RESULTS AND DISCUSSION

PART I:

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

Antimicrobial efficacy of 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) at different concentrations against *E. coli* O157:H7 ATCC 69373 in laboratory media (Tryptic Soy Broth) at 37°C through 24 hrs was studied, data are recorded in Table (1).

None of the additives had bactericidal effect at different concentrations at zero time but, the inhibition percentage was increased with increasing concentrations. Nearly, sodium lactate was more effective than others antimicrobials at zero, 12 and 24 hrs. This may be due to the effect of sodium lactate on decreasing water activity higher than organic salts at equimolar concentrations, a specific inhibition effect for the lactate ion on cell growth and reducing pH. The trend of our results was in agreement with other results as found by Lamkey *et al.* (1991), Shelef and Yang (1991), Shelef (1994), Naidu (1999), Kim *et al.* (2002) and McWilliam Leital and Stewart (2002).

From these data, sodium lactate was most effective at any concentration then 1% SL had bacterostatic effect on bacterial growth but, it was very effective at concentration $\geq$ 2%. A 3% SL had bactericidal effect against tested strain at 24 hrs (Fig. 1a).

This may be due to that lactate is one of the most effective organic salts in reducing numbers of viable *E. coli* O157:H7 strain (Hostema *et al.* 1993). These results are in agreement with Jordan *et al.* (1999), McWilliam Leital and Stewat (2002) and Whiling (2003).

Sodium acetate and sodium citrate had bacteriostatic effect at different concentrations against testsd strain. The inhibition percentage was increased with increasing the concentrations at zero, 12 and 24 hrs (Fig, 1a). While, after 24 hrs the cells were still a live, this may be due to relationship with the high acid tolerance of this pathogen strain. Sodium acetate was more effective than sodium citrate at any incubation time as indicated before (Benjamin and Datta, 1995; Conner and Kotrola, 1995 and Brudzinski and Harrison, 1998).

Tri-sodium phosphate had more effect than sodium acetate and sodium citrate. Tri-sodium phosphate was highly bacteriostatic effect at concentration from 1 to 3%, then the inhibition percentages were increased with increasing the concentration. Meanwhile, 4% TSP was bactericidal effect against test strain after 24 hrs incubation (Fig., 1a). This may be due to the effect of phosphate for chelating of metal ions in cell membranes which leads to caution deficiency with resulting loss of membrane integrity and inhibition of normal cell division. Phosphates can form stable complexes with metallic ions with resulting strong bound, these bound ions then become unavailable for metabolic functions of bacteria (Naidu, 1999). On the other hand, phosphates are polyvalent anions, they have a high degree of surface activity causing them to aggregate at cell surfaces when dissolved in water, this mechanism accounted for

the observed lysis of gram-negative bacteria and the growth inhibition of gram-positive bacteria as well as fungi. The trend of our results was in agreement with Kim and Slavik (1994), Taormina and Buechat (1999), Ramirez *et al.* (2001) and Korber *et al.* (2002).

Potassium sorbate and ascorbic acid have been shown to inhibit growth of yeasts, molds and many bacteria. Its activity against bacteria, however, is not as comprehensive as that against yeasts and molds (Sofos, 1989). Potassium sorbate and ascorbic acid were ineffective at different series levels concentrations against test strain at initial inoculums $1.30x10^5$. The inhibition percentage was increased with increasing the concentrations of potassium sorbate and a scobic acid (Fig, 1b) but, potassium sorbate was more effective than ascorbic acid against test strain at zero, 12, 24 hrs. Obtained results were in agreement with (Sofos and Busta, 1981; Liewn and Marth, 1985; Sofos, 1989 and 1992; El-Shenawy and Marth, 1988; Sofos and Busta, 1993; Kim and Slavik, 1994; Kasrazdah and Genigeorgis, 1995; Shaur and Cheng, 1996 and Cagri *et al.,* 2002).

Antimicrobial activity of BHA was high effective on microbial growth of *E. coli* O157:H7 ATCC 69373, this was reasoned that it may posses inhibitory powers against microorganisms (Fig, 1b). It was also thought that the antimicrobial effectiveness of this compound was warranted. The phenolic ring of BHA could interfere with the cell membrane or compete with coenzyme for protein, as suggested by Wyss (1948). Another possible mode of action could involve the ability of BHA to be a hydrogen donor, thus acting as a reducing agent as described by (Killindworth *et al.,* 1979).

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

Additives		Zero time		12 hrs		24 hrs	
		CFU ml^{-1}	IP%[$]	CFU ml^{-1}	IP%	CFU ml^{-1}	IP%
Control	0%	$1.53x10^5$	---	$2.18x10^7$	---	$3.45x10^7$	---
Sodium lactate	1%	$8.95x10^4$	41.50	$2.36x10^5$	98.92	$1.52x10^6$	95.59
	2%	$5.80x10^4$	62.09	$1.81x10^4$	99.92	$8.15x10^3$	99.98
	3%	$1.28x10^4$	91.63	$3.05x10^2$	99.99	0.00	100
	4%	$3.40x10^2$	99.78	0.00	100	0.00	100
Sodium acetate	1%	$1.01x10^5$	33.99	$2.32x10^6$	89.36	$1.84x10^7$	46.67
	2%	$9.70x10^4$	36.60	$1.81x10^6$	91.70	$1.06x10^7$	69.27
	3%	$5.45x10^4$	64.38	$8.30x10^5$	96.19	$2.48x10^6$	92.81
	4%	$5.30x10^4$	65.36	$2.81x10^5$	98.71	$1.88x10^5$	99.46
Sodium citrate	1%	$1.26x10^5$	17.56	$4.55x10^6$	79.13	$1.38x10^7$	60.00
	2%	$1.02x10^5$	33.33	$4.05x10^6$	81.42	$2.66x10^6$	92.29
	3%	$9.60x10^4$	37.25	$3.80x10^6$	82.57	$1.39x10^6$	95.97
	4%	$9.15x10^4$	40.20	$2.95x10^6$	86.47	$4.50x10^5$	98.70
Tri-sodium phosphate	1%	$1.03x10^5$	32.68	$1.03x10^6$	85.28	$1.38x10^5$	99.60
	2%	$6.60x10^4$	56.86	$1.31x10^5$	99.40	$4.55x10^2$	99.99
	3%	$1.96x10^4$	78.19	$1.35x10^3$	99.99	$2.60x10^2$	99.99
	4%	$1.49x10^3$	99.03	$5.25x10^2$	99.99	0.00	100.0
Control	0%	$1.30x10^5$	---	$1.86x10^6$	---	$4.95x10^7$	---
Potassium sorbate	1%	$1.18x10^5$	9.23	$1.02x10^6$	35.48	$2.42x10^7$	51.11
	2%	$9.30x10^4$	28.46	$2.12x10^5$	88.60	$1.64x10^6$	69.69
	3%	$6.60x10^4$	49.23	$1.61x10^5$	93.76	$2.75x10^5$	99.44
	4%	$4.85x10^4$	62.65	$4.25x10^4$	97.72	$1.03x10^5$	99.79
Ascorbic acid	0.1%	$1.21x10^5$	6.92	$1.36x10^6$	26.88	$1.17x10^7$	76.36
	0.2%	$9.40x10^4$	27.69	$1.05x10^6$	43.55	$1.38x10^6$	97.21
	0.3%	$7.80x10^4$	40.0	$7.25x10^5$	61.02	$1.08x10^6$	97.82
	0.4%	$5.20x10^4$	60.0	$5.80x10^4$	96.88	$6.50x10^5$	98.69
BHA	100 ppm	$9.20x10^4$	29.23	$8.35x10^5$	55.11	$4.52x10^6$	90.87
	200 ppm	$6.20x10^4$	52.31	$5.10x10^5$	72.58	$2.47x10^5$	99.50
	300 ppm	$2.86x10^4$	78.0	$1.27x10^5$	93.17	0.00	100.0
	400 ppm	$2.75x10^4$	78.85	$9.07x10^2$	99.95	0.00	100.0
BHT	100 ppm	$1.15x10^5$	11.54	$7.95x10^5$	57.26	$2.00x10^7$	59.60
	200 ppm	$1.03x10^5$	20.77	$6.95x10^5$	62.63	$1.72x10^6$	96.53
	300 ppm	$8.75x10^4$	32.69	$2.82x10^5$	84.84	$1.42x10^6$	97.13
	400 ppm	$8.50x10^4$	34.62	$1.63x10^5$	91.24	$1.92x10^5$	99.61

$: Inhibition percentage = $\frac{\text{Control count - treatment count}}{\text{Control count}} \times 100$

(Killindworth *et al.*, 1979)

CFU ml^{-1}: Colony forming unit ml^{-1}.

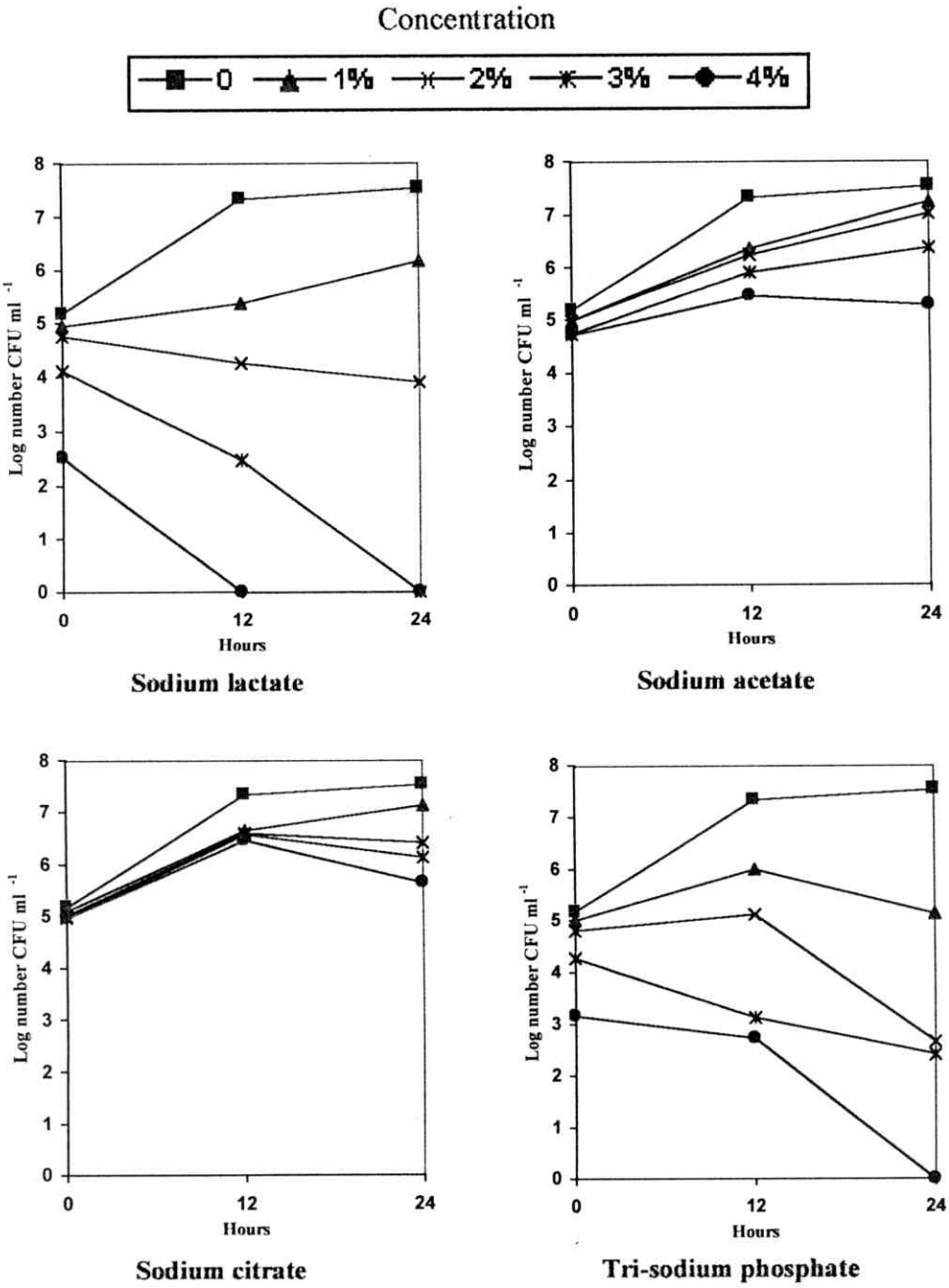


Fig. (1a): Effect of sodium lactate, sodium acetate, sodium citrate and tri-sodium phosphate at different concentrations against *E. coli* O157: H7 ATCC 69373 at 37°C.

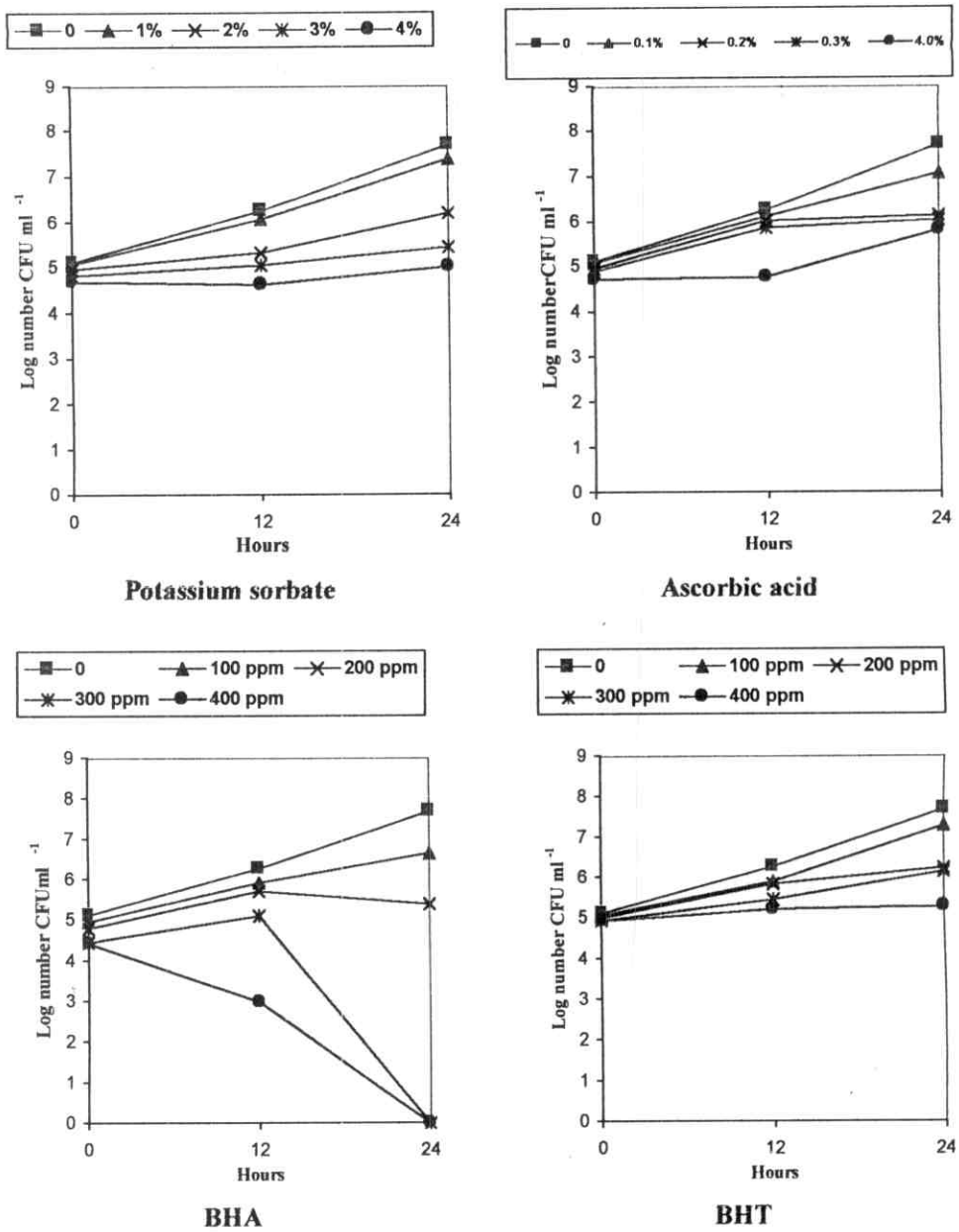


Fig. (1b): Effect of potassium sorbate, ascorbic acid, BHA and BHT at different concentrations against *E. coli* O157: H7 ATCC 69373 at 37°C.

BHA was more effective than BHT against test strain at initial inoculums $1.30x10^5$, these results are in agreement with (Lahellec *et al.,* 1981). BHT was ineffective at 100 to 400 ppm. While, BHA had bacteriostatic effect at 200 ppm then it had bactericidal effect with 300 ppm at 24 hrs incubation. The trend of our results are in agreement with reports by other researchers who had studied the antimicrobial property of phenolic antioxidants against various bacteria. Increasing the concentration of antioxidants had been shown to cause increased inhibition and/or death of various microorganisms in different laboratory media and model food systems (Chang and Branen, 1975; Oluski *et al.,* 1977; Shis and Harris, 1977; Robach and Pierson, 1979 and Grewal *et al.,* 1998).

The minimum inhibitory concentrations (concentration which inhibited the growth after 24 hrs) of sodium lactate, tri-sodium phosphate and BHA were 3, 4% and 300 ppm, respectively (at 24 hrs) (Table, 1). On the other hand, SA, SC, PS, AA and BHT hat no inhibiting effect at any concentrations. So it was decided to study the effect of more concentrations of these antimicrobials and antioxidants against *E. coli* O157:H7 ATCC 69373 at 37°C in TSB. The tested concentration were 5 & 6% for each of SA, SC and PS; 0.5 & 0.6% for AA and 500 & 600 ppm for BHT. The results were shown in Table (2) and Fig. (2). All tested additives had bacteriostatic effect by initial inoculums $3.4x10^5$ at zero time. In the same time, potassium sorbate and BHT had bactericidal effect at 6% and 500 ppm, respectively at 24 hrs. These results are in agreement with Gailani (1984).

2. Antimicrobial effect of combined antimicrobials and antioxidant against *E. coli* O157:H7 ATCC 69373 at 37°C:

Results in Tables (1 and 2) led to prepare ten mixtures of (SL, TSP, PS and BHA) with different concentrations which were presented previously in Table (A), section of materials and methods.

Effect of these mixtures against *E. coli* O157:H7 ATCC 69373 at 37°C in TSB was investigated to select the highly effective mixture with low concentration and data were shown in Table (3) and Fig. (3). Mixture (1) had slight bacteriostatic effect on growth of strain that the initial number was $4.10x10^6$ and became $1.40x10^6$, $7.85x10^6$ and $1.36x10^6$ at zero, 12, 24 hrs, respectively. A slight decrease was occurred in number of cells after 24 hrs. Effect of mixtures 2, 3 and 4 were increased with increasing of mixtures concentrations. This may be due to the accumulation effect of individual components of mixtures which caused a high effect on test strain. Inhibition percentage were 88.17, 98.78, 99.77 and 99.90% for mixtures 1, 2, 3 and 4 after 24 hrs incubation, respectively. Either mixture (5) or mixture (6) had bactericidal effect on test strain at 24 hrs. However, mixture (7) had bactericidal effect at 12 hrs. While, mixture (8) had more bactericidal effect on tested strain at zero time. Obtained results indicated that mixture (5) and (8) had bactericidal effect at 24 and zero hrs, respectively. These results are in agreement with (Stern *et al.*, 1979; Pierson *et al.*, 1980; Lahellec *et al.*, 1981; Morad *et al.*, 1982; Woolsey, 1998; Venkitanarayanan *et al.*, 1999 and Myung *et al.*, 2001). From these results, mixture

Table (2): Effect of more concentration of additives against *E. coli* O157:H7 ATCC 69373 in TSB at 37°C.

Additives		Zero time		12 hrs		24 hrs	
		CFU ml^{-1}	IP%	CFU ml^{-1}	IP%	CFU ml^{-1}	IP%
Control	0%	$3.4x10^5$	0.00	1.3610^7	0.00	$8.30x10^6$	0.00
Sodium acetate	5%	$1.29x10^5$	62.06	$2.87x10^5$	97.89	$1.85x10^5$	97.77
	6%	$1.10x10^5$	67.64	$1.78x10^5$	98.70	$1.51x10^5$	98.18
Sodium citrate	5%	$2.10x10^5$	38.24	$8.35x10^5$	93.87	$1.47x10^5$	98.23
	6%	$1.84x10^5$	45.88	$6.30x10^5$	95.37	$1.35x10^5$	98.37
Potassium sorbate	5%	$1.23x10^5$	63.82	$1.85x10^3$	99.99	$1.59x10^3$	99.98
	6%	$9.90x10^4$	70.88	$1.70x10^2$	99.10	0.00	100
Ascorbic Acid	0.5%	$1.53x10^5$	55.00	$3.90x10^5$	97.13	$6.10x10^4$	99.27
	0.6%	$1.41x10^5$	58.53	$1.28x10^5$	99.06	$3.45x10^4$	99.58
BHT	500 ppm	$2.14x10^5$	37.06	$2.14x10^5$	98.43	0.00	100
	600 ppm	$1.78x10^5$	47.65	$1.22x10^5$	99.10	0.00	100

CFU ml^{-1}: Colony forming unit ml^{-1}.

Table (3): Effect of combined antimicrobials and antioxidant against *E. coli* O15:H7 ATCC 69373 in TSB at 37°C:

Mixtures of additives#	Zero time		12 hrs		24 hrs	
	CFU ml^{-1}	IP%	CFU ml^{-1}	IP%	CFU ml^{-1}	IP%
control	$4.10x10^6$	0.00	$7.85x10^6$	0.00	$1.15x10^7$	0.00
M1	$1.40x10^6$	65.85	$2.78x10^6$	64.59	$1.36x10^6$	88.17
M2	$1.16x10^6$	71.71	$5.75x10^5$	92.68	$1.40x10^5$	98.78
M3	$2.59x10^5$	93.68	$3.15x10^4$	99.76	$2.64x10^4$	99.77
M4	$2.11x10^5$	94.85	$1.94x10^4$	99.75	$1.11x10^4$	99.90
M5	$3.60x10^3$	99.91	$3.30x10^3$	99.96	0.00	100
M6	$1.55x10^3$	99.96	$8.85x10^2$	99.99	0.00	100
M7	$6.85X10^2$	99.98	0.00	100	0.00	100
M8	0.00	100	0.00	100	0.00	100
M9	0.00	100	0.00	100	0.00	100
M10	0.00	100	0.00	100	0.00	100

CFU ml^{-1}: Colony forming unit ml^{-1}.
Mixtures are indicated previously in Table (A) in materials and methods.

