EFFECT OF FERMENTED SOY PASTES (MISO) ON *E.COLI* AND *E. COLI O157:H7* CELLS SURVIVAL DURING CURING OF GRILLED BORI FISH

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Abstract

The aim of this investigation was to study the behavior of inoculated test organisms *E. coli and E. coli O157* in grilled Bori. *E.coli* in uncured grilled Bori fish (control sample A) kept at 30◦C showed that highest numbers (approximately $99x10^8$ & $82x10^9$ cfu/g, respectively) reached after 2 days then completely spoiled. On contrast, in (control sample B) which cured with soy paste only without microbe, there was no microbial growth in all grilled fish kept at (30◦ and 4◦C) until 1, 3 days respectively.

All samples showed a decrease in their numbers of *E. coli* at room temperature after 2 days where paste (no.1) contained the lowest number reached to (90 cfu/g). Also, both the paste (no.3) and (no.4) reached to ($8.4 x10^2$ cfu/g) and ($9.9 x10^2$ cfu/g), respectively. Then, all samples spoiled at the third day of incubation. On the other hand, the samples when storage at 4◦C revealed some decrease for both *E. coli* and *E. coli O157* during all period from incubation time. The viable counts of *E. coli O157* which stored at 30◦C had higher numbers than other strain.

Both the soy paste samples (no. 11) and (no. 19) or (no. 22) scored the highest antimicrobial effect against both pathogens at room temperature after 2 days, and more effect of *E. coli* only when stored at 4°C after 7 days from beginning of the experiment, but cells of *E. coli O157* were slightly resistant. Whereas, data of treated sample (no. 15) scored a moderate decrease in cell counts at refrigerator temperature reaching the minimum approximately ($5x10^3$, $8.5X10^4$ cfu/g) respectively for both pathogens after 7 days from beginning of experiment.

The number of *E. coli* cells in the treated samples (21, 22 and 23) kept at 30◦C decreased sharply and reached to minimum level (9x10 and $6x10^2$ cfu/g) after 2 days for soy paste (no.22). But cells numbers of *E. coli O157* appeared more resistant than the other strain at the same conditions. Also, when the treated samples kept at refrigerator, the test organism decreased slightly in their numbers and reached to the lowest level at the end of period.

From the previous data, it could be concluded that the antimicrobial effect of different types of fermented soy pastes (miso) were higher for *E. coli* than *E. coli O157* indicating that the later strain was the more resistant.

Key words: Fermented soy pastes (miso), inhibition zone, grilled Bori fish, *E. coli* and *E. coli O157:H7.*

INTRODUCTION

Miso is a soybean paste made by the fermentation of soybean, with addition of rice or barely, using a mold *Asprgillus oryzae*. In traditional food, many microbial cultures are used for example *Asprgillus niger, Asprgillus flavus, Saccharomyces cervisia, and Mucor*. Miso is one of the three leading soybean produce in Japan, following (shouy) soy sauce and tofu (soy curd). Soy paste developed in China since 2500 years ago, now there is many several products of soy paste used in foods (Sujaya *et al.*, 2003). It was believed that miso introduced in Japan in the sixth century, the color of miso is various from a light yellow to very blackish brown. Production of traditional or oriental food as solid state fermentation is called (S.S.F) in East but in South Asia (Koji). These have a number of advantages, (i) presence of destriction desirable enzymes such as, amylase, lipase, protease and glucoamylase (ii) destroys of undesirable flavor and odors (iii) synthesize vitamins and antibiotics (Wang and Hesseltine, 1979).

Miso contained 41.5 % moisture, 11.8% protein , 6.1% fat, 28.0 carbohydrate, 2.5% curd fiber, 66.0 % Ca (mg), 2.74% Fe (mg), 3.22% Zn (mg), 0.8% thiamine (mg) , 0.25% riboflavin (USDA, 1986). Hsiang *et al., (*2005) mentioned that the miso is a traditional Japanese food being rich in the isoflavones aglycones, genistein and daidzein. The fermentation process increases availability of isoflavones in soy and convert the isoflavones precursor's genistin and daidzine to their active forms. Whereas, Yamabe *et al.,* (2007) observed that the longer storage period of miso for increasing isoflavones concentrations over three years. It was found that the main food source of isoflavons for the vegetarians were soy milk, soy isolate, soy sauce and soy miso. The content of isoflavones, mainly genistin and daizine, which considered as the most bioavailable phytoesterogen, were estimated with a total amounts of 1200-1400 µmol/100g (equal to daidzine 3040 µg/g or genistin 3996 µg/g). Miso has a health benefit, when feeding rats on soy miso a significant decrease in LDL and total cholaestrol and increasing in female hormones progesterone and estradiol were found (Zidan *et al.,* 2007). Soy miso and fermented soy products have been shown to be rich sources of micronutrients (antioxidant biofactor) with the potential to prevent various human diseases (Minamiyama *et al.,* 2003). Also, Jung (2006) added that the fermented food (miso) was the most effective in preventing cancer by decreasing tumor formation, and increasing natural killer cell activity in spleens.

