>0.05$) between TWB and ETWB was shown. ETWB was best treatment, because it obtained the highest score between all treatments. Multiple comparisons for different storage periods indicated that significant differences ($P<0.05$) in sensory characteristics at zero, 3 and 6 days.

PART III: Effect of frozen storage alone or accompanied with dipping treatments on Bolti fish:

1. Chemical composition of untreated and treated frozen Bolti fish:

- Moisture content was ranged from 78.64 to79.41% for all treatments. During subsequent frozen storage a decremental trend was shown. At 8 months the total loss in moisture contents were 4.95, 4.95, 2.30, 3.74 and 2.11% for RB, WWB, TWB, IWWB and ITWB treatments, respectively.

- Bolti fish samples contained 79.56 to 80.80% protein (on dry weight basis), such content consequently dropped during frozen storage period. At 8 months of frozen storage by loss being 7.29, 5.15, 3.99, 4.99 and 1.99 for RB, WWB, TWB, IWWB and ITWB treatments, respectively.
- Total lipid content was around 13.46% (on dry weight basis) at zero time of frozen storage then significantly ($P<0.05$) increased up to the end of storage periods.
- Ash content was ranged from 5.28 to 5.47 (on dry weight basis) at zero time. A slight increase in ash content of treated Bolti fish after dipping when compared with untreated ones was shown. However, frozen storage a significant ($P<0.05$) increase was observed.

2. Chemical freshness indices of untreated and treated frozen Bolti fish:

- The TVN ranged between 12.60 to 13.53 mg/100 g (on wet weight basis). The higher rate of TVN increasing during frozen storage was found in RB treatment when compared to other treatments.
- Trimethylamine values were ranged between 1.26 to 2.19 mg/100 g (on weight wet basis). A progressive increase in TMA during frozen storage period occurred till reached to a maximum value at 8 months of frozen storage in RB treatment. Dipped samples showed less rate of TMA rise during frozen storage.
- Thibarbituric acid values (as mg malonaldhyde/kg sample) were ranged from 0.22 to 0.45 mg malonaldhyde/kg. TBA values were 4.51, 3.98, 3.35, 3.48 and 2.76 RB, WWB,

TWB, IWWB and ITWB, respectively. The lower TBA values obtained in TWB and ITWB treatments than WWB and IWWB treatments when compared to RB treatment (at 8 months) one.

3. Physical properties of untreated and treated frozen Bolti fish:

- pH value was 6.36 to 6.18 for all treatments. Continuously increased during frozen storage till the end of the corresponding storage periods for each treatments was shown.
- The O.D. of gills extract ranged from 0.73 to 0.82 (as O.D. at 542 nm). A significant ($P<0.05$) decremental pattern was shown in O.D. of gills extract for all treatments. A highest O.D. of gills extract values were recorded with TWB and ITWB than WWB and IWWB treatment when compared to RB treatment.
- The optical density of Bolti fish muscles extract ranged from 0.305 to 0.503 (as O.D. at 420 nm). The incremental trend of TWB and ITWB was lower than that IWWB and WWB treatments when compared to RB sample at 8 months.
- Pigments concentration ranged from 0.93 to 1.09 (as O.D. at 540 nm) at zero time of frozen storage. The incremental trend of TWB and ITWB was lower than that WWB and IWWB treatments when compared to RB sample at 8 months.
- The RI of eye fluid was ranged from 1.3361 to 1.3394 before storage, then continuously increased till the end of storage

periods. The highest values was shown with RB treatments samples at 8 months of storage.

- The WHC value ranged from 9.40 to 11.23 (cm^2/0.3 g). During frozen storage of all treatments, the WHC was progressively decreased. While, it was improved in ITWB and TWB treatments.
- Plasticity ranged from 2.70 to 3.43 cm^2/0.3 g at zero time of frozen storage, a continuous decrease was recorded till the end of frozen storage for all treatments. At 8 months of storage, TWB and ITWB showed a higher plasticity than other treatments.
- The cooking loss value was around 13.59% at zero time of frozen storage. A continuous decreased is corresponding with time extended.