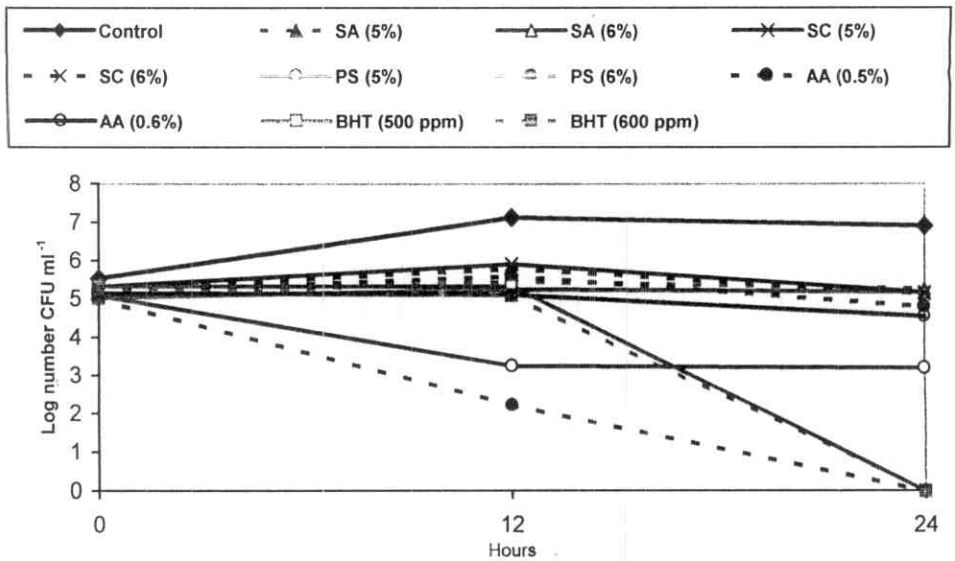


Fig. (2): Effect of more concentrations of antimicrobials and antioxidants against *E. coli* O157:H7 ATCC 69373 at 37°C.

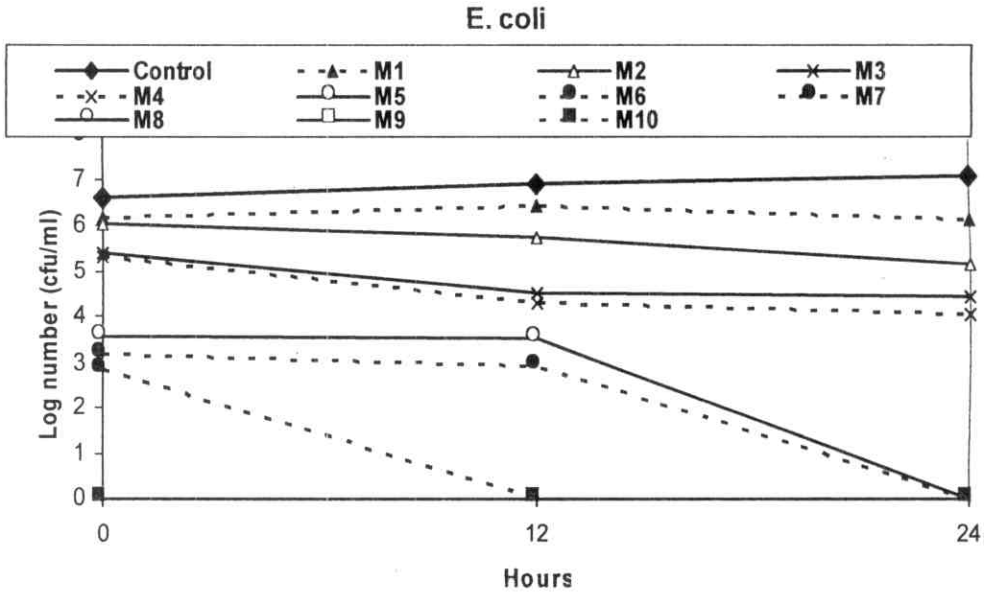


Fig. (3): Antimicrobial effect of combined antimicrobials and antioxidants against *E. coli* O157:H7 ATCC 69373 at 37°C.

loss in moisture content was 4.42, 1.46, 0.94, 1.90 and 1.51% for, RB, TWB, ETWB, ITWB and EITWB, respectively. While it was 3, 1.7, 3.2 and 2.21% for TWB, ETWB, ITWB and EITWB after 13 days, respectively. But, at the end storage period (18 days) it was 2.56 and 3.91% for ITWB and EITWB, respectively. The rate of moisture loss was more highly in RB than other treatments. This mainly due to the effect of dipping treatment (SL + TSP + PS + BHA) in improving the water holding capacity of fish protein (Hassan, 1980; Kirivchenia and Fennema, 1988; Reddy *et al.*, 1994 and Yasin, 1997).

Crude protein content is the second investigated parameter in the chemical composition of untreated and treated fish samples given in the same table. At the beginning of cold storage, no significant difference ($P>0.05$) occurred as a result of these treatments. Bolti fish samples contained 83.43 to 84.29% protein (on dry weight basis), such content consequently dropped during cold storage period. After 9 days of cold storage the loss being 9.55, 3.62, 3.01, 4.07 and 3.47% for RB, TWB, ETWB, ITWB and EITWB, respectively. However it was 9.61, 4.9, 8.78 and 4.53% for TWB, ETWB, ITWB and EITWB after 13 days, respectively. While it was 8.33 and 9.07% for ETWB and EITWB after 18 days, respectively. The rate of protein loss was more highly in RB than other all treatments. During subsequent cold storage, the samples were judged organoleptically to be spoiled (Table, 5), the less loss in crude protein content was shown in ETWB samples. The effect of dipping treatment (mainly TSP and SL) in improving the water holding capacity of fish protein and reducing the water activity could be a good explanation for less loss in crude protein from dipping samples

during cold storage (Hassan, 1980; Reddy, 1994; Shelef, 1994 and Yasin, 1997).

The total lipid content (calculated on dry weight basis) of different untreated and treated fish samples under investigation was also given in Table (5). At zero time of storage, slight increase in total lipid content was shown in both TWB and ETWB from RB while, slight decrease in total lipid content was ITWB and EITWB. Total lipid content was around 10% (on dry weight basis) at the beginning of cold storage then significantly ($P<0.05$) increased up to the end of various storage periods. However, the total lipid seemed to be slightly increased during cold storage in all samples. The more loss of nitrogenous compounds and moisture content during cold storage always were accompanied with increasing total lipid content in fish samples (on dry weight basis). These results coincide with those obtained by Cross (1980), Yasin (1997), Abou-Taleb and Ibrahim (2002) and Yasin (2003).

The ash content also, it was around 6.0% (on dry weight basis) in all of the investigated samples at zero time of cold storage. However, during cold storage a significant ($P<0.05$) increase was always observed. The increase percentages were 8.99, 12.0, 7.2, 7.3 and 6.04 for RB, TWB, ETWB, ITWB and EITWB after 9 days, respectively. A incremental trend was also shown till the end of storage periods. An apparent increase in ash content of chilled samples is due to the moisture loss under the same condition. This may be due to the effect of dipping treatment then occurred migration of antimicrobials to into the muscles (Mohamed, 1991).

Table (5): Chemical composition of untreated and treated Bolti fish during cold storage at 3±1°C (mean ± standard error).

Storage period (days)	Moisture content					Protein content ^{S}				
	RB	TWB	ETWB	ITWB	EITWB	RB	TWB	ETWB	ITWB	EITWB
Zero	78.74 ±0.16	78.59 ±0.28	77.66 ±0.16	78.48 ±0.24	78.35 ±0.55	83.55 ±0.55	83.94 ±0.28	83.43 ±0.91	84.29 ±0.75	83.49 ±1.54
3	78.55 ±0.14	78.47 ±0.02	77.60 ±0.04	77.96 ±0.14	78.07 ±0.21	82.94 ±0.74	83.48 ±0.33	83.16 ±0.16	83.54 ±0.83	83.24 ±0.92
6	76.70 ±0.11	77.94 ±0.09	76.99 ±0.31	77.02 ±0.14	77.20 ±0.20	79.43 ±0.35	82.60 ±0.28	81.90 ±1.60	81.64 ±1.74	82.02 ±1.79
9	75.26® ±0.23	77.44 ±0.23	76.93 ±0.23	76.99 ±0.19	77.17 ±0.09	75.57® ±2.76	80.90 ±0.49	80.82 ±1.41	80.86 ±1.44	80.59 ±0.93
11		77.26 ±0.28	76.67 ±0.14	76.83 ±0.18	77.09 ±0.23		79.61 ±0.88	79.87 ±0.98	80.22 ±1.20	80.01 ±0.88
13		76.23® ±0.18	76.34 ±0.23	75.98 ±0.22	76.62 ±0.18		75.87® ±0.76	79.33 ±0.66	76.89 ±1.14	79.71 ±0.76
15			76.23 ±0.36		76.56 ±0.14			78.76 ±0.46		79.70 ±0.96
17			76.13 ±0.18		75.55 ±0.28			77.82 ±0.24		77.26 ±0.66
18			75.67® ±0.28		75.29 ±0.16			76.48® ±0.56		75.92 ±0.34
LSD $^{@}_{(0.05)}$	0.64					3.40				

S: Calculated on dry weight basis. ®: At these point samples were organoleptically rejected.

@: To be used to compare between any two times within the same treatment.

RB : Raw Bolti fish untreated (control).

TWB : Whole Bolti fish was only washed, then dipped in a solution contained 2.7% SL + 3.6% TSP + 5.4% PS + 270 ppm BHA for 2 min. and drained.

ETWB : Whole Bolti fish was eviscerated, washed, then dipped in a solution contained 2.7% SL + 3.6% TSP + 5.2% PS + 270 ppm BHA for 2 min. and drained.

ITWB : Whole Bolti fish was only washed and inoculated with dipping in solution contained *E. coli* O157:H7 ATCC 69373 ($4.3x10^6$), then left for 15 min. and dipped in a solution contained 2.7% SL + 3.6% TSP + 5.4% PS + 270 ppm BHA, for 2 min. and drained.

EITWB : Whole Bolti fish was eviscerated, washed and inoculated with dipping in solution contain *E. coli* O157:H7 ATCC 69373 ($4.3x10^6$), then left for 15 min. and dipped in a solution contained 2.7% SL + 3.6% TSP + 5.4% PS + 270 ppm BHA for 2 min. and drained.

Table (5): Cont.

Storage period (days)	Total lipid content ^{S}					Ash content ^{S}				
	RB	TWB	ETWB	ITWB	EITWB	RB	TWB	ETWB	ITWB	EITWB
Zero	10.25 ±0.53	10.52 ±0.19	10.74 ±0.86	9.90 ±0.54	9.82 ±1.05	6.01 ±0.37	5.50 ±0.24	6.04 ±0.14	5.91 ±0.17	6.13 0.23
3	10.77 ±0.87	10.50 ±0.32	10.85 ±0.17	9.99 ±0.38	10.62 ±0.78	6.20 ±0.39	6.07 ±0.06	6.04 ±0.10	5.97 ±0.13	6.20 ±0.04
6	13.68 ±0.34	11.24 ±0.33	12.19 ±0.26	12.16 ±0.32	11.68 ±0.18	6.59 ±0.31	6.15 ±0.18	6.30 ±0.43	6.09 ±0.11	6.40 ±0.24
9	15.43® ±0.33	12.85 ±0.43	12.33 ±0.27	12.37 ±0.12	12.08 ±0.58	6.55® ±0.18	6.16 ±0.16	6.48 ±0.33	6.34 ±0.04	6.50 ±0.10
11		13.08 ±0.18	12.95 ±0.26	12.75 ±0.24	12.26 ±0.96		6.22 ±0.08	6.55 ±0.12	6.38 ±0.09	6.58 ±0.12
13		16.22® ±0.36	13.54 ±0.54	13.88 ±0.44	12.22 ±0.82		6.33® ±0.10	6.55 ±0.24	6.40 ±0.10	6.53 ±0.18
15			14.31 ±0.34		12.96 ±0.54			6.59 ±0.16		6.60 ±0.08
17			15.07 ±0.18		14.76 ±0.68			6.69 ±0.06		6.75 ±0.23
18			16.20® ±0.16		15.97 ±0.24			6.80® ±0.18		6.72 ±0.19
LSD$^{@}_{(0.05)}$	1.46					0.65				

S: Calculated on dry weight basis. ®: At these point samples were organoleptically rejected.

@: To be used to compare between any two times within the same treatment.

RB : Raw Bolti fish untreated (control).

TWB : Whole Bolti fish was only washed, then dipped in a solution contained 2.7% SL + 3.6% TSP + 5.4% PS + 270 ppm BHA for 2 min. and drained.

ETWB : Whole Bolti fish was eviscerated, washed, then dipped in a solution contained 2.7% SL + 3.6% TSP + 5.4% PS + 270 ppm BHA for 2 min. and drained.

ITWB : Whole Bolti fish was only washed and inoculated with dipping in solution contained *E. coli* OI57:H7 ATCC 69373 ($4.3x10^6$), then left for 15 min. and dipped in a solution contained 2.7% SL + 3.6% TSP + 5.4% PS + 270 ppm BHA, for 2 min. and drained.

EITWB : Whole Bolti fish was eviscerated, washed and inoculated with dipping in solution contained *E. coli* OI57:H7 ATCC 69373 ($4.3x10^6$), then left for 15 min. and dipped in a solution contained 2.7% SL + 3.6% TSP + 5.4% PS + 270 ppm BHA for 2 min. and drained.

Analysis of variance was carried out to detect the significant difference of the effect of both storage time and dipping treatment on the chemical composition of Bolti fish samples (Table, 6). Data indicated that there are significant differences ($P<0.05$) in moisture, protein, total lipid between either different treatments or different storage periods. A significant differences ($P<0.05$) were shown in ash content as affect by different storage time. While, no significant different ($P>0.05$) in ash as affected by different treatments. F value of interaction was significant in moisture whereas it was not significant in protein, lipid and ash contents.

Multiple comparisons for different treatments (Table, 7) indicated that no significant difference ($P>0.05$) in moisture content between RB, ETWB, ITWB and EITWB treatments. In the same time, significant difference ($P<0.05$) in protein and lipid contents between RB and other treatments. From the same table, no significant difference ($P>0.05$) in ash content for RB, ETWB, ITWB and EITWB treatments whereas significant different ($P<0.05$) between TWB and other treatments.

Multiple comparisons for different storage periods (Table, 8) indicated significant differences ($P<0.05$) in moisture, protein, lipid and ash contents at zero, 6 and 9 days. Not significant differences ($P>0.05$) in moisture, protein, lipid and ash contents at zero and 3 days.

In conclusion, the ETWB and EITWB treatments showed less changes in their chemical composition from TWB and ITWB treatment during cold storage when compared to RB treatment.

Table (6): Analysis of variance for chemical composition (calculated on dry weight basis) of untreated and treated Bolti fish stored at 3±1°C for 9 days.

Source of variance	D.F	F value and level of significancy			
		Moisture %	Protein %	Lipid %	Ash %
Time	3	58.67*	11.49*	33.41*	4.53*
Treatment	4	8.89*	2.61*	5.63*	1.85 NS
Time x treatment	12	5.61*	0.83 NS	1.96 NS	0.25 NS
Error	40	---	---	---	---
Total	59	---	---	---	---

D.F.: Degree of freedom
* Significant at 0.05
N.S.: Non significant

Table (7): Effect of different treatments on chemical composition (calculated on dry weight basis) of untreated and treated Bolti fish stored at 3+1°C for 9 days.

Treatments @	Components			
	Moisture %	Protein %	Total lipid %	Ash %
RB	77.30^{cb}	80.37^{b}	12.53^{a}	6.34^{a}
TWB	78.11^{a}	82.73^{a}	11.28^{b}	5.97^{b}
ETWB	77.30^{cb}	82.33^{a}	11.53^{b}	6.22^{ab}
ITWB	77.61^{b}	82.58^{a}	11.11^{b}	6.08^{ab}
EITWB	77.69^{b}	82.34^{a}	11.05^{b}	6.31^{a}
LSD (0.05)	0.32	1.70	0.73	0.33

@ : As tail of Table (5)
Means with the same latter in the same column are not significant different (P>0.05).

Table (8): Effect of different storage periods on chemical composition (calculated on dry weight basis) of Bolti fish stored at 3±1°C for 9 days.

Storage period (days)	Components			
	Moisture %	Protein %	Total lipid %	Ash %
0	78.36^{a}	83.74^{a}	10.25^{c}	5.92^{bc}
3	78.13^{a}	83.27^{a}	10.55^{c}	6.10^{b}
6	77.17^{b}	81.52^{b}	12.19^{b}	6.31^{ab}
9	76.76^{c}	79.75^{c}	13.01^{a}	6.41^{a}
LSD (0.05)	0.29	1.52	0.65	0.29

Means with the same latter in the same column are not significant different (P>0.05).

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

Chemical freshness indices (TVN, TMA and TBA) of different treated Bolti fish (*Oreochromis niloticus*) samples (RB, TWB, ETWB, ITWB and EITWB) at the zero time of storage period and during subsequent cold storage at 3±1°C were done, data are shown in Table (9).

2.1. Total volatile nitrogen (TVN):

The mean values of T.V.N. are given in Table (9). It could be noticed that the initial value of TVN in the different five investigated treatments 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 occurred with different rates depending on the initial treatments. The RB samples showed the highest incremental rate compared to other treatments. Similar trend was also noticed all over the storage periods. A high rate of increase in TVN content for RB sample was shown at the end of cold storage, so that RB samples were organoleptically rejected. On the other hand, the samples dipped in antimicrobials solution showed a different trend in TVN. Along life storage (18 days) were found for ETWB and EITWB at this time samples were rejected. The ETWB and EITWB had TVN values ranged between 28.00-29.4 mg TVN/100 g (on wet weight basis). However, the TVN values of about 30 mg TVN/100 g raw samples indicates spoilage of fish (Egyptian Standards, 2000). The progressive increase in TVN during cold storage might be attributed to the breakdown of

nitrogenous substances as a result of microbial activity and any autolytic enzymes found naturally in fish tissues as reported by Ibrahim and El-Zonfuly (1980), Yasin (1997) and Abou-Talb and Ibrahim (2002).

In conclusion, the higher rate of increasing in TVN during cold storage was found in RB samples which spoiled in the shortest time (9 days) compared to the other four treatments. The TVN could be considered as a good indicator describing fish quality during cold storage and correlated well with both microbial count (Tables, 17 and 18) and sensory evaluation (Table, 19) of the investigated treatments.

The proposed dipping solution (SL+TSP+PS+BHA) was found to be very effective in retarding protein breakdown by delaying and repressing microbial activity especially during the first 3 days of cold storage. Robach (1978) and Gelman *et al.* (1990) mentioned to the inhibitory effect of PS on the growth of most fish spoilers such as *Pseudomonas spp.* However, Dalgaard *et al.* (1993) reported that the increases in TVN during cold storage were coincided with small production of ammonia.

2.2. Trimethylamine (TMA):

Data tabulated in Table (9) revealed that the mean values of TMA of different investigated treatments at zero time of storage and during subsequent cold storage at 3±1°C. It could be noticed that the TMA values in the different five investigated treatments were ranged between 0.84 to 1.21 mg/100 g (on wet weight basis). During subsequent cold storage, significant differences ($P<0.05$) between these treatments were anticipated due to the antimicrobial effect of dipping solution. Even at the end of storage periods, where the

samples were judged organoleptically to be spoiled, i.e. at 9 days for RB treatment, 13 days for both TWB and ITWB and 18 days for both ETWB and EITWB treatments, the TMA values were 9.5, 9.29 and 9.57, 8.80 and 8.69 mg TMA/100 g (on wet weight basis), respectively. The TMA value of about 10 mg TMA/100 g (on wet weight basis) indicates spoilage of fish (Egyptian Standards, 2000). This indicates that the spoilage criterion of RB sample is mainly due to the off-odor produced upon the rise of TMA level. A long life for other treatment (13 days for TWB and ITWB) and (18 days for ETWB and EITWB). This may be due to the effect of dipping treatment which inhibited aerobic bacterial growth. Dipping in PS inhibited aerobic bacterial growth as well as sorbate being particularly effective in repressing aerobic microorganisms that are responsible for TMA formation (TMA producer-altermonas putrificans) which produce fish spoilage odor, on the other hand, sorbate was inhibitor for TMA-O reductase. In addition, the function of PS as an initial bactericidal followed by its bacteriostatic effect at spoilage state were considered. More addition the role of SL on reducing pH and BHA as antimicrobial agent. The explanation of such criteria was given by (Bremner & statham, 1983; Shaw *et al.*, 1983 and Yasin, 1997). These results are in harmony with (Ampola and Keller, 1985; Huss, 1988; Gennari and Campanini, 1991; Abou-Talb, 1993 and Yasin, 1997).

2.3. Thiobarbituric acid (TBA):

Thiobarbituric acid (TBA) determine the concentration of some oxidative products, i.e. malonaldehyde which is always found in fats exposed to oxidation deterioration.

Table (9): Freshness tests of untreated and treated Bolti fish during cold storage at 3±1°C (mean ± standard error).