The previous data confirmed by Masuda *et al.,* (1998) reported the number of *E. coli* to undetectable limit in Jerky kept at 4°C in soy sauce (fermented food) within 9 days compared to *E. coli 0157* in the marinating beef jerky with soy sauce. Soy sauce had antimicrobial effects against possible post processing contamination with the

pathogeneses *Listeria monocytogenes, E. coli 0157, and Salmonella sp.* Marinating by soy sauce may be possibly a means to decrease the microbial flora or post contamination with microorganisms to extend the shelf life of the cooked food (Abu Zaid, 2005).

Del-Rosario and Beuchat (1994) reported that *E. coli is* a gram negative bacterium that generally inhabits the intestinal tract of humans and animals. *Enterohemorrhagic E. coli 0157: H7* recognized as an important food borne pathogen, the consumption of undercooked ground beef sandwiches has been associated with out breaks of hemorrhagic colitis caused by *E. coli 0157: H7.* Food borne illnesses are caused by eating food contaminated with bacteria, parasites, or viruses, if meat have been contaminated during slaughtering or processing. *E. coli 0157:H7* as is a merging cause of food borne illnesses which cause symptoms, including diarrhea, fever, vomiting, abdominal cramps, and dehydration. In some people, especially children, hemolytic uric syndrome (HUS) can result from infection by a particular strain of *E. coli 0157: H7* which contaminate some foods like raw, undercooked meat, poultry and beef that is still pink, and can led to kidney failure and death (NDDIC, 2007). Yokoigawa *et al.,* (1999) concluded that difference between *E. coli & E. coli 0157: H7* and the non pathogenic strains was that incubation in the presence of 12.5% soy sauce (fermented food) allowed the growth of *E. coli 0157: H7* strain but reduced the viable cell numbers of non pathogenic *E. coli* strain.

The objective of this study is to minimize the cross contamination with *E. coli & E. coli 0157* in grilled Bori fish by curing with different types of soy paste (miso), and select the best treatment for application.

MATERIALS AND METHODS

1- Microorganism

- Mould strain:

Aspergillus oryzae (patent from Korea) was cultured on Malt agar media at 25C° for 2-3 days, then propagated on sterilized wheat bran, and kept in refrigerator until use in miso preparation.

- Pathogenic bacteria:

Two pathogenic bacteria used in this study (in grilled fish), The first strain *(Escherichia coli,* 25922) belonged to the American Type Culture Collection (ATCC) was obtained from culture collection of the Microbiology Department, Faculty of Agriculture, Cairo University. The second strain (*Escherichia coli O157)* was purchased from Cairo MIRCN, Faculty of Agriculture, Ain Shams University, Egypt. Cultures were

maintained on nutrient agar slants at 4°C and sub- cultured at 37°C in nutrient broth for 24 hrs before use.

2-**Media**

Malt agar medium was used for the enumeration of fungi, and violet red agar medium was prepared for the determination of *Escherichia coli and Escherichia coli* O157 (ISO, 2006).

3- **Preparation of miso:**

Miso prepared by two steps, first called Koji fermentation involves the spread of 1% wheat bran rich with fungi spores ($\simeq 10^6$ cfu/g) on barely or rice (steamed), the mixture kept it in incubator at 25°C for 3 days . The second step was mixing koji with soybean and blend stored in jars with 16% salt, storage time was maintained for one and two years. Table (1) shows the sample types used and storage period.

Table 1. Soy paste samples used in curing of grilled Bori fish as antimicrobial effect.

Samples	Barely: Soy koji %	Barely : Okara %	Storage time	Samples	Soy : Soy koji %	Storage time	Samples	Rice: Soy koji %	Storage time
1	1:1		year	10	2:1	year	17	3:1	year
2	5:1		year	11	1:1	year	18	2:1	year
3	2:1		year	12	1:5	year	19	1:5	year
4	1:1		2 years	13	5:1	year	20	3:1	year
5		1:1	2 years	14	1:3	year	21	1:3	year
6		1:2	2 years	15	3:1	year	22	5:1	year
7		2:1	2 years	16	100% soy	2 years	23	1:1	year
8	100% barely		2 years				24	100 % rice	2 years
9	100% okara		2 years						

Table (1) show the samples of soy paste used in curing of grilled Bori fish as antimicrobial effect. The samples divided into four parts according to their preparation. The parts consists of barely: soy koji (fermented soy), barely: okara, soy: soy koji and rice: soy koji %.