4. Microbiological quality attributes:

- Total mesophilic bacterial count (TMBC), Gram negative bacterial (GNB), Coliform group (CG) and *E. coli* count had considerable decreased during subsequent frozen storage. TWB and ITWB treatments had a highest decreasing in the microbial load.
- Interaction between frozen storage and dipping treatment causes decremental trend in microbial load (TMBC, GNB, CG and EC) of dipped samples. While, RB treatments had upward trend at second month but downward was shown as time extended. The response of scatter plots exhibited a high degree of association between the four-culture methods and the conventional plating methods for the enumeration of total mesophilic microbial loads (r^2 = 0.79), gram-negative

bacteria ($r^2 = 0.93$), coliform group ($r^2 = 0.86$) and *E. coli* ($r^2 = 0.76$). The association of the four-culture values with the results of the conventional plating methods suggested that the four-culture methods can be used to effectively enumerate the four critical bacterial group in fish over conventional plating methods.

- Proteolytic bacteria counts were 3.24, 3.17, 2.51, 2.90 and 2.80 log CFU g^{-1} for RB, WWB, TWB, IWWB and ITWB treatments, respectively (at zero time). With the progression of frozen storage, a variable trend in all treatments till the end of storage periods was observed. TWB and ITWB treatments had a lower bacterial load than other treatments.
- During subsequent frozen storage, variable trend in all treatments till the end of storage periods was observed in Lipolytic bacteria (LB) counts was recorded. RB treatments had a highest bacterial load when compared to all treatments at the end of storage periods.
- Moulds and yeasts (M&Y) was 2.20, 2.13 and 2.52 log CFU g^{-1} for RB, WWB and IWWB treatments, but not detected in TWB and ITWB at zero time of storage. During subsequent frozen storage, a slowly incremental trend was shown in M&Y counts of RB and IWWB treatments till the end of storage.

5. Sensory evaluation of untreated and treated frozen bolti fish:

5.1. Sensory evaluation of raw Bolti fish:

A significant differences($P<0.05$) in appearance, color of Bolti fish gills, texture, scales consistency, odor, sliminess, eye's

lustrous and overall acceptability between either different treatments or different storage periods. Multiple comparisons for different treatments indicated that there are significant differences ($P<0.05$) in sensory attributes between RB, WWB and TWB treatments means TWB treatment which obtained the highest values of parameters. Multiple comparisons for different storage periods clearly noticed that frozen storage time caused a significant differences ($P<0.05$) on sensory attributes of different treated samples at zero, 6 and 8 months. The expiration periods of the three Bolti fish samples not more 6 months for RB and WWB samples and not more 8 months for TWB samples.

5.2. Sensory evaluation of cooked Bolti fish:

This is no significant difference ($p>0.05$) between the three investigated treatments for appearance, texture, odor, taste, juiceness and overall acceptability at zero time of frozen storage. As the storage time prolonged a decremental trend was shown in all treatments. A significant differences ($P<0.05$) between TWB, RB and WWB treatments at 6 months of frozen storage was shown. The TWB had a highest score at zero time storage. RB and WWB treatments had a lowest score in appearance, texture, odor, taste, juiciness sliminess and overall acceptability when compared to TWB treatment at 6 months storage. The TWB treatments with stand frozen storage up to 8 months and their qualities were still acceptable, but RB and WWB treatments rejected at 8 months.

Conclusion

- Mixture contained 2.7% sodium lactate (SL) + 3.6% tri-sodium phosphate (TSP) + 5.2% potassium sorbate (PS) +270 ppm butylated hydroxyanisol (BHA) was more effective on microbial growth of either *Escherichia coli* O157:H7 ATCC 69373 or *Pseudomonase aeruginosa.*
- Chemical composition, freshness tests, physical properties, microbiological assay and sensory evaluation of different treated Bolti fish indicated that, it could be safely estimate the expiration periods of the three Bolti fish treatments to be as follows: (RB) samples: about 6 days, (TWB) samples: about 11 days and (ETWB) samples: about 17 days.
- According to mentioned data of freshness tests, physical properties, microbiological assay and sensory evaluation, whole fish with additional dipping treatment (TWB) and stored at 3±1°C could extend the shelf-life of Bolti fish 5 days more, whereas, when eviscerated fish and dipped in the same preservative solution, the expiration period was extended 11 days more.
- Chemical composition, Physical properties and sensory evaluation of different treated Bolti fish, it could be safely estimate the expiration periods of the three Bolti fish treatments to be as follows: (RB and WWB) samples: not more 6 months and (TWB) samples: not more 8 months.
- According to previously whole fish with additional dipping solution (TWB) and frozen storage at -18+1°C, extend the shelf-life of Bolti fish 2 months more when compared to either (RB and WWB) treatments.