Storage period (days)	TVN •					TMA •					TBA ►				
	RB	TWB	ETWB	ITWB	EITWB	RB	TWB	ETWB	ITWB	EITWB	RB	TWB	ETWB	ITWB	EITWB
Zero	13.53 ±0.47	13.07 ±0.93	13.07 ±0.47	14.00 ±0.00	13.53 ±0.47	0.98 ±0.08	1.07 ±0.12	1.03 ±0.05	1.21 ±0.09	0.84 ±0.08	0.50 ±0.05	0.57 ±0.07	0.53 ±0.09	0.58 ±0.06	0.78 ±0.06
3	16.33 ±0.47	15.27 ±0.77	14.47 ±0.47	15.40 ±0.00	14.93 ±0.47	3.13 ±0.55	2.52 ±0.28	1.96 ±0.43	2.33 ±0.47	1.73 ±0.33	1.76 ±0.15	1.50 ±0.02	1.01 ±0.03	1.07 ±0.06	1.02 ±0.03
6	23.80 ±0.81	18.20 ±0.81	16.33 ±0.47	16.80 ±0.81	17.27 ±0.47	7.84 ±0.43	4.94 ±0.41	3.83 ±0.37	3.55 ±0.41	3.47 ±0.34	1.97 ±0.25	1.52 ±0.20	1.13 ±0.12	1.39 ±0.05	1.17 ±0.11
9	29.40® ±0.81	20.13 ±0.53	17.73 ±0.47	20.53 ±0.47	19.13 ±0.47	9.50® ±0.40	6.68 ±0.55	5.09 ±0.45	6.35 ±0.45	5.27 ±0.47	3.29® ±0.17	1.59 ±0.10	1.66 ±0.11	1.66 ±0.12	1.61 ±0.19
11		233.33 ±0.68	20.53 ±0.81	22.40 ±0.36	21.47 ±0.36		7.65 ±0.48	5.93 ±0.28	7.51 ±0.26	6.30 ±0.41		2.60 ±0.12	1.91 ±0.18	2.03 ±0.09	1.88 ±0.08
13		28.93® ±0.56	21.93 ±0.66	28.00 ±0.58	23.33 ±0.48		9.29® ±0.38	6.67 ±0.43	9.57 ±0.16	6.94 ±0.56		4.03® ±0.08	2.10 ±0.15	4.16 ±0.12	2.12 ±0.20
15			22.87 ±0.66		24.73 ±0.56			7.84 ±0.16		7.79 ±0.38			2.39 ±0.10		2.50 ±0.09
17			25.20 ±0.38		26.60 ±0.53			7.93 ±0.58		8.10 ±0.18			3.26 ±0.07		2.74 ±0.16
18			28.47® ±0.81		28.93 ±0.78			8.80® ±0.40		8.69 ±0.55			4.07® ±0.09		3.99 ±0.14
LSD @ (0.05)	1.66					1.07					0.34				

• : Calculated as mg TVN/ 100 g fresh sample.

► : Calculated as mg malonaldyhde/ kg sample.

®: At these point samples were organoleptically rejected.

@: To be used to compare between any two times within the same treatment.

At the beginning of cold storage as seen in Table (9), the TBA values (expressed as mg malonaldhyde/kg) were ranged from 0.50 to 0.78 mg/kg. No significant difference ($P<0.05$) among all treatments was shown at zero time of cold storage. The highest TBA value that colorimetrically was measured for EITWB treatment at zero time compared to other treatments may be due to the interference of soaking compounds in the development of red color reaction between malonaldhyde and 2-TBA (Deng *et al.*, 1978).

During 9 days of storage at $3\pm1°C$, there is a significant difference ($P<0.05$) incremental pattern with different rates in the TBA value for RB, TWB and ETWB treatments. However, ETWB and EITWB treatments showed the lowest incremental pattern during cold storage then TWB and ITWB when compared to RB treatment. This may be due to treated this samples with (SL+TSP+PS+BHA) plays a significant role in preventing lipid oxidation during cold storage as discussed earlier (Sweet, 1973 and Yasin, 1997).

Analysis of variance for freshness tests of untreated and treated Bolti fish (Table, 10) indicated that there are significant differences ($P<0.05$) in TVN, TMA and TBA between either different treatments or different storage periods. F value of interaction was significant in TVN, TMA and TBA of treated Bolti fish samples.

Multiple comparisons for different treatments (Table, 11) indicated that there are significant differences ($P<0.05$) between RB and other treatments in TVN, TMA and TBA means that RB sample obtained the highest value. From data in the same table, it could be noticed that there is no significant differences ($P>0.05$)

Table (10): Analysis of variance for freshness tests of untreated and treated Bolti fish stored at 3±1°C for 9 days.

Source of variance	D.F.	F value and level of significancy		
		TVN	TMA	TBA
Time	3	182.55*	233.70*	112.96*
Treatment	4	51.73*	36.37*	29.78*
Time x treatment	12	13.54*	8.18*	8.84*
Error	40	--	--	--
Total	59	--	--	--

D.F.: Degree of freedom. * Significant at 0.05

Table (11): Effect of different treatments on freshness tests of (untreated and treated)* Bolti fish stored at 3±1°C for 9 days.

Treatments @	Freshness tests		
	TVN	TMA	TBA
RB	20.77^{a}	5.36^{a}	1.88^{a}
TWB	16.67^{b}	3.80^{b}	1.29^{b}
ETWB	15.40^{c}	2.97^{bc}	1.08^{c}
ITWB	16.68^{b}	3.36^{bc}	1.17^{bc}
EITWB	16.22^{bc}	2.83^{bc}	1.14^{bc}
LSD (0.05)	0.83	0.53	0.17

@ : As tail of Table (5)

Means with the same latter in the same column are not significant different (P>0.05).

Table (12): Effect of different storage periods on freshness tests of Bolti fish stored at 3±1°C for 9 days.

Storage period (days)	Freshness tests		
	TVN	TMA	TBA
0	13.44^{d}	1.03^{d}	0.59^{d}
3	15.28^{c}	2.33^{c}	1.27^{c}
6	18.48^{b}	4.33^{b}	1.44^{b}
9	21.38^{a}	6.58^{a}	1.96^{a}
LSD (0.05)	0.74	0.48	0.15

Means with the same latter in the same column are not significant different (P>0.05).

between TWB, ITWB and EITWB samples. also, no significant differences ($p>0.05$) between ETWB, and EITWB samples in TVN. No significant differences ($P>0.05$) between ETWB, ITWB, EITWB and TWB in TMA value whereas significant differences ($P<0.05$) were shown between ETWB and TWB treatments in TMA. Moreover, no significant differences ($p>0.05$) between either (ETWB, ITWB and EITWB) or (TWB, ITWB and EITWB) whereas a significant differences ($p<0.05$) were shown between ETWB and ITWB in TBA values. Results indicated that ETWB had a lowest score for TVN, TMA and TBA. So, this samples had a best quality.

Multiple comparisons for different storage periods (Table, 12) indicated that significant differences ($P<0.05$) were found in TVN, TMA and TBA as time extended.

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

The physical properties of different treated Bolti fish samples, i.e. pH value, O.D. for gills extract, muscles extract and pigments concentration as well as refractive index of eye fluids (RI), water holding capacity (WHC), plasticity and cooking loss were studied in this investigation as seen in Table (13).

3.1. pH value:

There are significant differences ($P<0.05$) in the pH values between the RB treatment and other treatments at the beginning of storage period (Table, 13), this is mainly due to the effect of dipping solution especially (SL). 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 period for each treatment. The incremental trend of the ETWB and EITWB treated were lower than that of TWB and ITWB when compared to RB at the end of cold storage for each. This may be due to the effect of dipping treatment on retarding the microbial load which may cause protein hydrolysis with the appearance of alkaline groups as indicated by Gelman *et al.* (1990), Stammen *et al.* (1990) and Yasin (1997).

Significant differences ($P<0.05$) were found in pH values at different storage time or between different treatments (Table, 14).

So, it could be concluded that the dipping treatment with (SL + TSP + PS + BHA) was more effective on reducing the rate of pH rise when compared to RB treatment. Such findings are correlated well with the other results of TVN, TMA and total microbial counts obtained in the present investigation and all these results were confirmed with each others.

3.2. Optical density of gills extract:

Data given in Table (13) showed that the effect of different treatments during subsequent cold storage at 3 ± 1°C on the O.D. of Bolti fish gills extract. At the beginning of cold storage the O.D. of gills extract was ranged from 0.77 to 0.90 (as O.D. at 542 nm). A slight decrease between eviscerated treatments and others was found. This may be due to the effect of evisceration process on destruction of some gill tissue cause remove some blood with washing water. A significant ($P<0.05$) decremental pattern was shown in O.D. of gills extract for all treatment with the progression of cold storage as seen in Table (13). This may be due to either the loss of gills ability to keep

their pigments inside its structure or pigments conversion and/or decomposition as a result of bacterial invasion. This could be confirmed on the basis that the higher bacterial count was accompanied with higher loss in the O.D. of gills extract (Khallaf, 1982 and Yasin, 1997).

Regarding to the five treatments, ETWB treatment it possessed the lower decremental trend at the end of cold storage. This may be a result of dipping treatment and its function or retarding the microbial load (Yasin, 1997).

3.3. Optical density of muscles extract:

The optical density of Bolti fish muscles extract as an indicator of fish quality was followed during cold storage at 3±1°C and data was recorded in Table (13). At the beginning of cold storage the O.D. of muscles extract was ranged from 0.19 to 0.23 (as O.D. at 420 nm). Also, there was no significant difference ($p>0.05$) between all treatments. A continuous increase was recorded till end of cold storage for all treatments. The incremental trend of the ETWB and EITWB treatments were lower than that of TWB and ITWB when compared to RB samples after 18 days. It is of interest to notice the possibility of using such parameter as an indicator of Bolti fish quality with the limiting value ranged from 0.19 to 0.89 (O.D. at 420 nm) as boarder line of rejection (the end of storage).

These data are in agreement with Yasin (1997), but are contradicted with Ghoneim (1974). This contradicted could be ascribed to the effect of crushed ice during thawing in leaching some pigments out of the muscles.

Table (13): Physical properties of untreated and treated Bolti fish during cold storage at 3±1°C (mean ± standard error).

Storage period (days)	pH					O.D of gills extract•				
	RB	TWB	ETWB	ITWB	EITWB	RB	TWB	ETWB	ITWB	EITWB
Zero	6.56 ±0.03	6.50 ±0.02	6.42 ±0.01	6.47 ±0.02	6.43 ±0.01	0.90 ±0.03	0.88 ±0.03	0.86 ±0.04	0.91 ±0.04	0.77 ±0.07
3	6.58 ±0.03	6.54 ±0.02	6.47 ±0.01	6.54 ±0.02	6.45 ±0.02	0.83 ±0.01	0.84 ±0.01	0.86 ±0.02	0.88 ±0.01	0.76 ±0.02
6	6.74 ±0.02	6.66 ±0.01	6.49 ±0.02	6.55 ±0.04	6.48 ±0.01	0.64 ±0.00	0.78 ±0.01	0.84 ±0.02	0.86 ±0.01	0.76 ±0.01
9	6.91® ±0.02	6.71 ±0.01	6.60 ±0.01	6.67 ±0.01	6.60 ±0.01	0.33® ±0.03	0.67 ±0.01	0.76 ±0.00	0.69 ±0.04	0.71 ±0.02
11		6.73 ±0.02	6.62 ±0.03	6.68 ±0.01	6.61 ±0.02		0.54 ±0.00	0.64 ±0.01	0.56 ±0.02	0.63 ±0.02
13		6.81® ±0.03	6.65 ±0.01	6.80 ±0.02	6.62 ±0.01		0.28® ±0.01	0.56 ±0.02	0.33 ±0.01	0.50 ±0.02
15			6.68 ±0.01		6.65 ±0.03			0.42 ±0.00		0.45 ±0.03
17			6.73 ±0.02		6.69 ±0.01			0.35 ±0.01		0.33 ±0.01
18			6.77® ±0.01		6.75 ±0.02			0.27® ±0.01		0.24 ±0.02
LSD@(0.05)	0.05					0.076				

• : Calculated as O.D at 542 nm.

®: At these point samples were organoleptically rejected.

@: To be used to compare between any two times within the same treatment.

Table (13): Cont.

Storage period (days)	O.D of muscles extract◄					Pigments concentration►				
	RB	TWB	ETWB	ITWB	EITWB	RB	TWB	ETWB	ITWB	EITWB
Zero	0.22 ±0.03	0.21 ±0.02	0.23 ±0.03	0.23 ±0.02	0.19 ±0.01	0.84 ±0.04	0.77 ±0.03	0.75 ±0.03	0.84 ±0.06	0.81 ±0.06
3	0.28 ±0.02	0.25 ±0.01	0.26 ±0.01	0.30 ±0.02	0.24 ±0.02	0.81 ±0.01	0.72 ±0.01	0.72 ±0.02	0.78 ±0.02	0.78 ±0.02
6	0.51 ±0.01	0.42 ±0.03	0.33 ±0.02	0.37 ±0.04	0.30 ±0.02	0.55 ±0.05	0.69 ±0.02	0.71 ±0.05	0.77 ±0.03	0.77 ±0.02
9	0.82® ±0.02	0.55 ±0.03	0.51 ±0.02	0.52 ±0.04	0.44 ±0.02	0.31® ±0.02	0.52 ±0.03	0.68 ±0.03	0.64 ±0.02	0.62 ±0.02
11		0.63 ±0.01	0.56 ±0.02	0.65 ±0.02	0.50 ±0.01		0.45 ±0.02	0.61 ±0.01	0.52 ±0.01	0.60 ±0.01
13		0. 83® ±0.02	0.62 ±0.02	0.80 ±0.03	0.60 ±0.01		0.26® ±0.02	0.51 ±0.03	0.29 ±0.01	0.47 ±0.02
15			0.72 ±0.03		0.67 ±0.02			0.41 ±0.01		0.42 ±0.2
17			0.78 ±0.01		0.76 ±0.03			0.32 ±0.02		0.36 ±0.03
18			0.89® ±0.02		0.81 ±0.01			0.24® ±0.02		0.26 ±0.02
LSD@(0.05)	0.068					0.09				

◄ : Calculated as O.D at 420 nm.

► : Calculated as O.D at 540 nm .

®: At these point samples were organoleptically rejected.

@: To be used to compare between any two times within the same treatment.

Table (13): Cont.

Storage period (days)	Refractive index of eye fluid					WHC *				
	RB	TWB	ETWB	ITWB	EITWB	RB	TWB	ETWB	ITWB	EITWB
Zero	1.3364 ±0.0000	1.3367 ±0.0003	1.3381 ±0.0003	1.3377 ±0.0003	1.3381 ±0.0003	7.53 ±0.037	6.87 ±0.43	8.97 ±0.52	7.23 ±0.18	7.43 ±0.29
3	1.3387 ±0.0003	1.3371 ±0.0003	1.3381 ±0.0003	1.3378 ±0.0003	1.3384 ±0.0000	8.07 ±0.45	7.13 ±0.54	7.60 ±0.76	6.80 ±0.35	7.43 ±0.58
6	1.3673 ±0.0149	1.3534 ±0.0068	1.3434 ±0.0035	1.3675 ±0.0082	1.3397 ±0.0028	11.00 ±0.78	8.37 ±0.12	7.47 ±0.12	6.50 ±0.21	6.47 ±0.33
9	1.4248® ±0.0057	1.3724 ±0.0035	1.3558 ±0.0037	1.3654 ±0.0035	1.3488 ±0.061	12.73® ±0.12	7.50 ±0.15	6.73 ±0.09	6.50 ±0.06	6.40 ±0.15
11		1.3828 ±0.0004	1.3608 ±0.0048	1.3797 ±0.0028	1.362 ±0.0057		7.30 ±0.28	6.57 ±0.36	6.30 ±0.26	6.37 ±0.16
13		1.3992® ±0.0012	1.3705 ±0.0028	1.3924 ±0.0008	1.3716 ±0.016		7.20® ±0.16	5.87 ±0.40	6.20 ±0.56	5.93 ±0.33
15			1.3828 ±0.0040		1.3819 ±0.0046			5.47 ±0.47		5.63 ±0.54
17			1.3988 ±0.0012		1.3936 ±0.0028			5.43 ±0.66		5.53 ±0.18
18			1.4176® ±0.0036		1.4162 ±0.0086			5.40® ±0.36		5.30 ±0.28
LSD@(0.05)	0.014					1.13				

*: Calculated as cm^2/0.3 g sample.
®: At these point samples were organoleptically rejected.
@: To be used to compare between any two times within the same treatment.

Table (13): Cont.

Storage period (days)	Plasticity *					Cooking loss (%)				
	RB	TWB	ETWB	ITWB	EITWB	RB	TWB	ETWB	ITWB	EITWB
Zero	3.57 ±0.13	3.07 ±0.48	3.37 ±0.18	3.27 ±0.15	3.33 ±0.12	9.50 ±1.03	10.69 ±1.03	11.24 ±0.74	9.74 ±0.85	11.00 ±0.75
3	3.47 ±0.24	3.30 ±0.15	3.27 ±0.15	3.80 ±0.15	3.33 ±0.08	14.18 ±0.56	11.22 ±0.59	11.83 ±0.56	12.65 ±0.60	12.84 ±0.23
6	3.13 ±0.03	3.23 ±0.03	3.23 ±0.03	3.27 ±0.03	3.0 ±0.06	19.08 ±0.91	12.13 ±0.40	11.77 ±0.14	12.39 ±0.26	12.15 ±0.19
9	2.87® ±0.09	3.7 ±0.03	3.27 ±0.09	3.3 ±0.09	3.47 ±0.07	23.59® ±0.31	12.10 ±0.17	11.55 ±0.22	11.86 ±0.25	11.12 ±0.42
11		3.10 ±0.12	3.23 ±0.06	3.13 ±0.10	3.37 ±0.15		11.29 ±0.68	10.28 ±0.48	11.15 ±0.76	10.10 ±0.57
13		3.70® ±0.08	3.17 ±0.10	3.13 ±0.03	3.23 ±0.12		10.03® ±0.88	9.83 ±0.76	9.44 ±0.36	9.30 ±0.62
15			3.07 ±0.03		3.20 ±0.26			8.86 ±0.64		8.96 ±0.58
17			2.97 ±0.16		3.13 ±0.10			8.58 ±0.59		8.71 ±0.28
18			2.93® ±0.09		3.03 ±0.08			7.88® ±0.36		8.22 ±0.31
LSD@(0.05)	0.61					1.68				

*: Calculated as cm^2/0.3 g sample.
®: At these point samples were organoleptically rejected.
@: To be used to compare between any two times within the same treatment.

3.4. Pigments concentration:

Data in Table (13) indicated that the pigments concentration of different treated Bolti fish samples. The pigments concentration was 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. During cold storage color deterioration of different treated Bolti fish was occurred. This may be due to many reasone, i.e. the oxidation of myoglobin and oxymyoglobin to metamyoglobin, a scape of some pigments (as water soluble protein) with drip (Shams El-Din, 1978) and the decomposition of pigments as a result of bacterial action (Khallaf, 1982). The decremental trend of the ETWB and EITWB treatments were lower than that of TWB and ITWB when compared to RB samples (samples were organoleptically rejected). Dipping treatment had an important effect on pigments concentration which may be due to the effect of dipping solution in improving the WHC of muscles, retarding oxidation process as well as due to its antibacterial action (Bremner and Statham, 1983).

3.5. Refractive index of fish eye fluid:

The refractive index of eye fluid (RI) of fish samples under investigation was followed during cold storage at $3\pm1°C$ in Table (13). No significant differences ($p>0.05$) were noticed in the RI values between all treatments of Bolti fish at a beginning of storage period.

The RI of eye fluids was ranged between 1.3364-1.3381 before storage then continuously increased till the end of storage periods to 1.4248, 1.3992, 1.4176, 1.3924 and 1.4162 for RB,

TWB, ETWB, ITWB and EITWB tratments, respectively. It is of interest to notice that storage time affects the refractive index and the highest value was appeared at the end of storage periods of different treated samples, when it was organoleptically rejected. However, the highest value was shown with RB samples after 9 days of cold storage. There are significant differences ($P<0.05$) was shown in all treatments during subsequent cold storage.

The aforementioned results indicate that RI of fish eye fluids was found to be affected mainly by autolysis and therefore could be used to identify their freshness (Shalaby, 1990). Similar trends were reported by (Ghoneim, 1974), (Khallaf, 1982), Verma *et al.* (1983), (Khallaf, 1986) and (Yasin, 1997).

3.6. Water holding capacity:

Data in Table (13) shows the WHC mean values of different untreated and treated Bolti fish stored at $3\pm1°C$. The WHC value was ranged from 6.87 to 8.97 $cm^2/0.3$ g. During subsequent cold storage of the five investigated treatments, the WHC was progressively increased in RB sample when compared to other treatments. This increase possibly due to protein denaturation and/or aggregatiom (Ali, 1986). From this table it could be seen that dipping treatment (SL + PS + TSP + BHA) led to marked and significant improvement in quality of Bolti fish tissues that WHC values were decreased during cold storage period. This is due to the role of phosphate salt and (SL) included in dipping solution on binding of cations with protein that could be considered as good explanation of fish tissue quality improvement (Hassan, 1980; Khallaf, 1982; Krivchenia and Fennema, 1988 and Yasin, 1997). In addition to, the role of

PS and BHA in retarding lipid oxidation products that formed and react with protein rendering it insoluble and consequently decrease of WHC value (Abd El-Razik, 1997).

Regarding to the statistical analysis of data tabulated in the same table, there are significant differences ($P<0.05$) in WHC between RB samples and other treatments during cold storage at 3 ± 1°C for 9 days. WHC of both (ETWB and EITWB) treatments was much more better than that of (TWB and ITWB) when compared to (RB) treatment at the end of cold storage 18, 13, 9 days, respectively. A strong relationship was shown between this results and cooking loss results in the same table (Maccallum *et al.*, 1964).

3.7. Plasticity:

The results given in Table (13) show the plasticity of different treated Bolti fish samples during cold storage at 3 ± 1°C. As far as plasticity measurement is concerned, the wide area indicate softness, while the smaller one indicates toughness.

The plasticity value was ranged from 3.07 to 3.57 cm^2/0.3 g at the beginning of cold storage. A continuous decrease was recorded till the end of cold storage for all treatments. These results were in accordance with other parameters used in the present work, i.e. proportional relation between the plasticity and WHC was noticed. After 9 days of cold storage ETWB and EITWB samples showed higher plasticity than TWB and ITWB samples when compared to RB samples, which may be due to the effect of dipping solution which cause increasing of protein solubility, retarding the formation of lipid oxidation products able to denature proteins and consequently reduce the tenderness (Hassan, 1980, Gelman *et al.*, 1990 and Yasin, 1997).

3.8. Cooking loss:

The cooking loss (%) was considered in the present investigation not only because of its relation with the WHC of fish muscles but also because of its relation with fish eating quality. The obtained results (Table, 13) showed that cooking loss was ranged from 9.50 to 11.24% in the investigated treatments at the beginning of cold storage. Cooking loss of RB samples was sharply increased as cold storage period was extended. Such results were positively correlated with that of the WHC in the same table.

After 9 days of cold storage the cooking loss of RB, TWB, ETWB, ITWB and EITWB were 23.59, 12.10, 11.55, 11.86 and 11.12%, respectively. The cooking loss was lower of both ETWB and EITWB than that of TWB and ITWB when compared to RB samples. A decremental trend was shown after 9 days till to the end of cold storage of each treatment. Low values were recorded for ETWB and EITWB at end of cold storage (18 days). This may be due to the effectiveness of dipping treatment in improving the WHC and protein characteristics.