4- Antimicrobial activity of soy paste

Inhibition Zone:

Paper – Disc plate technique was used to study the effect of soy paste on the bacterial growth by measuring the diameter (mm) of inhibition zone (Loo *et al.,* 1945).

5- Effect of seasoning by miso on the survival of pathogens

Curing of grilled Bori fish (without bone) with soy paste (10%w/w) which was then mixed well in sterilized foil plate with a glass rode for each sample. At the same time

the curing fish inoculated with different types of soy pastes (1,3,4,10,11,14,15,18,19,21,22, and 23) with two test organisms *Escherichia coli & Escherichia coli O157*, (1.0% v/w) separately and then stored for 7 days at 4◦C or 30°C. Samples for bacterial analysis were drawn – up periodically after zero time, 1, 2, 3, 4 and 7 days according to the procedure mentioned by APHA (1971).

RESULT & DISCUSSION

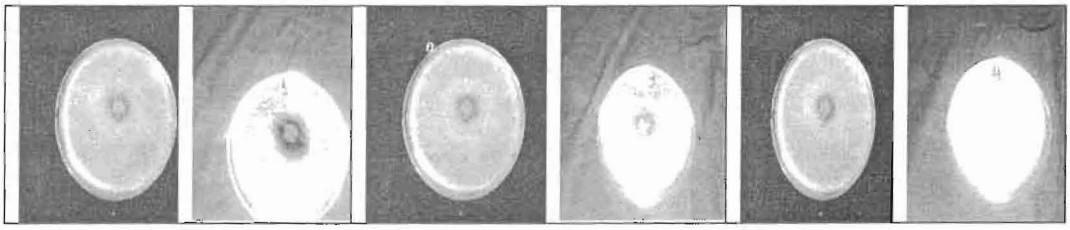
Table 2. Inhibition zone (mm) of different types of soy paste against *E. coli & E. coli O157.*

Soy paste types	Inhibition zone (mm)	
	Escherichia. Coli	Escherichia. coli *O157*
1	24	22
2	12	10
3	23	21
4	21	19
5	10	10
6	11	10
7	12	12
8	13	10
9	14	10
10	24	20
11	26	23
12	20	19
13	15	16
14	20	21
15	21	20
16	11	12
17	18	16
18	25	23
19	33	32
20	16	16
21	27	25
22	22	20
23	28	26
24	10	10

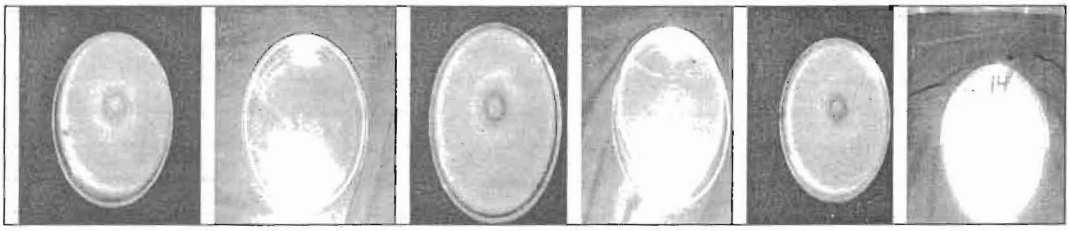
Table (2) show the bacterial inhibition zone (mm) of different types of soy paste against of *E. coli & E. coli O157* which used in marinating of grilled Bori fish. From the previous data, it was revealed that the antimicrobial effect of different types from soy paste were higher for *E. coli* than *E. coli O157: H7*, in other words *E. coli O157: H7* was more resistant than *E. coli* except for samples 7, 20 and 24 showing equal effect, whereas, samples no., 13, 14 and 16 were of lower inhibition zones than *E. coli O157: H7*. From the previous data, it could be noticed that the soy paste samples which consist of rice: soy have the greatest inhibition zone compared to others. The samples numbers were degraded ascending as follow, 19, 23, 21 and 18 after that come barely: soy koji (samples no. 1, 3 and 4) and then soy: soy koji (samples no. 10, 15

and 14) except of sample no. 11 which contains soy: soy koji and had high inhibition zone reached to 26 mm.