The release of tissues liquid during steam-cooking is dependent on the species and degree of freshness. Moreover, the moisture/protein ratio was a major factor that affects the cooking loss and cooking yield. When moisture/protein ratio increased; the cooking yield decreased and this trend causes a corresponding increase in cooking loss (Lyon *et al.*, 1978). Moisture/protein percentage for ETWB and EITWB were 98.94 and 99.17%, respectively then the cooking loss were 7.88 and 8.22%,respectively at 18 days. These results was corresponding

with data in Table (5) for moisture and protein and are in agreement with Yasin (1997).

Analysis of variance for physical properties of untreated and treated Bolti fish samples (Table, 14) indicated that there are significant differences ($P<0.05$) between different times (0, 3, 6 and 9 days) and different treatments in all physical properties except WHC which did not changes significantly ($p>0.05$) during the mentioned different times.

Multiple comparisons for different treatments (Table, 15) indicated that there are no significant differences ($P>0.05$) between either ETWB and EITWB or TWB and ITWB samples in pigment concentration and RI values. while, significant differences ($p<0.05$) between RB and other treatments was shown in pH, O.D of muscles, pigments concentration, WHC and cooking loss. While, no significant differences($p>0.05$) in plasticity was shown for all treatments. Also, no significant differences ($p>0.05$) between either (ETWB, TWB and EITWB) or (RB and ITWB) in O.D. of gills. A significant differences ($p<0.05$) in pigments concentration.

Multiple comparisons for different storage periods (Table, 16) indicated that significant differences ($P<0.05$) was shown for pH, O.D of gills, O.D of muscles and pigments concentration at zero, 3, 6 and 9 days. No significant differences ($p<0.05$) was shown in RI values between zero and 3 days whereas, significant differences ($P<0.05$) between 3, 6 and 9 days. From data in this table, there are no significant differences ($p>0.05$) in plasticity. Moreover, significant differences ($p<0.05$) in WHC and cooking loss between either zero or 9 days.

Table (14): Analysis of variance for physical properties of untreated and treated Bolti fish stored at 3±1°C for 9 days.

Source of variance	D.F.	F value and level of significancy							
		pH	O.D gills	O.D muscles	Pigments concentration	RI	WHC	plasticity	Cooking loss
Time	3	128.29*	72.24*	214.71*	56.31*	62.31*	2.49^{NS}	69.45*	36.72*
Treatment	4	78.29*	24.20*	26.62*	10.32*	17.17*	39.17*	29.65*	56.83*
Time x treatment	12	4.74*	11.99*	8.38*	7.03*	9.73*	11.97*	22.44*	19.96*
Error	40	--	--	--	--	--	--	--	--
Total	59	--	--	--	--	--	--	--	--

D.F.: Degree of freedom. * Significant at 0.05
N.S.: Non significant.

Table (15): Effect of different treatments on physical properties of untreated and treated Bolti fish stored at 3±1°Cfor 9 days.

Treatments @	Physical properties							
	pH	O.D of gills	O.D of muscles	pigments concentration	RI	WHC	Plasticity	Cooking loss
RB	6.69^{a}	0.68^{b}	0.45^{a}	0.63^{c}	1.3668^{a}	9.83^{a}	3.26^{a}	16.59^{a}
TWB	6.60^{b}	0.79^{a}	0.36^{b}	0.68^{b}	1.3499^{b}	7.47^{bc}	3.32^{a}	11.54^{b}
ETWB	6.50^{d}	0.83^{a}	0.33^{b}	0.75^{a}	1.3438^{c}	7.69^{b}	3.29^{a}	11.60^{b}
ITWB	6.56^{c}	0.63^{b}	0.36^{b}	0.76^{a}	1.3521^{b}	7.76^{b}	3.41^{a}	11.66^{b}
EITWB	6.50^{d}	0.75^{a}	0.29^{c}	0.75^{a}	1.3413^{c}	6.93^{c}	3.28^{a}	11.78^{b}
LSD (0.05)	0.03	0.038	0.034	0.047	0.007	0.56	0.31	0.84

@ : As tail of Table (5)
Means with the same latter in the same column are not significant different (P>0.05).

Table (16): Effect of different storage periods on physical properties of Bolti fish stored at 3±1°C for 9 days.

Storage period (days)	physical properties							
	pH	O.D of gills	O.D of muscles	pigments concentration	RI	WHC	Plasticity	Cooking loss
0	6.48^{d}	0.86^{a}	0.22^{d}	0.80^{a}	1.3374^{c}	7.61^{bc}	3.32^{a}	10.48^{c}
3	6.52^{c}	0.83^{a}	0.27^{c}	0.76^{b}	1.3380^{c}	7.41^{c}	3.43^{a}	12.54^{b}
6	6.58^{b}	0.78^{b}	0.39^{b}	0.70^{c}	1.3543^{b}	7.96^{ab}	3.17^{a}	13.50^{a}
9	6.70^{a}	0.63^{c}	0.57^{a}	0.55^{d}	1.3734^{a}	8.17^{a}	3.32^{a}	14.04^{a}
LSD (0.05)	0.02	0.034	0.030	0.040	0.006	0.50	0.27	0.75

Means with the same latter in the same column are not significant different (P>0.05).

4. Microbiological quality attributes of untreated and treated chilled Bolti fish:

Bacterial aspects of untreated and treated Bolti fish samples during cold storage at 3±1°C were enumerated, data was recorded in Table (17) and Fig. (5).

Four-culture method as a new technique was used as well as a conventional plating method to detect and enumerate the total mesophilic microbial load, gram-negative bacteria, coliforms and *E. coli* as indicator to monitor the microbiological quality and safety of chilled fish. Relationship between the results of the conventional plating methods and four culture methods for enumeration of four bacterial groups was done (Picture, 2).

Total mesophilic bacterial count (TMBC) was shown in Table (17) and Fig. (5). The initial TMBC of different treated were 3.98, 3.25, 3.45, 3.78 and 3.62 log CFU g^{-1} enumerating by conventional plating method and 4.01, 3.11, 3.41, 3.71 and 3.71 CFU g^{-1} enumerating by four-culture method for RB, TWB, ETWB, ITWB and EITWB, respectively. A considerable decrease of TMBC that noticed in treated sample when compared to RB at zero time of cold storage. A incremental trend in TMBC during subsequent cold storage was shown. After 9 days the initial TMBC was reached to 6.16, 4.60, 4.12, 4.87 and 4.12 log CFU g^{-1} (conventional plating methods) and 6.42, 4.61, 4.01, 4.91 and 4.31 log CFU g^{-1} (four-culture methods) for RB, TWB, ETWB, ITWB and EITWB, respectively. At this time, the RB samples had a highest TMBC also, it was organoleptically rejected. Similar incremental trend of all

treatments to the end of storage periods was shown. ETWB and EITWB had a lowest increasing in the total mesophilic microbial load.

Gram negative bacterial (GNB) of different treated Bolti fish was shown in the same table. The initial GNB were 3.54, 2.06, 2.56 , 2.59 and 2.68 log CFU g^{-1} (conventional plating methods) and 3.71, 2.20, 2.51, 2.51 and 2.51 log CFU g^{-1} (four culture methods) for RB, TWB, ETWB, ITWB and EITWB, respectively. A incremental rate in all treatments was shown. However, RB samples had more highly number of GNB than other treatments. A similar incremental trend with extending time was observed. ETWB and EITWB treatments had a lower increasing in GNB from TWB and ITWB when compared to RB samples at end of cold storage periods on either side methods. This results are in agreement with Kim and Hearnsberger, 1994 and Kim *et al.,* (1995).

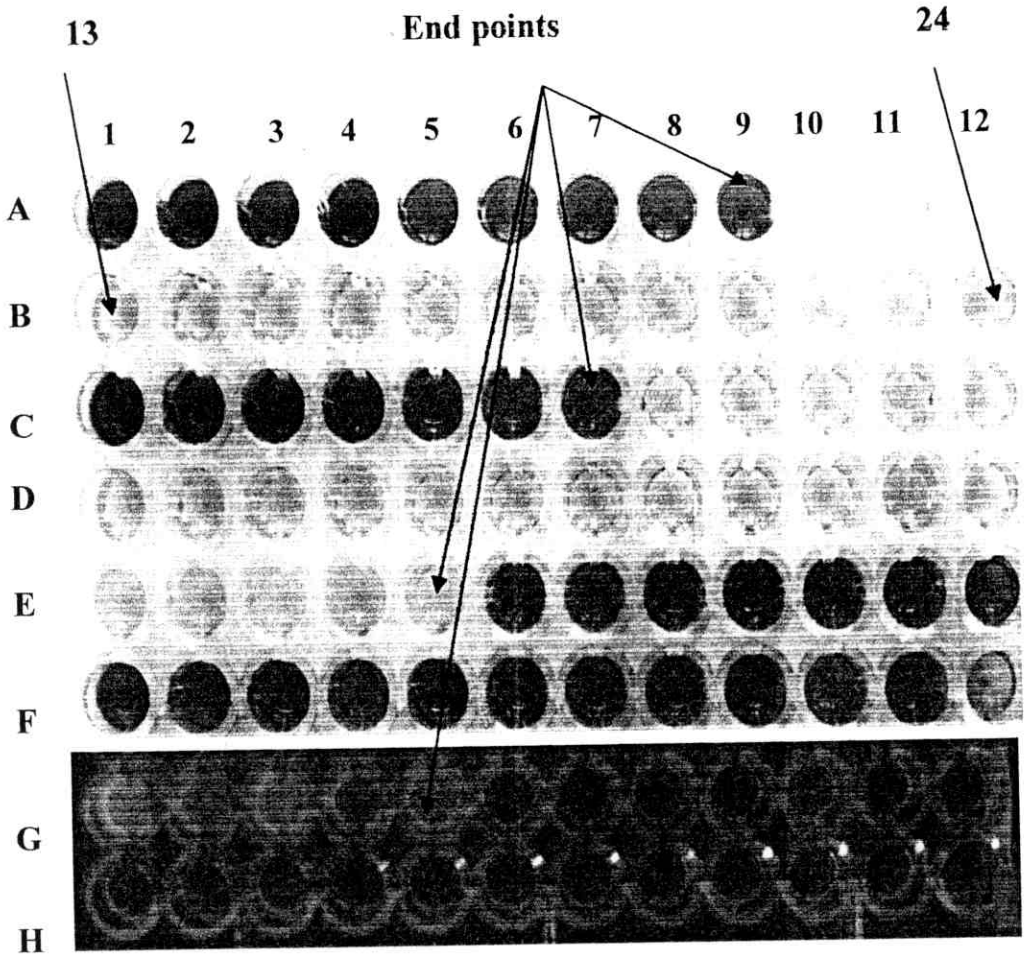
Data presented in Table (17) and Fig. (5) show the number of coliform group (CG) of various investigated samples during cold storage at 3±1°C. Rapidly increasing in RB samples, but slowly increasing in dipped treatment was shown after 9 days. At end of storage periods, the CG load of dipped treatment samples not reached to the CG load of RB samples. This changes was to accommodate the enumeration by both methods.

Change occurrence in *E. coli* profiles of different treated fish was presented in the same table. A high different between initial EC of RB samples and other dipped treatment due to the effect of dipping solution was more direct effect on *E. coli*. Regarding to the dipping treatment had lower growth of *E. coli* after 6 days of storage and even after 9 days when compared to

RB samples. A incremental trend was shown in all treatments at the end of storage periods. However, the ETWB and EITWB treatments showed the lowest incremental pattern during cold storage from TWB and ITWB treatment when compared to RB treatment.

In conclusion, from these data in Table (17), the interaction between cold storage and dipping treatment given low increase rate in microbial load (TMBC, GNB, CG and EC) for dipped samples. While, RB samples had a rapid increase rate in microbial load. The increasing in growth rate of dipped samples was observed after 9 days. The general incremental trend in microbial load during cold storage mainly due to the growth of psychrophilic bacteria (Abou-Taleb and Ibrahim, 2002). These results findings are correlated with the other results of physical and sensory properties obtained in the present investigation and in agreement with the results obtained by (Abou-Taleb, 1993 and Yasin, 1997).

The response of scatter plots exhibited a high degree of relationship 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* ($r^2 = 0.90$) (Fig. 5). These results are in agreement with Kang *et al.* (2003). The relationship between the four-culture values with the results of the conventional plating methods suggests that the four-culture methods can be used to enumerate the four critical bacterial group in fish over conventional plating methods.



Picture (2): Schematic diagram of typical four-culture method using a single microtiter for the enumeration of total mesophilic microbial loads (rows A and B), gram-negative bacteria (rows C and D), coliform counts (rows E and F) and *Escherichia coli* (rows G and H). The final positive reactions were determination and converted to using endpoint. The values indicated by the last well exhibiting a color or fluorescence were calculated using the formula $10x2^n$, where n = the number of the last well indicating growth. Example for total mesophilic microbial loads (rows A and B): endpoint (9) = $10x2^9 = 5.12x10^3$ CFU ml^{-1}, 3.71 $\log_{10}$ value.

Table(17): Logarithmic number of microbial aspects for untreated and treated bolti fish during cold storage at 3+1°C.

Storage period (days)	Micro-organisms	Treatments									
		RB		TWB		ETWB		ITWB		EITWB	
		A	B	A	B	A	B	A	B	A	B
Zero	TMB	3.98	4.01	3.25	3.11	3.45	3.41	3.78	3.71	3.62	3.71
	GN	3.54	3.71	2.06	2.20	2.56	2.51	2.59	2.51	2.68	2.51
	CG	3.54	3.41	2.31	2.51	2.39	2.51	2.48	2.51	2.40	2.51
	E. coli	3.24	3.11	2.02	2.20	2.36	2.51	2.20	2.20	2.32	2.51
3	TMB	4.82	4.61	3.66	3.71	3.54	3.41	4.14	4.31	3.78	3.71
	GN	4.40	4.31	3.11	3.11	3.28	3.41	3.22	3.11	3.10	3.11
	CG	4.13	4.01	2.47	2.51	2.45	2.51	2.94	3.11	2.68	2.81
	E. coli	3.78	3.71	2.32	2.20	2.23	2.20	2.40	2.51	2.48	2.51
6	TMB	5.97	5.82	3.79	3.71	3.68	3.71	4.07	4.31	3.91	4.01
	GN	4.92	4.91	3.15	3.11	3.46	3.41	3.29	3.41	3.08	3.11
	CG	4.20	4.31	2.63	2.81	2.61	2.81	2.96	3.11	2.76	2.81
	E. coli	3.95	3.01	2.38	2.51	2.43	2.51	2.46	2.51	2.48	2.51
9	TMB	6.16®	6.42	4.60	4.61	4.12	4.01	4.87	4.91	4.12	4.31
	GN	5.00®	4.91	3.22	3.11	3.62	3.71	3.81	3.71	3.69	3.41
	CG	4.88®	4.91	3.78	3.71	3.60	3.41	3.79	3.71	3.47	3.41
	E. coli	4.18®	4.01	3.02	3.11	2.78	2.81	3.20	3.11	2.86	2.81
11	TMB			4.76	4.61	4.16	4.31	4.92	4.91	4.18	4.31
	GN			3.93	3.71	3.64	3.71	3.95	5.20	3.85	3.71
	CG			3.74	3.71	3.27	3.11	3.75	3.71	3.72	3.71
	E. coli			3.55	3.41	2.94	2.81	3.72	3.71	3.11	3.11
13	TMB			5.19®	5.20	4.26	4.31	4.99	5.20	4.31	4.31
	GN			4.00®	4.01	3.70	3.71	3.91	4.01	3.86	4.01
	CG			3.74®	3.71	3.40	3.41	3.82	3.71	3.71	3.71
	E. coli			3.62®	3.71	2.95	2.81	3.80	3.71	3.08	3.11
15	TMB					4.29	4.31			4.36	3.61
	GN					3.81	4.01			3.91	4.01
	CG					3.54	3.71			3.80	3.71
	E. coli					2.99	3.11			3.12	3.01
17	TMB					4.75	4.91			4.97	5.20
	GN					3.90	3.71			4.05	4.01
	CG					3.67	3.71			3.96	4.01
	E. coli					3.02	3.11			3.90	3.71
18	TMB					5.15®	5.20			5.26	5.51
	GN					3.98®	3.01			4.18	4.31
	CG					3.80®	3.71			3.99	4.02
	E. coli					3.07®	3.11			3.31	3.41

A : Enumerate by conventional plating methods.
B : Enumerate by four-culture methods.
® : At these point samples were organoleptically rejected.

TMB: Total mesophilic bacteria.
GN : Gram-negative bacteria.
CG : Coliform group.

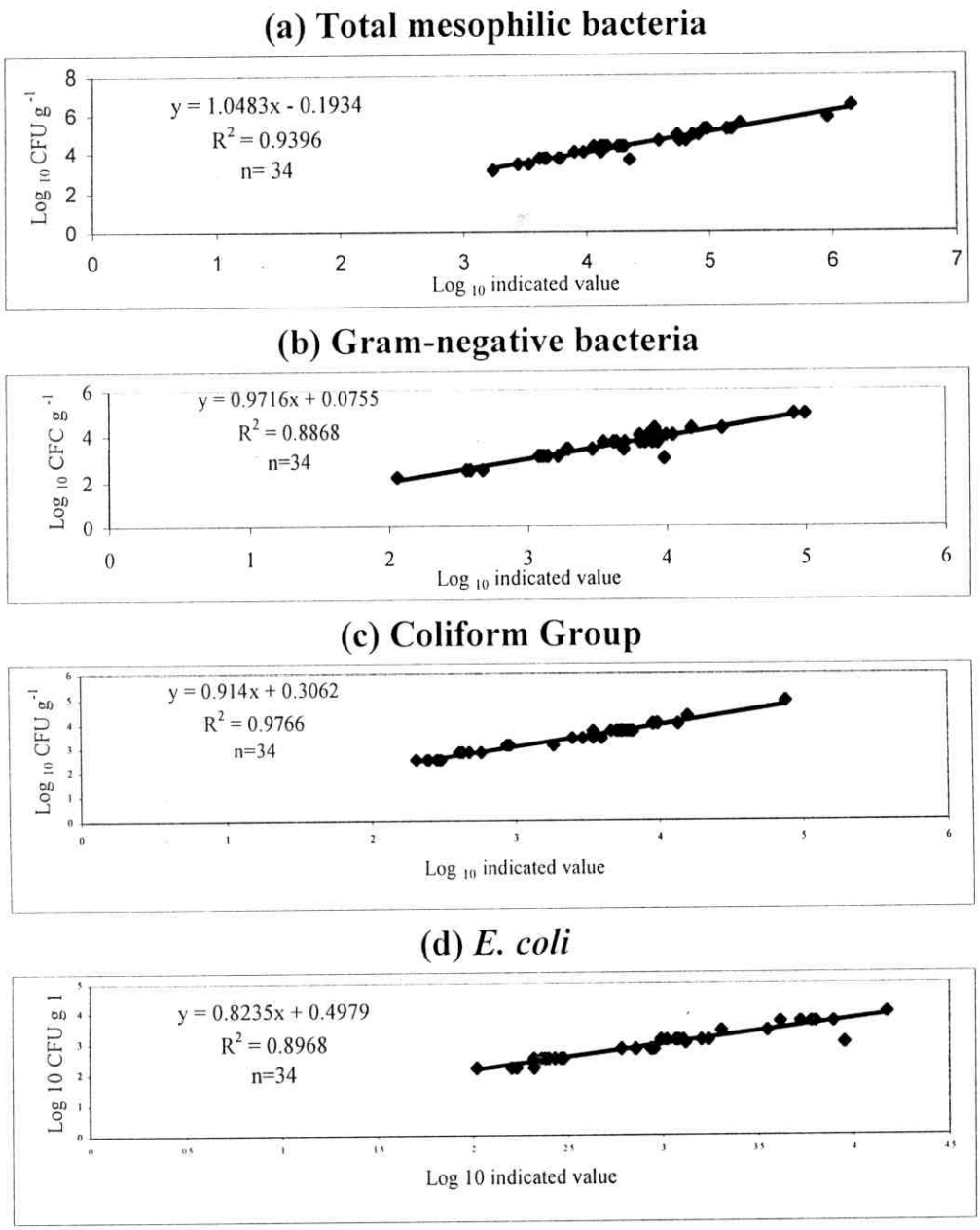


Fig. (5): The linear relationship between the results of four-culture methods (log_{10} of the reciprocal endpoint dilution) and those of the conventional plating methods (log_{10} CFU g-1) for the enumeration of (a) total mesophilic microbial load, (b) gram-negative bacteria, (c) coliform counts (d) and *E. coli* for chilled untreated and treated Bolti fish.

Clearly, the four-culture methods can be applied to the enumeration of four critical bacterial groups in fish. Moreover, this assay has minor advantage: (1) it requires about 0.04% of media compared to the media used in conventional plating methods; (2) the liquid media used in four-culture methods not require melting and tempering of media after autoclaving; (3) A small area is needed for operation and incubation; (4) it reduces the cost of labor and start up equipment; (5) one microtiter plate can be used, instead of several petri dishes, for the duplicate plating of several dilutions and (6) waste can be reduced by sterilized and disposed of with ease. A possible disadvantage of the four-culture method is difficulty in isolating colonies. However, most food plants need just to monitor the numbers of bacterial groups as indicator test; they do not need to isolate and identify the micro-organisms (Kang and Gray, 2000). In conclusion, this method will facilitate sanitation monitoring at fish-processing plants by simplifying procedures.

Data presented in Table (18) shows psychrophilic bacterial count (PsB) of different treated Bolti fish during cold storage at 3±1°C. Regarding to a high effect of dipping treatment on reducing (PsB) of dipped samples when compared to RB samples was shown at zero time. An increamental trend was observed in all treatments, but dipped samples had lowest rate when compared to RB samples. After 9 days, RB was more high than dipped treatments. Psychrophilic counts were progressively increased with the prolonged cold storage at 3±1°C. This is due to the proteolytic enzymes that hydrolyze fish proteins, polypeptides and amino acids, which were a good nutrition

intermediate compounds for multiplying psychrophilic bacterial (El-Akeel, 1988).

Proteolytic bacteria (PB)was considered in the present investigation, data was recorded in the same table. At the beginning of cold storage, the initial PB 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. With the progression of cold storage, general incremental trend in PB during cold storage coincided with increasing in psychrophilc counts then to run into 4.62, 3.51, 3.46, 3.78 and 3.36 log CFU g^{-1} for RB, TWB, ETWB, ITWB and EITWB, respectively after 9 days. An incremental trend in all treatment till the end of storage was observed. This is mainly due to the growth of psychophilic bacteria (Yasin, 2003).

Lipolytic bacteria (LB) counts of various investigated samples were shown in the same table. At zero time of storage, the initial LB counts were
2.18, 2.00, 2.40, 2.90 and 2.18 log CFU g^{-1} for RB, TWB, ETWB, ITWB and EITWB, respectively. During subsequent cold storage, an incremental trend in LB counts was recorded. However, this trend was slower than in the case of (PB) at the same conditions. This finding is in agreement with Farag *et al.* (1983). Also, incremental trend in LB counts during subsequent cold storage till end of storage periods.

Moulds and yeasts (M&Y) were also counted in this investigation, data was recorded in Table (18). The initial M&Y count was 2.35 log CFU g^{-1} for RB sample, but it was not detected in other dipped treatments at zero time of storage. During subsequent cold storage, a slowly incremental trend

Table (18): Logarithmic number of physcrophilic, proteolytic, Lipolytic, *E. coli* O157:H7 ATCC 69373 bacteria and molds & yeasts for untreated and treated Bolti fish during cold storage at 3+1°C.

Storage period (days)	Micro-organisms	Treatments				
		RB	TWB	ETWB	ITWB	EITWB
Zero	Ps.C	3.58	2.41	2.38	2.67	2.42
	P.B	3.41	2.85	2.40	2.88	2.48
	L.B	2.18	2.00	2.40	2.90	2.18
	E.C.	-	-	-	2.43	2.62
	M&Y	2.35	ND	ND	ND	ND
3	Ps.C	4.18	3.83	3.13	3.00	2.71
	PB	3.61	3.31	3.13	2.91	3.92
	LB	3.65	3.03	3.38	3.71	2.94
	E.C.	-	-	-	1.85	1.74
	M&Y	2.53	ND	ND	ND	ND
6	Ps.C	5.28	3.86	3.69	3.03	2.73
	PB	4.57	3.35	3.16	3.26	2.98
	LB	3.66	3.05	3.53	3.63	3.14
	E.C.	-	-	-	ND	ND
	M&Y	2.59	ND	ND	ND	ND
9	Ps.C	6.31®	4.70	4.56	4.18	3.31
	PB	4.62®	3.51	3.46	3.78	3.36
	LB	3.92®	3.11	3.43	3.67	3.18
	E.C.	-	-	-	ND	ND
	M&Y	2.72®	ND	ND	ND	ND
11	Ps.C		4.75	4.58	4.22	3.46
	PB		4.65	3.54	3.84	3.42
	LB		3.15	3.50	3.72	3.22
	E.C.		ND	ND	ND	ND
	M&Y		ND	ND	ND	ND
13	Ps.C		4.91®	4.70	4.30	4.57
	PB		4.75®	3.62	3.87	3.45
	LB		4.21®	3.53	3.78	3.23
	E.C.		-	ND	ND	ND
	M&Y		ND®	ND	ND	ND
15	Ps.C			4.72		4.84
	PB			3.72		3.80
	LB			3.54		3.43
	E.C.			ND		ND
	M&Y			ND		ND
17	Ps.C			4.99		5.00
	PB			3.78		3.92
	LB			3.51		4.60
	E.C.			ND		ND
	M&Y			ND		ND
18	Ps.C			5.01®		5.13
	PB			3.84®		3.97
	LB			3.67®		3.81
	E.C.			ND®		ND
	M&Y			ND®		ND

PsC : Psycrophilic cunt. PB : Proteolytic bacteria. LB : Lipolytic bacteria.
E.C: *E. coli* O157:H7 ATCC 69373. M&Y : Moulds and yests. ND : Not detected.
® : At these point samples were organoleptically rejected.

was shown in Y&M counts till reached to 2.72 log CFU/g for RB samples after 9 days. This is may be due to the effect of cold storage.

On the other hand, *E. coli* O157:H7 ATCC 69373 was counted in inoculated samples to study the effect of dipping solution on retarding inoculated strain. The initial *E. coli* O157:H7 counts were 2.43 and 2.62 CFU g^{-1} for ITWB and EITWB, respectively, immediately after dipping for 2 min. in solution contains (SL + TSP + PS + BHA). A decremental trend during storage at 3±1°C for 3 days was shown, then the strain was not detected after 6 days of cold storage and during subsequent cold storage till end of storage periods.

In conclusion, TMBC, GNB, CG and EC as well as PsB, PB, LB had lowest rate of growth in dipped treated samples and M&Y were not detected when compared to this growth in RB samples which was the highest in microbial load. For inoculated samples, *E. coli* O157:H7 ATCC 69373 strain disappeared after 6 days of cold storage. This mainly due to the antimicrobial effect of dipping solution (SL + TSP + PS + BHA) on retarding the microbial load and mainly more effectiveness against *E. coli* O157:H7 ATCC 69373 which was discussed previously in part (I).

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

5.1. Sensory evaluation of raw Bolti fish:

Means of numerical scores of sensory evaluation characters and the standard error as well as L.S.D. were

calculated and tabulated in Table (19). From these data, it could be followed the appearance, color of gills, texture, scale consistency, odor, slimness, eye lustrous and overall acceptability as affected by different treatments (RB, TWB and ETWB) during cold storage at 3±1°C till the end of storage periods.

The first item in Table (19) illustrated the appearance of Bolti fish during storage at 3±1°C. At the beginning of cold storage (zero time), no significant difference ($P>0.05$) was found between the samples (RB, TWB and ETWB). The appearance value was ranged from 9.67 to 9.78 for all treatments. During subsequent cold storage a decremental trend was shown in appearance score of all treatments till the end of storage periods. A significant difference ($p<0.05$) was shown in all treatments after 9 days, it could be noticed that ETWB was the best treatment from other samples. This may be due to the effect of scaling process and the dipping treatment to improve the appearance by inhibition the microbial load and discoloration of surfaces (O'connor *et al.*, 1993; Marshall and Kim, 1996 and Yasin, 1997).

Data illustrated in Table (19), reflect the color of Bolti fish gills throughout cold storage at 3±1°C. At the beginning of cold storage the color of gills values were around 9.17 and no significant difference ($P>0.05$) between all treatment was recorded. With the progression of cold storage all treatments were affected from a concomitant deterioration in gills color. Less rates of color deterioration was found in ETWB sample than TWB sample when compared to RB sample. These findings are confirmed with the data of the optical density of gills extract

(Table, 13). Such accordance between objective and subjective evaluations indicate the success of the panelists in finding out most changes occurred in Bolti fish during cold storage. This results are in agreement with Yasin (1997 and 2003).

The texture of fresh Bolti fish samples is firm when pressed by finger, a formed gap is clearly noticed, then quickly disappeared. The texture of various samples under investigation could be followed as shown in Table (19). No significant difference ($P>0.05$) between all treatments and the values of texture were in range 8.89-9.28 at the beginning of cold storage. During subsequent cold storage the values of texture were decreased as the time extended. The disappearance of the formed gap was slow (unaccepted) in RB samples (9 days) and more long life for TWB samples (13 days). Meanwhile, the ETWB samples had firm texture (rapid gap disappearance) owing to the role of dipping treatment in improving fish texture during storage. These findings are confirmed with the data of optical density of muscles extract (Table, 13) and the results are in agreement with those obtained by Yasin (1997).

Data in Table (19) show the mean score of scales consistency as indicator of freshness and quality and handling for RB and TWB samples during cold storage at 3 ± 1°C. At zero time of storage the values were 9.22 for RB and 9.00 for TWB. Also, no significant difference ($P>0.05$) between the two samples at zero time of cold storage. During subsequent cold storag a decremental trend was shown as time extended. Moreover, a significant difference ($P<0.05$) was shown between treatments after 9 days of cold storage. These results were accompanied with both appearance and sliminess which may be

due to the microbial growth. The improvement scales consistency of TWB may be due to the effect of dipping solution on inhibition the microbial growth.

As seen in Table (19), odor could be detected during cold storage at 3±1°C. At zero time, no significant difference ($P>0.05$) between different samples was found. During subsequent cold storage, the dipped samples ETWB had a higher scores than that of TWB when compared to RB samples and a significant difference ($P<0.05$) between dipped and undipped samples was recorded. These findings are confirmed with the data of TVN and TMA (Table, 9). The onset of spoilage was easily detected by undesirable odor (off-odor) that mainly due to the formation of ammonia, indol and other amines as metabolites induced by the action of spoilage bacteria on fish tissues during storage (Reddy *et al.*, 1995). After 9 days of storage the best treatments were ETWB and TWB due to the effect of dipping solution (SL + TSP + PS + BHA) in reducing bacterial load as discussed in (Table, 17 and 18). These results are in agreement with (Shaw *et al.*, 1983; Ampala and Keller, 1985; Thakur and Patel, 1994 and Yasin, 1997).

Table (19) shows the average scores of sliminess for different treated Bolti fish during cold storage at 3±1°C. No significant difference ($P>0.05$) between all treatments was recorded at the beginning of cold storage. The values of sliminess were in the range 8.17-8.68. A decrease rate and a significant difference ($P<0.05$) was recorded in all treatments. The RB samples had a higher decremental rate than TWB and ETWB samples. This is mainly due to the effect of dipping

solution on retarding the microbial load (Thakur and Patel, 1994 and Yasin, 1997).

Table (19) shows the average scores of the sensory evaluation of eye's lustrous of different treated Bolti fish, there is no significant difference ($P>0.05$) between the three investigation treatments at zero time of storage. The values of eye lustrous were ranged from 9.06 to 9.39 at the beginning of cold storage. As cold storage time prolonged, a decrement trend in the score of eye's lustrous was occurred in all of treated samples till the point of complete deterioration after 9 days (RB), 13 days (TWB) and 18 days (ETWB). A slight less decremental trend could be noticed in case of ETWB and TWB samples when compared to RB samples. This result is agrees with findings concerning the refractive index of eye fluid (Table, 13). This mainly due to the effect of dipping treatment in retarding many deterioration changes. These results are in accordance with the other freshness parameters given in this investigation and are in agreement with Yasin (1997).

Data given in Table (19) shows the mean score of overall acceptability of differently treated Bolti fish during cold storage at $3\pm1°C$. the values were ranged from 91.17 to 92.67 and no significant difference ($P>0.05$) was recorded at the beginning of cold storage. During cold storage, a decremental trend was shown as the storage time prolonged. Moreover, a significant difference ($P<0.05$) between samples because the differences were found in all characteristics. However, dipping fish in antimicrobial solution had the best quality when compared to that of (RB) samples.

Table (19): Sensory evaluation of untreated and treated raw Bolti fish during cold storage at 3±1°C (mean ± standard error).

Storage period (days)	RB	TWB	ETWB	RB	TWB	ETWB
	Appearance			Color		
Zero	9.72±0.25	9.78±0.28	9.67±0.32	9.17±0.20	9.28±0.17	9.06±0.28
3	8.56±0.42	8.56±0.42	8.72±0.32	7.56±0.39	8.72±0.29	8.61±0.35
6	6.61±0.40	7.89±0.18	8.17±0.25	5.89±0.39	8.39±0.33	7.95±0.28
9	.28±0.52®	6.89±0.50	8.00±0.32	3.50±0.49®	5.39±0.50	6.67±0.46
11		5.50±0.48	7.55±0.26		5.22±0.38	6.45±0.28
13		.50±0.36®	7.04±0.40		3.67±0.47®	6.00±0.39
15			6.72±0.52			4.56±0.16
17			6.06±0.24			4.17±0.39
18			4.83±0.20®			3.78±0.44®
$LSD^{@}_{(0.05)}$	1.00			1.00		
	Texture			Scales consistency		
Zero	9.22±0.24	9.28±0.19	8.89±0.21	9.22±0.23	9.00±0.24	-
3	8.28±0.25	8.22±0.25	8.89±0.15	7.39±0.55	7.83±0.50	-
6	5.67±0.30	8.13±0.28	8.61±0.17	5.72±0.37	7.05±0.37	-
9	2.83±0.61®	7.06±0.34	7.67±0.21	.00±0.44®	6.89±0.51	-
11		5.83±0.28	7.28±0.14		6.00±0.48	-
13		4.28±0.47®	7.11±0.28		4.39±0.23®	-
15			6.39±0.23			-
17			4.95±0.15			-
18			3.88±0.18®			-
$LSD^{@}_{(0.05)}$	0.81			1.16		

® : At these point samples were organoleptically rejected.

@: To be used to compare between any two times within the same treatment.

Table (19): Cont.

Storage period (days)	RB	TWB	ETWB	RB	TWB	ETWB
	Odor			Sliminess		
Zero	9.22±0.21	9.39±0.19	9.72±0.13	8.17±0.32	8.68±0.36	8.25±0.37
3	8.22±0.40	8.89±0.17	9.17±0.12	6.92±0.45	7.92±0.31	7.67±0.40
6	5.50±0.22	8.61±0.21	8.83±0.19	4.83±0.65	7.52±0.68	7.51±0.60
9	1.50±0.43®	5.95±0.21	8.22±0.19	2.92±1.08®	7.00±0.49	7.50±0.48
11		3.39±0.18	7.67±0.17		6.51±0.51	7.33±0.27
13		2.11±0.26®	7.00±0.15		4.93±0.47®	6.58±0.36
15			6.00±0.13			5.75±0.51
17			5.38±0.21			4.42±0.47
18			.45±0.17®			4.00±0.37®
$LSD^{@}_{(0.05)}$	0.67			1.54		
	Eye lustrous			Overall acceptability		
Zero	9.39±0.19	9.06±0.22	9.17±0.20	92.67±1.39	92.00±1.14	91.17±1.38
3	6.94±0.55	8.11±0.29	8.67±0.18	77.33±3.78	81.42±2.28	88.17±1.53
6	5.39±0.25	7.33±0.31	8.33±0.17	55.57±2.79	78.83±2.27	87.67±1.54
9	3.17±0.42®	6.28±0.46	7.17±0.40	11.25±4.53®	69.83±2.64	80.17±2.75
11		5.95±0.27	6.78±0.37		68.83±4.18	78.00±2.78
13		.39±0.49®	6.44±0.31		48.42±3.58®	64.42±3.14
15			5.5±0.23			59.00±1.78
17			5.50±0.55			55.67±4.23
18			4.11±0.47®			43.33±2.52®
$LSD^{@}_{(0.05)}$	0.91			7.50		

® : At these point samples were organoleptically rejected.

@: To be used to compare between any two times within the same treatment.

Analysis of variance for sensory evaluation of untreated and treated Bolti fish (Table, 20) proved that there are 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.

Multiple comparisons for different treatments (Table, 21) indicated the significant differences ($P<0.05$) in sensory attributes between ETWB and RB treatments. Also, a same trend was shown between TWB and RB treatments. While, the ETWB was better than TWB treatments because, it obtained a highest values indicating the best quality.

Multiple comparisons for different treatments of untreated and treated Bolti fish (Table, 22) indicated the significant differences ($P<0.05$) in sensory characteristics with increasing storage periods.

In conclusion, obtained data for chemical, freshness, physical, microbiological and organoleptic changes of different treated Bolti fish indicated that, it could safely estimate the expiration periods of the three Bolti fish samples 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) could extend the shelf-life of Bolti fish 5 days more, whereas, when eviscerated fish and dipped as proposed in the present thesis (SL + TSP + PS + BHA) the expiration period was extended 11 days more.

Table (20): Analysis of variance for sensory evaluation of untreated and treated raw Bolti fish stored at 3±1°C for 9 days.

Source of variance	D.F.	F value and level of significancy							
		Appearance	Color of gills	Texture	Scales consistency	Odor	Sliminess	Eye's lustrous	Overall acceptability
Time	3	151.40*	52.09*	71.56*	35.96*	163.60*	16.11*	65.41*	114.04*
Treatment	2	15.47*	18.16*	30.20*	16.32*	89.83*	5.26*	25.60*	75.34*
Time x treatment	6	9.62*	5.13*	18.58*	9.64*	43.37*	2.11^{NS}	8.40*	39.89*
Error	132	--	--	--	--	--		--	--
Total	143	--	--	--	--	--		--	--

D.F.: Degree of freedom. * Significant at 0.05
N.S.: Non significant.

Table (21): Effect of different treatments on sensory evaluation of untreated and treated raw Bolti fish stored at 3±1°C for 9 days.

Treatments @	Sensory evaluation							
	Appearance	Color of gills	Texture	Scales consistency	Odor	Sliminess	Eye's lustrous	Overall acceptability
RB	7.04c	6.53b	6.50b	6.33b	6.11c	5.71b	6.23c	59.21c
TWB	8.28b	7.95a	8.22a	7.69a	8.21b	7.78a	7.69b	80.52b
ETWB	8.89a	8.07a	8.52a	-	8.98a	7.73a	8.34a	86.80a
LSD (0.05)	0.50	0.50	0.41	0.58	0.33	0.77	0.50	3.75

@ : As tail of Table (5)
Means with the same latter in the same column are not significant different (P>0.05).

Table (22): Effect of different storage periods on sensory evaluation of raw Bolti fish stored at 3±1°C for 9 days.

Storage period (days)	Sensory evaluation							
	Appearance	Color of gills	Texture	Scales consistency	Odor	Sliminess	Eye's lustrous	Overall acceptability
0	9.72a	9.17a	9.13a	9.11a	9.44a	8.37a	9.21a	91.95a
3	8.61b	8.30b	8.46b	7.61b	8.76b	7.50b	7.91b	82.31b
6	7.89c	7.41c	7.54c	6.38c	7.65c	6.62c	7.02c	74.02c
9	6.06d	5.19d	5.85d	4.95d	5.22d	5.81d	5.54d	53.75d
LSD (0.05)	0.58	0.57	0.47	0.67	0.39	0.89	0.52	4.33

Means with the same latter in the same column are not significant different (P>0.05).

5.2. Sensory evaluation of cooked Bolti fish:

Sensory attributes of untreated and treated Bolti fish samples after fried were evaluated, data were recorded in Table (23). There is no significant difference ($P<0.05$) between the three investigated treatments RB, TWB and ETWB at zero time storage. As the storage time prolonged a decremental trend was shown in all investigated characteristics for all treatments.

During subsequent cold storage, a significant differences ($p<0.05$) in appearance for RB treatment and other treatments was recorded after 3 and 6 days. While, no significant difference ($P>0.05$) between TWB and ETWB treatments was recorded. The ETWB treatment had lower decremental rate than TWB sample till the end of storage periods.

As seen in the same table average of taste scores was around 8.47 at zero time of storage. A significant difference ($p<0.05$) in taste for RB treatment and other treatments after 3 and 6 days was recorded. This may be due to the role of dipping treatment in improving the taste. Also, the ETWB treatment had lower decremental rate than TWB treatment till the end of cold storage when compared to RB treatment.

Data given in the same table shows the mean scores of texture ranged from 8.29 to 8.54. A significant difference ($p<0.05$) between RB treatment and other treatments after 3 and 6 days was recorded. While, no significant difference ($p>0.05$) between TWB and ETWB treatments for 6 days, at the end of cold storage periods, the ETWB was the best treatment between all treatments. These results were confirmed with the data of the optical density of muscles extract as an indicator of quality.

Table (23) illustrated the juiciness numbers that average scores was around 8.47. A significant difference ($P<0.05$) was found between RB and other treatments at 3 days. The ETWB had lower decremental pattern than TWB when compared to RB treatment. These findings are confirmed with the data of moisture content see Table (5) and WHC (Table, 13). This mainly due to the effect of dipping solution (SL + TSP + PS + BHA) in improving WHC and binding the water by SL and TSP (Shelef and Yang, 1991 and Yasin, 1997).

Data given in the same table shown the mean scores of odor for different untreated and treated Bolti after fried fish during cold storage. At the beginning of cold storage, the value of odor was 8.13, 8.25 and 8.29 for RB, TWB and ETWB treatments, respectively. A significant difference ($p<0.05$) between RB treatment and other treatments was recorded during storage for 3 and 6 days. A decremental trend was shown for all treatments and the ETWB samples had lower decrease rate than TWB when compared to RB sample. These findings are confirmed with the data of freshness test especially TVN (Table, 9).

Overall acceptability of Bolti after fried fish throughout cold storage at 3 ± 1°C was also evaluated, data are recorded in Table (23). The values of overall acceptability were in range 87.42 to 89.75. With the progression of cold storage, a significant difference ($P<0.05$) between RB and other treatments after 3 days was observed. The lowest rate for decrease of overall acceptability was found in ETWB samples when compared to other treatments.

Table (23): Sensory evaluation of untreated and treated bolti fish after cooking during cold storage at 3±1°C (mean ± stander error).

Storage period (days)	RB	TWB	ETWB	RB	TWB	ETWB
	Appearance			Taste		
Zero	8.50±0.19	8.83±0.17	9.00±0.19	8.58±0.19	8.50±0.28	8.67±0.28
3	6.84±0.41	8.42±0.25	8.38±0.53	7.34±0.25	8.29±0.31	8.42±0.33
6	5.21±0.35	8.38±0.25	8.19±0.21	5.50±0.20	7.63±0.20	8.17±0.15
9		7.46±0.17	8.08±0.23		6.09±0.19	8.08±0.18
11		6.21±0.28	8.04±0.47		5.79±0.23	7.88±0.33
13			7.96±0.17			7.75±0.21
15			7.33±0.36			7.50±0.16
17			6.88±0.19			6.17±0.28
$LSD^{@}_{(0.05)}$	0.86			0.70		
	Texture			Juiciness		
Zero	8.29±0.18	8.54±0.30	8.46±0.34	8.67±0.22	8.29±0.30	8.46±0.30
3	6.93±0.33	8.29±0.26	8.34±0.23	7.12±0.39	8.21±0.35	8.33±0.34
6	4.85±0.37	7.75±0.19	8.29±0.11	5.07±0.39	7.92±0.36	8.29±0.32
9		6.84±0.21	8.17±0.28		7.21±0.27	8.25±0.29
11		5.90±0.16	7.88±0.19		6.42±0.31	7.83±0.17
13			7.42±0.33			7.29±0.38
15			7.33±0.16			6.96±0.36
17			6.29±0.27			6.25±0.21
$LSD^{@}_{(0.05)}$	0.75			0.94		
	Odor			Overall acceptability		
Zero	8.13±0.15	8.25±0.28	8.29±0.33	87.42±1.72	88.08±2.49	89.75±0.3.09
3	7.38±0.33	8.21±0.29	8.13±0.31	75.00±3.82	86.42±2.27	87.17±1.81
6	6.13±0.28	7.75±0.18	8.12±0.14	55.92±3.09	82.17±1.74	85.00±1.19
9		7.42±0.33	8.09±0.27		75.75±2.76	83.25±1.78
11		6.92±0.21	7.67±0.17		62.83±3.58	76.67±3.12
13			7.13±0.21			73.75±1.86
15			6.58±0.16			72.12±2.46
17			6.38±0.29			61.83±3.44
$LSD^{@}_{(0.05)}$	0.74			6.97		

@: To be used to compare between any two times within the same treatment.