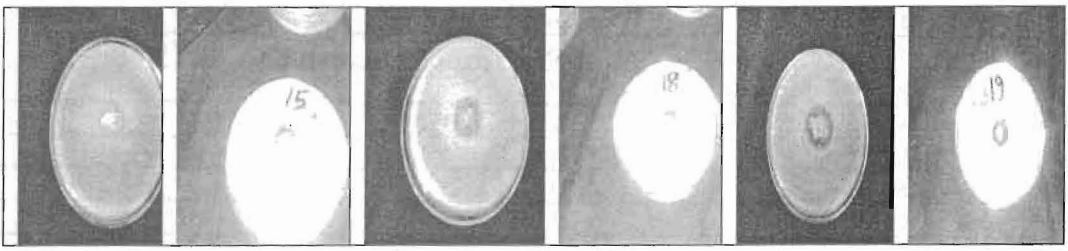
The following images show the bacterial inhibition zones (mm) of some types of soy pastes against both *E. coli and E. coli O157.*



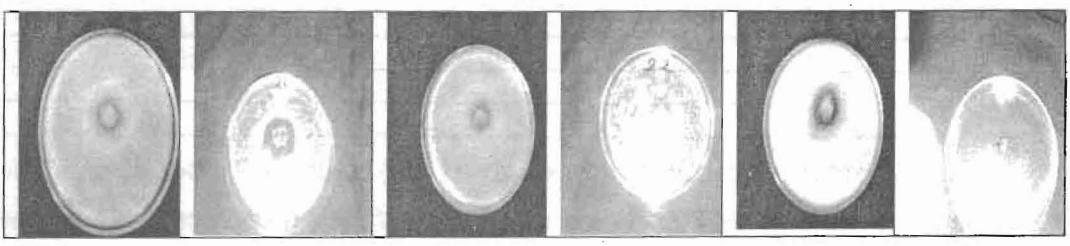
(1) *(1)|* *(3)* *(3)|* *(4)* *(4)|*



(10) *(10)|* *(11)* *(11)|* *(14)* *(14)|*



(15) *(15)|* *(18)* *(18)|* *(19)* *(19)|*



(21) *(21)|* *(22)* *(22)|* *(23)* *(23)|*

Codes between brackets refer to *E. coli.,* codes with dash referred to *E. coli O157.*

Table 3. Effect of curing with soy pastes on the viability of *E. coli & E. coli O157* inoculated in grilled Bori fish kept at 30 & 4◦C as compared with control.

Storage time (Days)	Viable Counts (cfu/g)			
	Escherichia coli kept at		*Escherichia coli O157* kept at	
	30◦C	4 ◦C	30◦C	4 ◦C
Control (microbe only) no. (A)				
Zero time	$34x10^5$	$34x10^5$	$34x10^5$	$34x10^5$
1 day	$40x10^7$	$80x10^5$	$90x10^8$	$80x10^5$
2 days	$99 x10^8$	$65x10^4$	$82x10^9$	$35x10^5$
3 days	Sp	$45x10^4$	Sp	$55x10^5$
7 days	Sp	$35x10^4$	Sp	$35x10^4$
Control (soy paste only) no. (B)				
Zero time	ND	ND	ND	ND
1 day	ND	ND	ND	ND
2 days	$66 x10^2$	ND	$17x10^3$	ND
3 days	Sp	ND	Sp	ND
7 days	Sp	$39 x10^2$	Sp	$15x10^2$
Soy paste no. (1)				
Zero time	$40x10^5$	$40x10^5$	$40x10^5$	$40x10^5$
1 day	$44x10^3$	$26x10^4$	$6x10^4$	$46x10^5$
2 days	$9x10^1$	$12x10^4$	$16x10^3$	$62x10^5$
3 days	Sp	$5X10^3$	Sp	$5X10^4$
7 days	Sp	$65x10^2$	Sp	$15x10^4$
Soy paste no. (3)				
Zero time	$25 x10^5$	$25 x10^5$	$25x10^5$	$25x10^5$
1 day	$59x10^3$	$26x10^4$	$56x10^4$	$76x10^4$
2 days	$84x10^1$	$12x10^4$	$89x10^2$	$32x10^4$
3 days	Sp	$25X10^3$	Sp	$15X10^4$
7 days	Sp	$15x10^3$	Sp	$67x10^3$
Soy paste no. (4)				
Zero time	$64x10^5$	$64x10^5$	$64x10^5$	$64x10^5$
1 day	$40x10^3$	$17x10^4$	$90x10^3$	$38x10^4$
2 days	$99 x10^1$	$25x10^4$	$86x10^2$	$35x10^4$
3 days	Sp	$55x10^3$	Sp	$45x10^4$
7 days	Sp	$35x10^3$	Sp	$55x10^3$

ND: Not Detected Sp: Spoilage of sample

Control Sample (A): Grilled Bori fish + microbe only.