Previous data indicated that, ETWB was a best treatment followed by TWB treatment when compared to RB treatment. This may be due to the effect of dipping solution on retarding the microbial load and improving the quality of Bolti fish (Yasin, 1997).

Analysis of variance for sensory evaluation of untreated and treated Bolti fish after cooking is presented in Table (24) which indicated that there are significant differences ($P<0.05$) in sensory attributes of different treated Bolti fish after cooked, between either different treatments or different storage periods.

Multiple comparisons for different treatments (Table, 25) showed significant differences ($P<0.05$) between each of ETWB and RB, and TWB and RB treatments. No significant difference ($P>0.05$) between TWB and ETWB was shown. ETWB was best treatment, that it obtained the highest score between all treatment.

Multiple comparisons for different storage periods (Table, 26) indicated presence of significant differences ($P<0.05$) in sensory characteristics at zero, 3 and 6 days.

Table (24): Analysis of variance for sensory evaluation of untreated and treated cooked Bolti fish stored at 3±1°C for 9 days.

Source of variance	D.F.	F value and level of significancy					
		Appearance	Texture	Odor	Taste	Juiciness	Overall acceptability
Time	2	47.27*	69.00*	26.82*	26.82*	29.73*	4.118*
Treatment	2	26.09*	41.73*	24.74*	16.73*	10.83*	5.203*
Time x treatment	4	18.56*	28.08*	8.70*	2.09^{NS}	1.08^{NS}	1.41^{NS}
Error	99	--	--	--	--	--	--
Total	107	--	--	--	--	--	--

D.F.: Degree of freedom. * Significant at 0.05
N.S.: Non significant.

Table (25): Effect of different treatments on sensory evaluation of untreated and treated cooked Bolti fish stored at 3±1°C for 9 days.

Treatments @	Sensory evaluation					
	Appearance	Texture	Odor	Taste	Juiciness	Overall acceptability
RB	6.82^{b}	7.14^{b}	6.69^{b}	6.95^{b}	7.21^{b}	72.78^{b}
TWB	8.54^{a}	8.14^{a}	8.19^{a}	8.14^{a}	8.07^{a}	85.56^{a}
ETWB	8.52^{a}	8.42^{a}	8.36^{a}	8.36^{a}	8.18^{a}	87.31^{a}
LSD (0.05)	0.50	0.41	0.44	0.54	0.43	4.02

@ : As tail of Table (5)
Means with the same latter in the same column are not significant different (P>0.05).

Table (26): Effect of different storage periods on sensory evaluation of cooked Bolti fish stored at 3±1°C for 9 days.

Storage period (days)	Sensory evaluation					
	Appearance	Texture	Odor	Taste	Juiciness	Overall acceptability
0	8.78^{a}	8.58^{a}	8.43^{a}	8.47^{a}	8.22^{a}	88.42^{a}
3	7.88^{b}	8.02^{b}	7.85^{b}	7.89^{b}	7.91^{a}	82.86^{b}
6	7.26^{c}	7.10^{c}	6.96^{c}	7.09^{c}	7.33^{b}	74.36^{c}
LSD at 0.05	0.50	0.41	0.44	0.54	0.43	4.02

Means with the same latter in the same column are not significant different (P>0.05).

PART III:

Effect of frozen storage alone or accompanied with dipping treatment on Bolti fish:

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

Chemical composition of differently treated Bolti fish (*Oreochromis niloticus*) samples raw Bolti fish undipped (RB), whole Bolti fish was washed and dipped in water for 2 min. (WWB), whole Bolti fish was washed, dipped in a solution contained 2.4% (SL) + 3.2% (TSP) +4.8% (PS) +240 ppm (BHA) for 2 min and drained (TWB), whole Bolti fish was washed, inoculated with dipping in solution contain *E.coli* OI57:H7 ATCC 69373 ($4.85x10^5$) then left for 15 min. after that dipped in water for 2 min. and drained (IWWB) and whole Bolti fish was washed, inoculated with dipping in solution contain *E.coli* OI57:H7 ATCC 69373 ($4.85x10^5$) then left for 15 min. after that dipped in the same preservatives solution formula and drained (ITWB) at the beginning of storage period and during subsequent frozen storage at -18±1°C, that mentioned earlier was shown in Table (27).

It could be noticed that at zero time, the moisture content was 78.64, 79.41, 79.02, 79.22 and 78.99 for RB, WWB, TWB, IWWB and ITWB, respectively. During subsequent frozen storage a decremental trend was shown. After 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, respectively. The reason of such loss in moisture content may be due to the sublimation of ice crystals and/or separating drip during thawing

process in addition to the decrease of WHC for fish muscles during frozen storage period. These results go parallel with that of Khallaf (1986), Harrison and Morgaret (1989), Yasin (1997), Glannini (2001) and Tseng, *et al.*, (2003).

The role of dipping treatment (especially, TSP and SL) that retarded the moisture loss during storage and improved the WHC of fish protein (Krivchenia and Fennema, 1988 and Yasin, 1997).

The statistical analysis indicated that both time of storage and type of treatment influenced the moisture content of frozen Bolti fish.

A downward pattern was also recorded and given in the same table for Bolti fish protein content during subsequent frozen storage period (8 months). Bolti fish samples contained 79.56 to 80.80% protein (on dry weight basis), such content consequently dropped during frozen storage period. After 8 months of frozen storage loss were 7.29, 5.15, 3.99, 4.99 and 1.99 for RB, WWB, TWB, IWWB and ITWB, respectively. A slight losses in protein content during frozen storage is mainly due to the partial hydrolysis of proteins by natural meat enzymes such as (cathepsins) and bacterial enzymes that produced before freezing (Shehata, 1974 and El-Ghazali, 1981). However, more slightly lower loss in protein content was observed in TWB and ITWB than WWB and IWWB samples, when compared to RB one. The effect of dipping treatment (mainly TSP and SL) in reducing the amount of separation drip and improving the WHC of fish protein and reducing the water activity could be a good explanation for less loss in crude proteins. These results are in

accordance with that of Hassan (1980), Khallaf (1982), Corey *et al.* (1989) and Yasin (1997).

Upward trend of lipid content in Bolti tissues was recorded in the same table. Total lipid content was around 13.46% (on dry weight basis) at the beginning of frozen storage then significantly ($P<0.05$) increased up to the end of storage periods. However, the total lipid seemed to be slightly increased during frozen storage in all samples. Higher lipid content was found in RB, WWB and IWWB treatments than TWB and ITWB ones. These results have gone inversely with those of moisture and protein content as shown before. These results are in accordance with those obtained by Cross (1980), Corey *et al.* (1987) and Yasin (1997).

Statistical analysis was carried out and the differences was found to be significant, i.e. both storage time as well as the suggested treatment had their effect on the Bolti fish lipid content.

Data in Table (27), also indicated that Bolti fish ash content was affected by either treatments or long term frozen storage. Ash content was in range 5.28 to 5.47 (on dry weight basis) at zero time. The suggested dipping treatment in this investigation (SL + TSP + PS + BHA) caused slight increase in ash content of treated Bolti fish after dipping when compared with untreated ones. However, during frozen storage a significant ($P<0.05$) increase in ash content was observed. The increase percentage were 40.72, 33.58, 18.10, 35.07 and 15.44% for RB, WWB, TWB, IWWB and ITWB, respectively after 8 months. An apparent increase in ash content of frozen samples is due to the moisture loss under the same condition.

Table (27): Chemical composition of untreated and treated Bolti fish during frozen storage at –18±1°C for 8 months (mean ± standard error).

Storage period (months)	Moisture content					Protein content $				
	RB	WWB	TWB	IWWB	ITWB	RB	WWB	TWB	IWWB	ITWB
Zero	78.64 ±0.34	79.41 ±0.17	79.02 ±0.09	79.22 ±0.42	78.99 ±0.15	80.52 ±0.50	80.37 ±0.63	80.80 ±0.25	80.38 ±0.85	79.56 ±0.94
2	77.49 ±0.19	77.97 ±0.46	78.76 ±0.41	78.61 ±0.41	78.49 ±0.25	78.77 ±0.49	78.43 ±0.63	80.04 ±0.69	79.32 ±1.18	79.27 ±0.25
4	76.43 ±0.19	76.62 ±0.42	78.04 ±0.27	77.60 ±0.42	78.24 ±0.07	76.78 ±0.43	77.43 ±0.88	79.49 ±1.54	79.04 ±0.56	79.23 ±0.37
6	75.45 ±0.19	75.85 ±0.27	77.68 ±0.30	76.99 ±0.29	77.56 ±0.30	75.76 ±0.15	76.72 ±0.69	78.78 ±0.23	77.75 ±0.44	78.14 ±1.04
8	74.75® ±0.21	75.48® ±0.25	77.20 ±0.22	76.26 ±0.14	77.32 ±0.40	74.65® ±0.07	76.23® ±0.34	78.57 ±0.56	76.37 ±0.16	77.98 ±0.60
LSD@(0.05)	0.83					1.90				

$: Calculated on dry weight basis.
®: At these point samples were organoleptically rejected.
@: To be used to compare between any two times within the same treatment.
RB : Raw Bolti fish undipped .
WWB : Whole Bolti fish washed, then dipped in water for 2 min.
TWB : Whole Bolti fish washed, then dipped for 2 min. in a solution contained 2.4% (SL) + 3.2% (TSP) +4.8% (PS) +240 ppm (BHA) and drained.
IWWB : Whole Bolti fish washed, inoculated with dipping in solution contained *E. coli* OI57:H7 ATCC 69373 ($4.85x10^5$) then left for 15 min. after that dipped in water for 2 min. and drained.
ITWB : Whole Bolti fish washed, inoculated with dipping in solution contained *E. coli* OI57:H7 ATCC 69373 ($4.85x10^5$) then left for 15 min. after that dipped in the same preservatives solution formula and drained.

Table (27): Cont.

Storage period (months)	Total lipid content ^{S}					Ash content ^{S}				
	RB	WWB	TWB	IWWB	ITWB	RB	WWB	TWB	IWWB	ITWB
Zero	14.01 ±0.25	13.18 ±0.14	13.09 ±0.11	13.25 ±0.28	13.75 ±0.36	5.28 ±0.04	5.42 ±0.25	5.47 ±0.04	5.36 ±0.04	5.44 ±0.19
2	15.14 ±0.43	14.51 ±0.07	14.07 ±0.43	14.79 ±0.83	14.97 ±0.29	5.86 ±0.29	5.62 ±0.23	5.78 ±0.46	5.56 ±0.62	5.57 ±0.09
4	16.39 ±0.23	15.66 ±0.25	14.15 ±0.15	14.87 ±0.19	14.11 ±0.17	6.38 ±0.53	6.23 ±0.56	6.12 ±0.44	6.14 ±0.33	5.77 ±0.56
6	16.91 ±0.33	15.89 ±0.09	14.66 ±0.19	15.29 ±0.15	14.70 ±0.13	6.87 ±0.30	6.74 ±0.31	6.41 ±0.14	7.06 ±0.24	6.06 ±0.22
8	17.19® ±0.12	16.65® ±0.03	14.71 ±0.33	16.14 ±0.05	15.03 ±0.29	7.43® ±0.21	7.24® ±0.26	6.46 ±0.22	7.24 ±0.08	6.28 ±0.17
LSD$^{@}_{(0.05)}$	0.89					0.91				

S: Calculated on dry weight basis.
®: At these point samples were organoleptically rejected.
@: To be used to compare between any two times within the same treatment.
RB : Raw Bolti fish undipped .
WWB : Whole Bolti fish washed, then dipped in water for 2 min.
TWB : Whole Bolti fish washed, then dipped for 2 min. in a solution contained 2.4% (SL) + 3.2% (TSP) +4.8% (PS) +240 ppm (BHA) and drained.
IWWB : Whole Bolti fish washed, inoculated with dipping in solution contained *E. coli* O157:H7 ATCC 69373 ($4.85x10^5$) then left for 15 min. after that dipped in water for 2 min. and drained.
ITWB : Whole Bolti fish washed, inoculated with dipping in solution contain *E. coli* O157:H7 ATCC 69373 ($4.85x10^5$) then left for 15 min. after that dipped in the same preservatives solution formula and drained.

Analysis of variance was carried out to detect the significant difference of the effect of both storage time and dipping treatment on the chemical composition of Bolti fish samples (Table, 28) which indicated that there are significant differences ($P<0.05$) in moisture, protein and lipid contents between either different treatments or different storage periods. A significant difference ($P<0.05$) was shown in ash content as affect by different storage time while, no significant difference ($P>0.05$) in ash as affect by different treatments.

Multiple comparisons for different treatments (Table, 29) indicated that there are no significant differences ($P>0.05$) in moisture and protein contents means of TWB and ITWB, which obtained the highest values. A similar trend was shown in lipid and ash content means of TWB and ITWB which obtained the lowest values. A significant differences ($P<0.05$) between RB treatment and other treatments were shown in moisture, protein and lipid content.

Multiple comparisons of different storage periods for chemical composition (Table, 30) indicated that all differences in moisture, protein, lipid and ash are significant ($P>0.05$) and the highest values were recorded at zero time in moisture and protein while, it recorded in lipid and ash at 8 months of frozen storage. It is noticed that moisture and protein contents decreased with increasing storage periods. Dipping treatment reduced the rate of hydrolysis in protein content after 8 months. Although, there is no direct effect of these treatments on lipid and ash content. The main reason for such significance is attributed to that, both changes in moisture and protein contents had their effect on total lipid and ash contents.

Table (28): Analysis of variance for chemical composition (calculated on dry weight basis) of untreated and treated Bolti fish stored at -18±1°C for 8 months.

Source of variance	D.F	F value and level of significancy			
		Moisture %	Protein %	Lipid %	Ash %
Time	4	*76.46	*23.89	*44.58	*78.66
Treatment	4	*27.84	*7.26	*13.47	0.98 NS
Time x treatment	16	*2.44	1.05 NS	1.55 NS	0.78 NS
Error	50	---	---	---	---
Total	74	---	---	---	---

D.F.: Degree of freedom. * Significant at 0.05
N.S.: Non significant.

Table (29): Effect of different treatments on chemical composition (calculated on dry weight basis) of untreated and treated Bolti fish stored at -18±1°C for 8 months.

Treatments @	Components			
	Moisture %	Protein %	Total lipid %	Ash %
RB	76.55^{d}	77.30^{bc}	15.93^{a}	6.36^{a}
WWB	77.07^{c}	77.84^{b}	15.18^{b}	6.25^{a}
TWB	78.14^{a}	79.54^{a}	14.14^{d}	6.05^{ab}
IWWB	77.74^{b}	78.57^{ab}	14.87^{b}	6.27^{a}
ITWB	78.12^{a}	78.84^{a}	14.51^{bc}	5.82^{b}
LSD (0.05)	0.37	0.85	0.40	0.41

@ : As tail of Table (27)
Means with the same latter in the same column are not significant different (P>0.05).

Table (30): Effect of different storage periods on chemical composition (calculated on dry weight basis) of Bolti fish stored at -18±1°C for 8 months.

Storage period (months)	Components			
	Moisture %	Protein %	Total lipid %	Ash %
0	79.06^{a}	80.33^{a}	13.46^{d}	5.40^{c}
2	78.26^{b}	79.17^{b}	14.70^{c}	5.68^{c}
4	77.39^{c}	78.39^{b}	15.04^{c}	6.13^{b}
6	76.71^{d}	77.43^{c}	15.50^{b}	6.63^{a}
8	76.20^{e}	76.76^{c}	15.94^{a}	6.93^{a}
LSD (0.05)	0.37	0.85	0.40	0.41

Means with the same latter in the same column are not significant different (P>0.05).

In conclusion, the TWB and ITWB treatments showed less changes in their chemical composition than WWB and IWWB treatment during frozen storage when compared to RB treatment. This is mainly due to the effect of dipping solution (Yasin, 1997).

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

Chemical freshness indices (TVN, TMA and TBA) of different treated Bolti fish at the beginning of storage period and during subsequent frozen storage at 3±1°C, were done, data were shown in Table (31).

2.1. Total volatile nitrogen (TVN):

The TVN parameter is an indicator for fish protein breakdown Table (31). It could be noticed that the initial value of TVN in the different five investigated treatments were ranged between 12.60 to 13.53 mg/100 g (on wet weight basis). A parallel relationship between TVN value and frozen storage time was recorded, i.e. the longer of frozen storage period, the higher values of TVN were observed. The higher rate of TVN increasing during frozen storage was found in RB samples when compared to other treatments. In the same time it should be noticed the role of dipping treatments which retarding the rate of TVN formation in comparison with undipped ones. This is may be due to the function of dipping solution in retarding protein breakdown as mentioned earlier.

These results go in harmony with those of Corey *et al.* (1987), Abdalla *et al.* (1989) and Yasin (1997 and 2003).

Table (31): Freshness tests of untreated and treated Bolti fish during frozen storage at –18±1°C for 8 months (mean ± standard error).

Storage period (months)	TVN •					TMA •					TBA ►				
	RB	WWB	TWB	IWWB	ITWB	RB	WWB	TWB	IWWB	ITWB	RB	WWB	TWB	IWWB	ITWB
Zero	13.07 ±0.47	13.53 ±0.47	13.53 ±0.93	13.07 ±0.93	12.60 ±0.81	1.26 ±0.08	1.96 ±0.29	2.01 ±0.41	2.19 ±0.61	1.77 ±0.37	0.45 ±0.01	0.29 ±0.03	0.28 ±0.06	0.22 ±0.05	0.25 ±0.07
2	16.80 ±0.81	14.93 ±0.47	14.00 ±0.81	15.40 ±0.81	14.00 ±0.81	3.27 ±0.47	2.80 ±0.00	2.77 ±0.43	2.43 ±0.20	3.03 ±0.23	0.74 ±0.01	0.69 ±0.02	0.50 ±0.05	0.65 ±0.03	0.49 ±0.03
4	22.87 ±0.47	17.73 ±0.47	16.80 ±0.81	18.67 ±0.47	17.13 ±0.53	5.13 ±0.47	4.67 ±0.47	3.27 ±0.47	4.67 ±0.47	4.20 ±0.81	1.24 ±0.01	1.07 ±0.16	0.70 ±0.02	1.20 ±0.07	0.71 ±0.04
6	27.53 ±1.23	23.33 ±1.23	19.13 ±0.47	22.40 ±0.81	19.60 ±0.81	7.93 ±0.47	7.00 ±0.81	6.07 ±0.47	6.53 ±0.93	6.30 ±0.40	2.81 ±0.25	2.34 ±0.51	1.87 ±0.10	2.34 ±0.18	1.64 ±0.10
8	32.90® ±0.38	28.00® ±0.81	24.27 ±0.47	27.53 ±0.47	25.20 ±0.81	9.77® ±0.47	9.33® ±0.47	7.47 ±0.47	8.87 ±0.47	8.17 ±0.62	4.51® ±0.18	3.98® ±0.36	3.35 ±0.31	3.48 ±0.40	2.76 ±0.19
$LSD^{@}_{(0.05)}$	2.09					1.41					0.53				

®: At these point samples were organoleptically rejected.
@ : To be used to compare between any two times within the same treatment.
• : Calculated as mg /100g on (wet weight basis).
► : Calculated as mg malonaldyde /Kg sample

2.2. Trimethylamine (TMA):

Table (31) reflect the TMA values of different treated Bolti fish during frozen storage. It could be noticed that the TMA values were ranged between 1.26 to 2.19 mg/100 g (on wet weight basis). Similar findings were shown earlier for TVN values which were recorded with TMA values. It could be noticed that a progressive increase in TMA all over the frozen storage period occurred till reached to a maximum value at 8 months of storage at -18±1°C in RB samples. The formation of TMA in fish tissues is due to the conversion of TMAO to TMA by non-enzymic process, by native tissue enzymes or by bacterial enzymes which are still active even at low temperature as reported by Shehata, 1974 and Kelleher *et al.,* 1981. Bolti fish samples dipped in (SL + TSP + PS + BHA) solution prior to freezing process showed less change of TMA rise during frozen storage. This is may be due to the effect of dipping solution then inhibited aerobic bacterial growth and more effects as mentioned earlier.

2.3. Thiobarbituric acid (TBA):

The values of TBA at zero time and during 8 months of frozen storage were shown in Table (31).

At the beginning of frozen storage the TBA values (as mg malonaldhyde/kg sample) were ranged from 0.22 to 0.45 mg/kg. The corresponding TBA values become 4.51, 3.98, 3.35, 3.48 and 2.76 for RB, WWB, TWB, IWWB and ITWB, respectively. The lower TBA values obtained in TWB and ITWB than WWB and IWWB when compared to RB samples (after 8 months) ones could be attributed to the effect of dipping solution in retarding the autoxidation process (Yasin, 1997).

Table(32): Analysis of variance for freshness tests of untreated and treated Bolti fish stored at -18±1°C for 8 months.

Source of variance	D.F.	F value and level of significancy		
		TVN	TMA	TBA
Time	4	306.63*	165.27*	266.80*
Treatment	4	38.20*	4.53*	12.99*
Time x treatment	16	4.26*	1.32 NS	2.03*
Error	50	--	--	--
Total	74	--	--	--

D.F.: Degree of freedom. *: significant at 0.05
N.S.: Non significant.

Table (33): Effect of different treatments on freshness tests of untreated and treated Bolti fish stored at -18±1°C for 8 months.

Treatments @	Freshness tests		
	TVN	TMA	TBA
RB	22.63^{a}	5.57^{a}	1.95^{a}
WWB	19.51^{b}	5.15^{a}	1.67^{b}
TWB	17.55^{c}	4.31^{b}	1.33^{c}
IWWB	19.41^{b}	4.94^{ab}	1.58^{b}
ITWB	17.71^{c}	4.69^{b}	1.17^{c}
LSD (0.05)	0.94	0.63	0.24

@ : As tail of Table (27)
Means with the same latter in the same column are not significant different (P>0.05).

Table (34): Effect of different storage periods on freshness tests of Bolti fish stored at -18±1°C for 8 months.

Storage period (months)	Freshness tests		
	TVN	TMA	TBA
0	13.16^{e}	1.84^{e}	0.30^{e}
2	15.03^{d}	2.86^{d}	0.61^{d}
4	18.64^{c}	4.39^{c}	0.98^{c}
6	22.40^{b}	6.77^{b}	2.20^{b}
8	27.58^{a}	8.72^{a}	3.62^{a}
LSD (0.05)	0.94	0.63	0.24

Means with the same latter in the same column are not significant different (P>0.05).

Analysis of variance of freshness tests for untreated and treated Bolti fish (Table, 32) indicated that there are significant differences ($P<0.05$) in TVN, TMA and TBA between either different treatments or different storage periods.

Multiple comparisons for different treatments (Table, 33) indicated significant differences ($P<0.05$) in TVN, TMA and TBA.

Multiple comparisons for different storage periods (Table, 34) indicated that a significant differences ($P<0.05$) were shown with the time increasing time. The lowest values was recorded at zero time. While, the highest values was recorded in 8 months of frozen storage.

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

The physical properties of different treated Bolti fish samples, i.e. pH value, O.D. of gills extract, O.D of muscles extract and pigments concentration as well as refractive index of eye fluids (RI), water holding capacity (WHC), plasticity and cooking loss (%) were studied in this investigation as seen in Table (35).

3.1. pH value:

The pH value measured at different storage periods. The initial values of pH at zero time were 6.36, 6.32, 6.19, 6.48 and 6.18 for RB, WWB, TWB, IWWB and ITWB, respectively. Dipping process led to decrease pH value after dipping treatments mainly due to the effect of dipping solution especially (SL). During further frozen storage, a continuous increase in the pH value was noticed till the end of storage period. The

incremental trend of TWB and ITWB treatments were lower than that of WWB and IWWB treatments when compared to RB treatment at the end of frozen storage. There was a significant difference ($P<0.05$) in pH values vs time of storage at -18±1°C/8 months. So, it could be concluded that the dipping treatment with (SL + TSP + PS + BHA) was more effective on reducing the rate of pH rise when compared to RB treatment. Such findings are correlated well with the other results of TVN, TMA and total microbial counts obtained in the present investigation and all these results were confirmed with Yasin (1997).

3.2. Optical density of gills extract:

The O.D. of gills extract was measured in this study to explain the effect of either the applied treatments or storage time at -18±1°C on untreated and treated Bolti fish, obtained data were given in Table (35). At the beginning of frozen storage, the O.D. of gills extracts ranged from 0.73 to 0.82 (as O.D. at 542 nm). A highest O.D. of gills extract values were recorded with TWB and ITWB than WWB and IWWB when compared to RB samples with the time as extended. This may be due to the loss of gills ability to keep their pigments inside its structure or pigments conversion and/or decomposition as a result of bacterial invasion. This could be confirmed on the basis that the higher bacterial count was accompanied with higher loss in the O.D. of gills extract (Khallaf, 1982 and Yasin, 1997).

3.3 Optical density of muscles extract:

The optical density of Bolti fish muscles extract was also followed during 8 months of frozen storage at -18±1°C as seen in Table (35). At zero time of frozen storage the O.D. of muscles extract was ranged from 0.305to 0.503 (as O.D. at 420 nm). With

extending the storage period the values were around 0.61, 0.77, 0.82 and 0.90 at 2, 4, 6 and 8 months, respectively. The incremental trend could be arranged in ascending manner for TWB, ITWB, IWWB and WWB when compared to RB samples after 8 months. These results were confirmed with texture (Table, 41). It means that the O.D. of muscles extract for TWB and ITWB was improved by dipping solution when compared to RB, WWB and IWWB samples. This data was in agreement with Yasin (1997) and contradicted with Ghoneim (1974).

3.4. Pigments concentration:

Pigments concentration as affected by used dipping solution as well as long term frozen storage at -18±1°C was considered in this study (Table, 35). The pigments concentration was ranged from 0.93 to 1.09 (as O.D. at 540 nm) at zero time of frozen storage. During subsequent frozen storage the pigment concentration was generally decreased with the time increasing. During frozen storage color deterioration of different treated Bolti fish was occurred which may be due to oxidation of myoglobin and oxymyoglobion to metamyoglobin, a scape of some pigment (as water soluble protein) with drip (Shams El-Din, 1978) and the decomposition of pigments as a result of bacterial action (Khallaf, 1982). The increase rates were 0.084, 0.095, 0.073, 0.090 and 0.081/month for RB, WWB, TWB, IWWB and ITWB, respectively. The TWB and ITWB had less increasing from RB when compared to WWB and IWWB samples.

The high value of pigments concentration was found in TWB and ITWB treated because the dipping treatment improved the WHC as recorded in the same table.

Table (35): Physical properties of untreated and treated Bolti fish during frozen storage at −18±1°C for 8 months (mean ± standard error).

Storage period (months)	pH					O.D of gills •				
	RB	WWB	TWB	IWWB	ITWB	RB	WWB	TWB	IWWB	ITWB
Zero	6.36 ±0.01	6.32 ±0.03	6.19 ±0.04	6.48 ±0.01	6.18 ±0.06	0.820 ±0.057	0.734 ±0.006	0.855 ±0.030	0.804 ±0.019	0.818 ±0.041
2	6.58 ±0.06	6.52 ±0.08	6.34 ±0.04	6.64 ±0.04	6.41 ±0.09	0.588 ±0.014	0.648 ±0.025	0.667 ±0.012	0.676 ±0.011	0.727 ±0.009
4	6.65 ±0.04	6.52 ±0.06	6.44 ±0.04	6.76 ±0.03	6.44 ±0.06	0.427 ±0.013	0.459 ±0.018	0.590 ±0.012	0.515 ±0.007	0.665 ±0.009
6	6.79 ±0.02	6.73 ±0.09	6.54 ±0.05	6.82 ±0.05	6.56 ±0.06	0.377 ±0.011	0.404 ±0.012	0.415 ±0.012	0.398 ±0.018	0.408 ±0.011
8	6.87® ±0.02	6.81® ±0.04	6.62 ±0.05	6.82 ±0.01	6.62 ±0.04	0.233® ±0.034	0.279® ±0.031	0.364 ±0.028	0.330 ±0.043	0.383 ±0.047
LSD@(0.05)	0.14					0.071				

• : Calculated as O.D at 542 nm.
® : At these point samples were organoleptically rejected.
@: To be used to compare between any two times within the same treatment.

Table (35): Cont.

Storage period (months)	O.D of muscles ◄					Pigments concentration ►				
	RB	WWB	TWB	IWWB	ITWB	RB	WWB	TWB	IWWB	ITWB
Zero	0.398 ±0.001	0.352 ±0.028	0.368 ±0.022	0.503 ±0.049	0.305 ±0.053	0.995 ±0.002	1.054 ±0.014	0.934 ±0.003	1.050 ±0.003	1.091 ±0.001
2	0.662 ±0.002	0.563 ±0.003	0.481 ±0.006	0.744 ±0.004	0.587 ±0.004	0.763 ±0.003	0.837 ±0.003	0.757 ±0.006	0.730 ±0.004	0.806 ±0.003
4	0.793 ±0.009	0.689 ±0.011	0.536 ±0.010	0.801 ±0.016	0.607 ±0.013	0.595 ±0.009	0.691 ±0.014	0.680 ±0.010	0.550 ±0.015	0.760 ±0.012
6	0.839 ±0.022	0.786 ±0.017	0.675 ±0.027	0.859 ±0.017	0.763 ±0.014	0.469 ±0.016	0.498 ±0.008	0.512 ±0.013	0.426 ±0.032	0.578 ±0.012
8	0.971® ±0.028	0.874® ±0.031	0.863 ±0.042	0.979 ±0.016	0.809 ±0.048	0.327® ±0.050	0.291® ±0.013	0.350 ±0.043	0.327 ±0.035	0.440 ±0.041
LSD @ (0.05)	0.070					0.057				

◄ : Calculated as O.D at 420 nm.
►: Calculated as O.D at 540 nm .
® : At these point samples were organoleptically rejected.
@: To be used to compare between any two times within the same treatment.

Table (35): Cont.

Storage period (months)	RI					WHC *				
	RB	WWB	TWB	IWWB	ITWB	RB	WWB	TWB	IWWB	ITWB
Zero	1.3390 ±0.0003	1.3381 ±0.0003	1.3361 ±0.0003	1.3391 ±0.0012	1.3394 ±0.0012	10.17 ±0.28	10.00 ±0.26	9.73 ±0.38	9.40 ±0.45	11.23 ±0.22
2	1.3588 ±0.0052	1.3526 ±0.0034	1.3414 ±0.0006	1.3501 ±0.0057	1.3391 ±0.0003	10.83 ±0.28	11.97 ±0.48	10.27 ±0.12	11.73 ±0.48	11.33 ±0.28
4	1.3714 ±0.0055	1.3694 ±0.0031	1.3591 ±0.0003	1.3755 ±0.0062	1.3521 ±0.0028	11.07 ±0.26	11.30 ±0.00	11.23 ±0.43	12.10 ±0.32	9.10 ±0.36
6	1.3794 ±0.0015	1.3754 ±0.0010	1.3671 ±0.0023	1.3791 ±0.0023	1.3704 ±0.0025	12.23 ±0.37	12.57 ±0.43	11.50 ±0.61	12.43 ±0.20	8.07 ±0.20
8	1.4304® ±0.0101	1.4042® ±0.0136	1.3874 ±0.0042	1.4211 ±0.0161	1.3887 ±0.0064	13.33® ±0.41	12.80® ±0.38	11.03 ±0.12	13.13 ±0.34	8.57 ±0.32
$LSD^{@}_{(0.05)}$	0.0159					0.98				

▪ : Calculated as cm^2/0.3g sample.

® : At these point samples were organoleptically rejected.

@: To be used to compare between any two times within the same treatment.

Table (35): Cont.

Storage period (months)	Plasticity *					Cooking loss(%)				
	RB	WWB	TWB	IWWB	ITWB	RB	WWB	TWB	IWWB	ITWB
Zero	2.70 ±0.30	3.13 ±0.19	3.03 ±0.12	3.43 ±0.09	3.40 ±0.10	13.61 ±0.23	13.43 ±0.42	13.36 ±0.23	13.63 ±0.54	13.91 ±0.43
2	2.87 ±0.24	2.80 ±0.32	2.47 ±0.20	2.50 ±0.15	2.63 ±0.27	16.58 ±0.10	15.32 ±1.51	14.55 ±0.70	15.93 ±0.41	14.36 ±0.75
4	2.77 ±0.20	2.40 ±0.35	2.27 ±0.18	2.43 ±0.24	2.87 ±0.13	18.20 ±0.71	17.95 ±0.21	15.59 ±0.31	18.01 ±0.18	15.91 ±0.15
6	2.53 ±0.20	2.20 ±0.10	2.53 ±0.26	2.33 ±0.12	2.57 ±0.24	21.22 ±0.69	20.59 ±0.45	17.44 ±0.30	21.21 ±0.69	17.95 ±0.20
8	2.37® ±0.18	2.03® ±0.07	2.53 ±0.12	2.30 ±0.17	2.47 ±0.07	25.12® ±0.69	23.26® ±0.86	19.28 ±0.62	22.49 ±0.42	18.65 ±0.43
LSD @ (0.05)	0.57					1.62				

▪ : Calculated as cm^3/0.3g sample.
® : At these point samples were organoleptically rejected.
@: To be used to compare between any two times within the same treatment.

Table(36): Analysis of variance for physical properties of untreated and treated Bolti fish stored at -18±1°C for 8 months.

Source of variance	D.F.	F value and level of significancy							
		pH	WHC	Plasticity	RI	O D muscles	O D gills	Pigments concentration	Cooking loss
Time	4	60.44*	16.19*	12.02*	110.26*	255.29*	317.76*	837.78*	163.88*
Treatment	4	30.22*	33.63*	13.40*	10.83*	28.02*	14.28*	27.13*	26.75*
Time x treatment	16	0.50 NS	12.16*	1.26 NS	1.73 NS	2.42*	2.97*	4.77*	3.52*
Error	50	--	--	--	--	--	--	--	--
Total	74	--	--	--	--	--	--	--	--

D.F.: Degree of freedom. * Significant at 0.05
N.S.: Non significant.

Table (37): Effect of different treatments on physical properties of untreated and treated Bolti fish stored at -18±1°C for 8 months.

Treatments @	Physical properties							
	pH	O.D of gills	O.D of muscles	pigments concentration	RI	WHC	Plasticity	Cooking loss
RB	6.65^{a}	0.489^{c}	0.733^{b}	0.630^{c}	1.3758^{a}	11.53^{a}	2.65^{a}	18.95^{a}
WWB	6.58^{b}	0.505^{c}	0.653^{c}	0.674^{b}	1.3680^{b}	11.73^{a}	2.51^{b}	18.11^{b}
TWB	6.43^{c}	0.578^{a}	0.605^{d}	0.747^{a}	1.3582^{c}	10.75^{b}	2.57^{a}	15.92^{c}
IWWB	6.70^{a}	0.545^{b}	0.777^{a}	0.617^{c}	1.3730^{a}	11.76^{a}	2.60^{a}	$18.26a^{b}$
ITWB	6.44^{c}	0.600^{a}	0.654^{c}	0.735^{a}	1.3580^{c}	9.66^{c}	2.79^{a}	16.16^{c}
LSD (0.05)	0.06	0.032	0.031	0.025	0.0071	0.44	0.25	0.72

@ : As tail of Table (27)
Means with the same latter in the same column are not significant different (P>0.05).

Table (38): Effect of different storage periods on physical properties of Bolti fish stored at -18±1°C for 8 months.

Storage period (months)	Physical properties							
	pH	O.D of gills	O.D of muscles	pigments concentration	RI	WHC	Plasticity	Cooking loss
0	6.31^{c}	0.806^{a}	0.405^{e}	1.025^{a}	1.3383^{a}	10.11^{c}	3.14^{a}	13.59^{e}
2	6.50^{b}	0.661^{b}	0.607^{d}	0.778^{b}	1.3484^{b}	11.23^{ba}	2.65^{b}	15.35^{d}
4	6.56^{b}	0.531^{c}	0.765^{c}	0.655^{c}	1.3655^{b}	10.96^{b}	2.55^{b}	17.13^{c}
6	6.69^{a}	0.400^{d}	0.824^{b}	0.497^{d}	1.3743^{c}	11.36^{a}	2.43^{b}	19.68^{b}
8	6.75^{a}	0.318^{e}	0.899^{a}	0.347^{e}	1.4064^{d}	11.77^{a}	2.34^{b}	21.76^{a}
LSD (0.05)	0.06	0.032	0.031	0.025	0.0071	0.44	0.25	0.72

Means with the same latter in the same column are not significant different (P>0.05).

improving the fish tissue quality (Corey *et al.*, 1987 and Yasin, 1997).

Analysis of variance of physical properties for untreated and treated Bolti fish (Table, 36) indicated that there are significant differences ($P<0.05$) between either different treatments or different storage periods for all characteristics.

Multiple comparisons for different treatments (Table, 37) indicated that values of pH, O.D. of gills, O.D. of muscles, pigments concentration, RI, WHC, plasticity and cooking loss recorded significant differences ($P<0.05$) for TWB and ITWB when compared to other treatments.

With respect to the effect of different storage periods on the physical properties of untreated and treated Bolti fish, data presented in (Table, 38) showed significant differences ($P<0.05$) in all parameters with increasing storage time.

4. Microbiological quality attributes of untreated and treated frozen Bolti fish:

Bacterial aspects of untreated and treated Bolti fish samples during frozen storage at -18±1°C were enumerated, data were recorded in Table (39).

Four-culture method as a new technique used as well as a conventional plating method to detect and enumerate the total mesophilic microbial load, gram-negative bacteria, coliforms and *E. coli* as indicator to monitor the microbiological quality and safety of frozen fish. Relationship between the results of the conventional plating methods and four culture methods for enumeration of four bacterial groups was done.

Total mesophilic bacterial count (TMBC) was shown in Table (39). The initial TMBC of different treated Bolti fish were 3.91, 3.64, 2.39, 3.45 and 2.61 log CFU g^{-1} enumerating by conventional plating methods and 3.71, 3.41, 2.51, 3.42 and 2.51 log CFU g^{-1} enumerating by four-culture methods for RB, WWB, TWB, IWWB and ITWB, respectively. Upward trends were shown till second months of frozen storage then the downward trend was taken place till the end of storage periods in RB, WWB and IWWB. On the other hand, the downward trend was taken place in TWB and ITWB since zero storage time till the end of storage periods.

Gram negative bacterial (GNB) of different treated Bolti fish was shown in the same table. The initial GNB were 3.12, 3.13, 2.13, 3.38 and 2.63 log CFU/g (conventional plating methods) and 3.11, 3.11, 2.20, 3.41 and 2.51 log CFU g^{-1} (four-culture methods) for RB, TWB, ETWB, ITWB and ITWB, respectively. Upward trends were shown till second months of frozen storage then the downward trend was taken place till the end of storage periods in RB, WWB and IWWB. On the other hand, the downward trend was taken place in TWB and ITWB since zero storage time till the end of storage periods (Kim and Hearnsberger, 1994 and Kim *et al.,* 1995).

Data presented in Table (39) show the number of coliform group (CG) of various investigated samples during frozen storage at -18±1°C. Upward trends were shown till second months of frozen storage then the downward trend was taken place till the end of storage periods in RB, WWB and IWWB. On the other hand, the downward trend was taken place in TWB

and ITWB since zero storage time till the end of storage periods in colifirm group.

Dipped treatments led to reduce *E. coli* (EC) compared to RB treatment that dipping solution had more direct effect on *E. coli*. Upward trends were shown till second months of frozen storage then the downward trend was taken place till the end of storage periods in RB, WWB and IWWB. On the other hand, downward trend was taken place in TWB and ITWB since zero storage time till the end of storage periods.

In conclusion, from these data in Table (39), frozen storage and dipping treatment gave slight decrease rate in microbial load (TMBC, GNB, CG and EC) for dipped samples. While, RB, WWB and IWWB samples had a slight increase rate in microbial load. These results are in agreement with the results obtained by (Yasin, 1997 and Giannini, 2001).

The response of scatter plots exhibited a high degree of relationship 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* ($r^2 = 0.76$) (Fig. 6). These results in agreement with Kang *et al.* (2003). The relationship between the four-culture values with the results of the conventional plating methods suggests that the four-culture methods can be used to enumerate the four critical bacterial group in fish over conventional plating methods.

In conclusion, as previously mentioned in part II, this method will facilitate sanitation monitoring at fish-processing plants by simplifying procedures.

Table (39): Logarithmic number of microbial aspects for untreated and treated Bolti fish during frozen storage at $-18\pm1^{\circ}C$.

Storage period (months)	Micro-organisms	RB		WWB		TWB		IWWB		TWB	
		A	B	A	B	A	B	A	B	A	B
Zero	TMB	3.91	3.71	3.64	3.41	2.39	2.51	3.45	3.42	2.61	2.51
	GN	3.12	3.11	3.13	3.11	2.13	2.20	3.38	3.41	2.63	2.51
	CG	2.89	2.81	2.55	2.51	2.27	1.90	2.94	2.81	2.33	2.20
	E. coli	2.54	2.51	2.34	2.20	2.23	1.90	2.43	2.51	2.28	2.20
2	TMB	4.41	4.01	3.98	3.71	3.26	2.81	3.45	4.31	3.31	3.11
	GN	3.46	3.41	3.41	3.41	2.53	2.51	3.14	3.11	2.56	2.51
	CG	2.93	2.81	3.08	3.11	2.50	2.51	2.95	2.81	2.53	2.51
	E. coli	2.41	2.51	2.25	2.20	2.42	2.51	2.88	2.81	2.33	2.20
4	TMB	3.82	3.61	3.73	3.41	3.10	3.41	4.12	4.01	3.53	3.41
	GN	3.39	3.41	3.31	3.41	2.57	2.51	3.11	3.11	2.51	2.51
	CG	2.82	2.81	3.15	3.11	2.67	2.51	2.75	2.81	2.67	2.83
	E. coli	2.80	2.81	2.25	2.51	2.54	2.20	2.61	2.51	2.59	2.51
6	TMB	3.65	3.71	3.82	3.71	3.82	3.71	3.96	4.01	3.64	3.41
	GN	3.44	3.41	3.14	3.11	2.75	2.51	2.76	2.81	2.47	2.20
	CG	2.61	2.51	3.13	3.11	2.66	2.51	2.75	2.81	2.45	2.20
	E. coli	2.48	2.51	2.63	2.20	2.54	2.20	2.62	2.51	2.18	2.20
8	TMB	3.44®	3.11	3.30®	2.81	3.02	3.11	3.54	3.41	3.21	3.11
	GN	3.26®	3.10	2.92®	2.81	2.59	2.20	2.63	2.51	2.27	2.20
	CG	2.56®	2.51	2.94®	2.81	2.57	2.20	2.51	2.51	2.23	2.20
	E. coli	2.60®	2.51	2.26®	2.20	2.18	1.90	2.13	2.20	2.16	2.20

A : Enumerate by conventional plating methods.
B : Enumerate by four-culture methods.

TMB: Total mesophilic bacteria.
GN : Gram-negative bacteria.
CG : Coliform group.

®: At these point samples were organoleptically rejected.

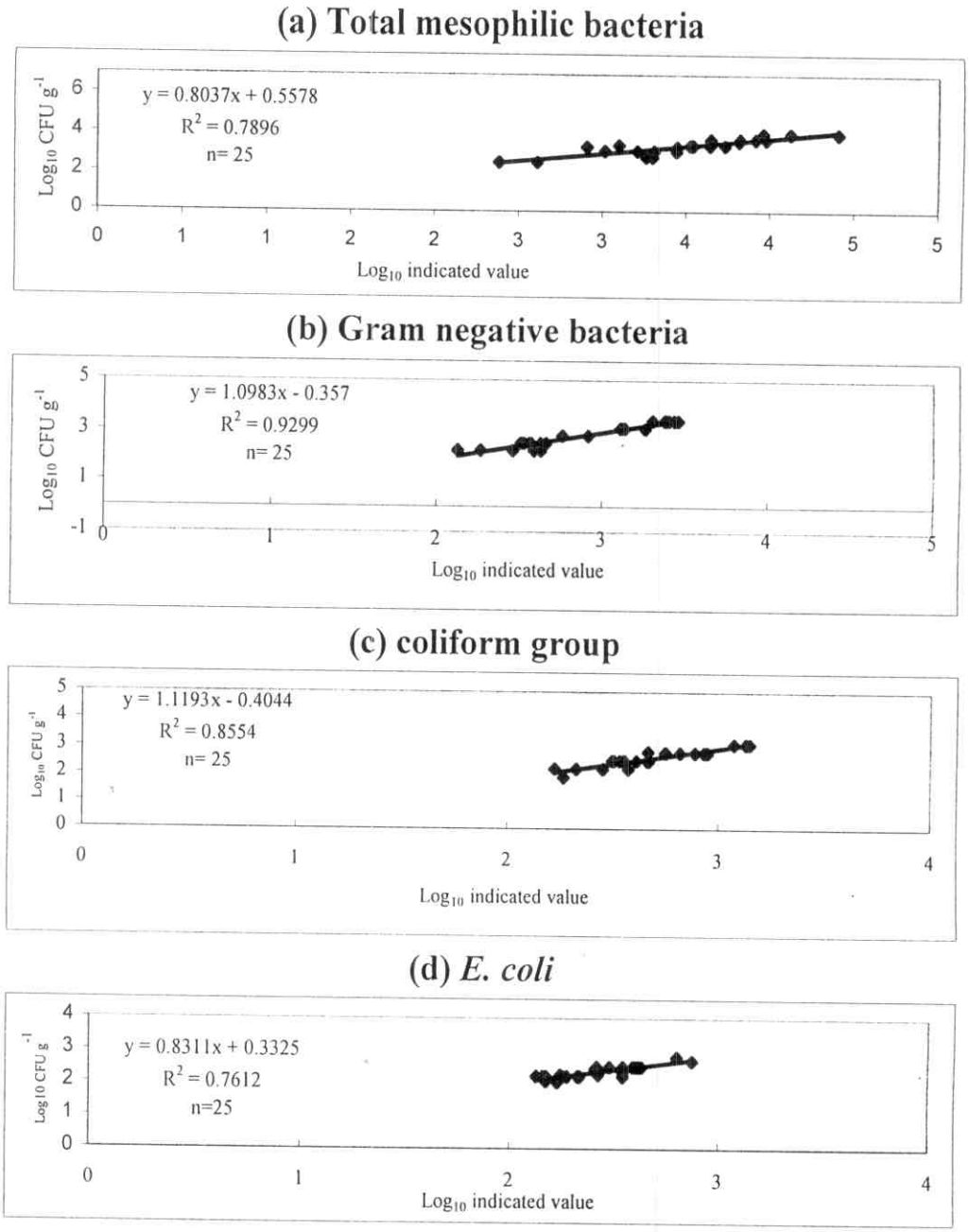


Fig. (6): The linear relationship between the results of four-culture methods (log_{10} of the reciprocal endpoint dilution) and those of the conventional plating methods (log_{10} CFU g-1) for the enumeration of (a) total mesophilic microbial load, (b) gram-negative bacteria, (c) coliform counts and (d) *E. coli* for frozen untreated and treated Bolti fish.

Proteolytic bacteria (PB) was considered in the present investigation, data were recorded in Table (40). At the beginning of frozen storage, the initial PB counts were 3.24, 3.17, 2.51, 290 and 280 log CFU g^{-1} for RB, WWB, TWB, IWWB and ITWB, respectively. With the progression of frozen storage, general decremental trend was shown in all treatments bacteria (Badawy, 1988 and Yasin, 2003).

Lipolytic bacteria (LB) counts of various investigated samples were shown in the same table (Table, 40). At zero time of storage, the initial LB counts were 2.61, 2.66, 2.08, 2.30 and 2.18 log CFU g^{-1} for RB, WWB, TWB, IWWB and ITWB, respectively. During subsequent frozen storage, general decremental trend was shown in all treatments till end of frozen periods (Badawy, 1988 and Yasin, 2003).

Moulds and yeasts (M&Y) were also counted in this investigation, data was recorded in Table (40). The intial M&Y counts were 2.20, 2.13 and 2.52 log CFU g^{-1} for RB, WWB and IWWB, respectively. While, M&Y were not detected in TWB and ITWB treatments at zero time of storage. During subsequent frozen storage, a slowly incremental trend was shown in Y&M counts till reached to 2.91 log CFU g^{-1} for RB samples after 8 months.

On the other hand, *E. coli* O157:H7 ATCC 69373 was counted in inoculated samples to study the effect of dipping solution on retarding our inoculated strain. The initial *E. coli* O157:H7 counts were 3.10 and 2.41 log CFU g^{-1} for IWWB and ITWB, respectively, immediately after dipping for 2 min. in solution contains (SL + TSP + PS + BHA). A decremental trend during storage at $-18\pm1°C$ for 2 Months was shown, then the

Table (40): Logarithmic number of physcrophilic, proteolytic, lipolytic, *E. coli* O157.:H7 ATCC 69373 bacteria and moulds & yeasts for untreated and treated bolti fish during frozen storage at -18+1°C.

Storage period (months)	Micro-organisms	Treatments				
		RB	WWB	TWB	IWWB	ITWB
Zero	LB	2.61	2.66	2.08	2.30	2.18
	PB	3.24	3.17	2.51	2.90	2.80
	M&Y	2.20	2.13	ND	2.52	0.00
	E.C.	---	---	---	3.10	2.41
2	LB	2.85	2.88	2.49	2.75	2.33
	PB	3.29	3.12	2.89	2.60	2.34
	M&Y	2.41	2.16	ND	2.27	ND
	E.C.	---	---	---	3.09	2.39
4	LB	2.71	2.62	2.38	2.89	2.52
	PB	3.94	3.45	2.67	3.91	2.31
	M&Y	2.24	2.47	ND	2.20	0.00
	E.C.	---	---	---	3.21	0.00
6	LB	2.67	2.61	2.61	2.80	2.45
	PB	3.94	3.90	2.61	3.09	2.75
	M&Y	3.12	2.26	ND	3.00	ND
	E.C.	---	---	---	3.22	ND
8	LB	2.64®	2.56®	2.22	2.55	2.29
	PB	3.29®	3.82®	2.57	3.08	2.64
	M&Y	2.91®	ND®	ND	2.73	ND
	E.C.	---	---	---	3.13	ND

PB : Proteolytic bacteria.
LB : Lipolytic bacteria.
ND : Not detected.
® : At these point samples were organoleptically rejected.
E. coli O157:H7 ATCC 69373.
M&Y : Moulds and yeasts.

strain was not detected at 4 months of frozen storage and during subsequent frozen storage till end of storage periods. This mainly due to the antimicrobial effect of dipping solution (SL + TSP + PS + BHA) on retarding the microbial load and mainly more effectiveness against *E. coli* O157:H7 ATCC 69373 which was discussed in part (I). While, the number of *E. coli* O157:H7 ATCC 69373 was unaffected by frozen storage (Bolton *et al.*, 2002).

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

5.1. Sensory evaluation of raw Bolti fish:

The sensory attributes of RB, WWB and TWB samples were studied. Appearance, color of gills, texture, scales consistency, odor, sliminess, eye's lustrous and overall acceptability were considered as freshness indices. The boarder line of acceptability of these sensory was consider as being 4 (Yasin, 1997), data were recorded in Table (41).

Appearance is one of the most important characters for consumption and handling of fish, it was ranged from 8.89 to 8.99. No significant difference ($P>0.05$) between all treatments at zero time storage. While, at 6 and 8 months a significant differences were observed between all treatment at 8 months the RB and WWB samples had a lower score and organoleptically rejected when compared to TWB samples (still accepted).

The color of Bolti fish gills was also organoleptically evaluated throughout the storage period. As seen in the same table, it was around 8.74 before storage. No significant difference ($p>0.05$) was shown between all treatments at zero time.

Table (41): Sensory evaluation of untreated and treated raw Bolti fish during frozen storage at −18±1°C for 8 months (mean ± standard error).

Storage period (months)	RB	WWB	TWB	RB	WWB	TWB
	Appearance			Color of gills		
Zero	8.89±0.25	9.17±0.23	8.99±0.44	8.66±0.32	8.50±0.28	9.06±0.27
6	6.89±0.50	7.72±0.45	8.50±0.17	6.06±0.47	6.22±0.39	7.83±0.34
8	2.39±0.61®	3.61±0.45®	5.56±0.39	1.83±0.72®	3.56±0.61®	4.17±0.44
$LSD^{@}_{(0.05)}$	1.15			1.26		
	Texture			Scales consistency		
Zero	9.06±0.17	9.28±0.19	8.82±0.32	9.06±0.24	9.39±0.28	8.83±0.41
6	6.06±0.41	7.00±0.37	7.94±0.28	6.72±0.51	7.50±0.41	7.89±0.29
8	1.50±0.39®	3.39±0.25®	4.83±0.26	1.39±0.54®	4.17±0.47®	5.50±0.55
$LSD^{@}_{(0.05)}$	0.86			1.19		
	Odor			Sliminess		
Zero	9.06±0.19	8.78±0.21	8.50±0.39	8.25±0.59	8.83±0.34	9.00±0.43
6	4.39±0.49	6.67±0.52	8.06±0.42	4.92±0.58	5.83±0.67	7.00±0.87
8	1.39±0.41®	3.00±0.35®	4.94±0.28	2.50±1.08®	3.08±0.61®	5.00±0.39
$LSD^{@}_{(0.05)}$	1.06			1.85		
	Eye's lustrous			Overall acceptability		
Zero	8.68±0.25	8.83±0.19	8.83±0.42	90.67±2.17	91.42±1.88	87.33±2.59
6	5.61±0.29	5.89±0.33	6.83±0.39	67.50±5.17	72.33±4.60	86.25±2.33
8	1.39±0.45®	3.39±0.46®	4.83±0.39	13.92±3.50®	34.08±2.25®	44.50±1.84
$LSD^{@}_{(0.05)}$	1.02			8.82		

®: At these point samples were organoleptically rejected.

@: To be used to compare between any two time within the same treatment.

A continuously decreased in color of Bolti fish score for all treatments till the end of storage period with the same findings that noticed earlier in appearance. A lowest value was recorded with RB and WWB samples (organoleptically rejected) when compared to TWB which still accepted. These data agree with those of O.D. of gills extract (Table, 35). These results are in agreement with Yasin (1997).

Data in Table (41) of sensory attributes of three treated Bolti fish indicated changes in texture score during the whole storage period that became less than its initial score. No significant difference ($P>0.05$) between all treatments was shown at zero time. Textural toughening of fish muscles is a major problem during frozen storage of some kinds of fish. The formation of hydrogen hydrophobic and disulfide bonds has been suggested as the cause of insolublization of myofibrillar proteins during frozen storage of Greenland halibut (Lim and Haard, 1984). During frozen storage of fish, muscle protein under go denaturation due to the formation of ice crystals, dehydration and increase in salt concentration of changes in pH following removal of water by the ice formation (Suzuki, 1981). How is pretty that TWB had highest texture score at 8 months of frozen storage when compared with other ones.

Scales consistency as indicator of freshness, quality and handling of RB, WWB and TWB during frozen storage at -18±1°C was warranted in the same table. At the beginning of storage, value was in range 8.83 to 9.39. At, 8 months of storage a lowest score was recorded with RB samples when compared to TWB samples. This results were harmony with appearance, texture and sliminess may be due to the microbial growth under

thawing. The improvement scales consistency of TWB may be due to the effect of dipping solution on inhibition the microbial growth.

As seen in Table (41) the average score of different treated Bolti fish odor could be detected during frozen storage at -18±1°C for 8 months. A decreamental trend was shown in all treatments as the time extended. The dipped samples TWB had a higher score than that of WWB when compared to RB samples and significant difference ($p<0.05$) between treated and untreated samples was recorded. These findings are confirmed with the data of TVN and TMA (Table, 31). At 8 months of frozen storage, RB and WWB had a lowest value to causes refusing the samples but at this time TWB was still accepted.

Table (41) shows the average scores of sliminess for different treated Bolti fish during frozen storage. No significant difference ($p>0.05$) between all treatments was recorded at the beginning of storage. The value of sliminess was in range 8.25 to 9.00. A decrease rate and a significant difference ($p<0.05$) was observed in all treatment at 8 months. The RB and WWB samples had a higher decremental trend of sliminess rate than TWB treatment. This mainly due to the effect of dipping solution on retarding the microbial load.

Regarding to eye's lustrous of RB, WWB and TWB samples, the lowest scores given by panelists were noticed at 8 months of storage. In the same time, the TWB scores were higher than those of RB and WWB ones. In addition to, RB and WWB were rejected but TWB not rejected. The TWB no rejection limits (less than 4) are given even at 8 months storage.

These data are coincided with the RI of fish eye fluid (Table, 35).

Overall acceptability was also orgnaoleptically evaluated throughout the storage period. As seen in the same table it was around 89.81% and no significant difference ($p>0.05$) was recorded at the beginning of frozen storage. A decremental trend was shown as the storage time prolonged. Moreover, RB and WWB samples were rejected as the cause of the changes which were found in odor, texture, color of gills and others. However, TWB had a best quality at 8 months then still accepted by panelists.

Analysis of variance of the obtained data (Table, 42) proved that there are significant differences ($P<0.05$) in appearance, texture, odor, eye's lustrous, color of gills, scales consistency, sliminess and overall acceptability between either different treatment or different storage periods.

Multiple comparisons for different treatments (Table, 43) indicated there is significant differences ($P<0.05$) in sensory attributes between RB and WWB, and RB and TWB samples means TWB samples which obtained the highest values of parameters.

Multiple comparisons for different storage periods in (Table, 44) 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.

In conclusion, by studying all the obtained data of chemical, Physical and sensory changes of different treated Bolti fish, we could be safely estimate the expiration periods of the three Bolti fish treatments to be as follows: RB and WWB

Table (42): Analysis of variance for sensory evaluation of untreated and treated raw Bolti fish during storage at -18±1°C for 8 months.

Source of variance	D.F.	F value and level of significancy							
		Appearance	Color of gills	Texture	Scales consistency	Odor	Sliminess	Eye's lustrous	Overall acceptability
Time	2	103.41*	104.28*	284.32*	120.25*	168.13*	36.70*	179.83*	286.61*
Treatment	2	9.80*	11.09*	13.54*	11.17*	8.36*	3.83*	5.64*	17.91*
Time x treatment	4	5.40*	1.35*	10.15*	8.37*	8.43*	2.08^{NS}	4.67*	8.30
Error	99	--	--	--	--	--	--	--	--
Total	107	--	--	--	--	--	--	--	--

D.F.: Degree of freedom. * Significant at 0.05
N.S.: Non significant.

Table (43): Effect of different treatments on sensory evaluation of untreated and treated raw Bolti fish stored at -18±1°C for 8 months.

Treatments @	Sensory evaluation							
	Appearance	Color of gills	Texture	Scales consistency	Odor	Sliminess	Eye's lustrous	Overall acceptability
RB	6.06^{c}	5.52^{b}	5.54^{c}	5.72^{b}	4.95^{c}	5.22^{c}	5.23^{c}	57.36^{c}
WWB	6.83^{b}	6.09^{b}	6.56^{b}	7.02^{a}	6.15^{b}	5.91^{b}	6.04^{b}	65.94^{b}
TWB	7.68^{a}	7.02^{a}	7.20^{a}	7.41^{a}	7.17^{a}	7.00^{a}	6.83^{a}	72.69^{a}
LSD (0.05)	0.66	0.73	0.49	0.69	0.61	1.07	0.59	5.09

@ : As tail of Table (27)
Means with the same latter in the same column are not significant different at (P>0.05).

Table (44): Effect of different storage periods on sensory evaluation of raw Bolti fish stored at -18±1°C for 8 months.

Storage periods (month)	Sensory evaluation							
	Appearance	Color of gills	Texture	Scales consistency	Odor	Sliminess	Eye's lustrous	Overall acceptability
0	9.02^{a}	8.74^{a}	9.05^{a}	9.09^{a}	8.78^{a}	8.69^{a}	8.78^{a}	89.81^{a}
6	7.70^{b}	6.70^{b}	7.00^{b}	7.37^{b}	6.37^{b}	5.92^{b}	6.11^{b}	75.36^{b}
8	3.85^{c}	3.19^{c}	3.24^{c}	3.69^{c}	3.11^{c}	3.53^{c}	3.20^{c}	30.83^{c}
LSD(0.05)	0.66	0.73	0.49	0.69	0.61	1.07	0.59	5.09

Means with the same latter in the same column are not significant different (P>0.05).

samples: not more 6 months and TWB samples: not more 8 months.

According to previously whole fish with additional dipping solution (TWB) extend the shelf-life of Bolti fish 2 months more when compared to either RB and WWB treatments. This is mainly due to the effect of dipping solution (SL + TSP + PS +BHA). These results are in agreement with (Khallaf, 1986 and Yasin, 1997).

5.2. Sensory evaluation of cooked Bolti fish:

Sensory evaluation of untreated and treated Bolti fish samples after thawing in refrigeration at overnight, eviscerated, washed, dipped in spices mixture and flower coated then fried in cotton seed oil were done, data was recorded in Table (45). This is no significant difference ($p>0.05$) between the three investigated treatments for appearance, texture, odor, taste, juiciness and overall acceptability at zero time of frozen storage. As the storage time prolonged a decremental trend was shown in all treatments. The RB and WWB treatments had a lowest score in texture, odor, taste, juiciness and overall acceptability as well as appearance when compared to TWB samples at 6 months storage. The TWB had a highest score in taste at zero time storage and during storage periods, as the cause of dipping solution effect on improvement the taste by SL which gave acid taste then favored by panelists. This may be due to the effect of dipping solution (SL + TSP + PS + BHA) on reducing the loss of protein content, improvement of freshness tests and physical parameters as well as retarding the microbial growth (Khallaf, 1986; Dellenbarger, *et ai.,* 1994; Marshall, 1996; Jahnche and Gooch, 1997; Yasin, 1997 and Ou *et al.*, 2002).

Table (45) : Sensory evaluation of untreated and treated Bolti fish after cooking during frozen storag at –18±1°C for 8 months (means ± standard error).

Storage period (months)	RB	WWB	TWB	RB	WWB	TWB
	Appearance			Taste		
Zero	8.96±0.22	8.79±0.23	9.04±0.21	8.75±0.27	8.75±0.36	9.38±0.18
6	5.79±0.63	6.67±0.22	8.00±0.34	5.25±0.53	5.83±0.40	7.17±0.35
$LSD^{@}_{(0.05)}$	0.97			1.04		
	Texture			Juiciness		
Zero	8.71±0.20	8.67±0.25	9.00±0.28	9.00±0.25	8.46±0.28	8.29±0.32
6	5.13±0.41	5.79±0.39	7.54±0.37	5.42±0.48	5.67±0.41	7.08±0.38
$LSD^{@}_{(0.05)}$	0.92			1.02		
	Odor			Overall acceptability		
Zero	8.71±0.25	8.79±0.28	8.71±0.24	89.83±1.91	90.67±2.13	92.75±1.04
6	5.50±0.48	5.54±0.37	6.83±0.38	52.42±3.42	57.08±3.38	74.25±3.00
$LSD^{@}_{(0.05)}$	0.97			7.43		

@: To be used to compare between any two time within the same treatment.

Analysis of variance for data of sensory evaluation for untreated and treated Bolti fish after cooking (Table, 46) indicated that there are significant differences ($P<0.05$) in appearance, texture, odor, taste, juiciness and overall acceptability between either different treatments or different storage periods.

Multiple comparisons for different treatments (Table, 47) indicated that there are no significant differences ($P>0.05$) in sensory attributes of RB and WWB samples. Significant differences ($P<0.05$) was shown between either TWB and RB or WWB and RB samples. Meanwhile, the TWB treatment had a highest score of sensory evaluation parameters. The high score of characteristics were appeared in TWB treatment. On the other hand, less fish quality was noticed firstly in RB samples then WWB samples.

Multiple comparisons for different storage periods in Table (48) indicated that there are a significant differences ($P<0.05$) on sensory attributes of cooked treated samples at zero and 6 months.

In conclusion, frozen storage of Bolti fish greatly retarded deteriorate changes in fish tissues. The TWB samples with stand frozen storage up to 8 months and their qualities were still acceptable, but RB and WWB samples were rejected at 8 months. Of course, the quality of fish shows downward pattern during storage periods. However, the expiration period of frozen fish at $-18\pm1°C$ in most standards were about 6 months (Egyptian standards, 1991) taking in consideration that the quality of frozen fish is less than the fresh ones. All determined chemical quality parameters either protein breakdown products

Table (46): Analysis of variance for sensory evaluation of untreated and treated cooked Bolti fish stored at -18±1°C for 8 months.

Source of variance	D.F.	F value and level of significancy					
		Appearance	Texture	Odor	Taste	Juiciness	Overall acceptability
Time	1	40.55*	55.07*	76.26*	72.30*	55.84*	152.61*
Treatment	2	6.07*	3.53*	21.9*	6.15*	9.6*	11.33*
Time x treatment	2	6.20*	1.53 NS	2.01 NS	2.65 NS	5.92*	6.94*
Error	66	--	--	--	--	--	--
Total	71	--	--	--	--	--	--

D.F.: degree of freedom. * significant at 0.05
N.S.: Non significant.

Table (47): Effect of different treatments on sensory evaluation of untreated and treated cooked Bolti fish stored at -18±1°C for 8 months.

Treatments @	Sensory evaluation					
	Appearance	Taste	Texture	juiciness	Odor	Overall acceptability
RB	7.38^b	7.00^b	6.92^b	7.02^b	6.61^b	71.13^b
WWB	7.73^b	7.29^b	7.23^b	7.07^b	7.17^b	73.88^b
TWB	8.52^a	8.28^a	8.27^a	7.69^a	7.77^a	83.50^a
LSD (0.05)	0.56	0.60	0.53	0.59	0.56	4.29

@ : As tail of Table (27)
Means with the same latter in the same column are not significant different (P>0.05).

Table (48): Effect of different storage periods on sensory evaluation of cooked Bolti fish stored at -18±1°C for 8 months.

Storage period (months)	Sensory evaluation					
	Appearance	Taste	Texture	juiciness	Odor	Overall acceptability
0	8.93^a	8.96^a	8.79^a	8.58^a	8.74^a	91.08^a
6	6.82^b	6.08^b	6.15^b	6.06^b	5.96^b	61.25^b
LSD (0.05)	0.68	0.73	0.65	0.72	0.69	5.25

Means with the same latter in the same column are not significant different (P>0.05).

(TVN and TMA) or lipid deterioration products (TBA) remain at accepted limits up to 8 months for TWB and 6 months for RB and WWB (If compared to cold storage fish). Similarly, physical quality parameters as well as microbiologically, frozen Bolti fish had good quality up to 6 month and remains acceptable up to the 8^{th} month for TWB of frozen storage. The same previous conclusion could also be confirmed by sensory evaluation.

It could be recommended that the expiration period for dipping in (2.4% SL + 3.2% TSP + 4.8% PS + 240 ppm BHA) frozen Bolti fish is about 8 months, whereas untreated frozen fish could safely be estimated to be about 6 months.