Control Sample (B): Grilled Bori fish + paste only.

Table (3) results show the effect of curing grilled Bori fish with soy paste types 1, 3, and 4 on the number of both pathogens *(E. coli & E. coli O157)* when kept at room temperature (30◦C) and refrigerator (4◦C) for zero, 1, 2, 3 and 7 days as compared with control samples (A) which contains microbe only and (B) which contains soy paste only. The previous data showed the behavior of *E. coli* in control sample when compared that with cells of *E. coli O157,* both had same trend of change and *E. coli O157* samples provided that sample A kept at 30◦C showed highest numbers than *E. coli* cells and reached to ($82x10^9$cfu/g) after 2 days.

All samples showed decrease in their numbers of *E. coli* at room temperature after 2 days where paste (no.1) contains the lowest number reached to (90 cfu/g). Meanwhile, all samples spoilage occurred at the third day of incubation. On the other hand, the samples when stored at 4◦C revealed some decrease for both *E. coli* and *E. coli O157* during all periods of incubation time. The viable counts of *E. coli O157* during storage at 30 ◦C showed higher numbers than the other strain. In this investigation the control sample no. A (grilled Bori fish + *E. coli* microbe) increased rapidly reached to the maximum number (99 $x10^8$ cfu/g) after 2 days of incubation at 30◦C then sample spoiled, while control sample no. B (grilled Bori fish + paste) showed no detected viable cells with in 2 days of incubation at the same temperature. The control sample A kept at 4◦C showed some decrease in numbers from $34x10^5$ to $35x10^4$. In contrast, control sample B at same temperature remained free from any viable counts until 7 days of incubation ($39X10^2$ at 7^{th} day).

It could be noticed that, the antibacterial activity of soy paste (no.1) was stronger than other types against *E. coli* cells at 30◦C whereas, sample (no. 4) was stronger than other types against *E. coli O157* cells. While, at 4◦C there was no difference or slight decrease in the activity. The viable counts of *E. coli O157* were higher than *E.coli* cells for all paste types used in this study.

Table 4. Effect of curing with soy paste on the viability of *E. coli & E. coli O157* inoculated in grilled Bori fish kept at 30 and 4◦C as compared with control.

Storage time (Days)	Viable Counts (cfu/g)			
	Escherichia coli kept at		*Escherichia coli O157* kept at	
	30◦C	4 ◦C	30◦C	4 ◦C
Soy paste no (10)				
Zero time	$90x10^6$	$90x10^6$	$90x10^6$	$90x10^6$
1 day	$84x10^3$	$26x10^6$	$56x10^4$	$20x10^6$
2 days	$59x10^1$	$12x10^6$	$79x10^2$	$52x10^5$
3 days	Sp	$67X10^4$	Sp	$35X10^5$
7 days	Sp	$64x10^4$	Sp	$56x10^4$
Soy paste no (11)				
Zero time	$56x10^6$	$56x10^6$	$56x10^6$	$56x10^6$
1 day	$29x10^3$	$26x10^6$	$46x10^4$	$80x10^6$
2 days	$5X10^1$	$16x10^4$	$39x10^2$	$30x10^5$
3 days	Sp	$44x10^4$	Sp	$45X10^4$
7 days	Sp	$95x10^3$	Sp	$75x10^4$
Soy paste no (14)				
Zero time	$66x10^6$	$66x10^6$	$66x10^6$	$66x10^6$
1 day	$49x10^4$	$8x10^6$	$34x10^4$	$70x10^6$
2 days	$69 x10^1$	$65x10^6$	$56x10^2$	$35x10^6$
3 days	Sp	$15x10^5$	Sp	$55x10^5$
7 days	Sp	$5x10^4$	Sp	$55x10^4$
Soy paste no (15)				
Zero time	$90x10^5$	$86x10^5$	$90x10^5$	$86x10^5$
1 day	$84x10^3$	$18x10^5$	$56x10^4$	$88x10^5$
2 days	$59x10^1$	$15x10^4$	$79x10^2$	$45x10^5$
3 days	Sp	$15x10^4$	Sp	$75x10^4$
7 days	Sp	$5x10^3$	Sp	$85x10^3$
Soy paste no (18)				
Zero time	$40x10^5$	$40x10^5$	$40x10^5$	$40x10^5$
1 day	$44x10^3$	$26x10^4$	$6x10^3$	$46x10^4$
2 days	$79x10^1$	$12x10^4$	$60x10^2$	$62x10^4$
3 days	Sp	$5X10^4$	Sp	$5X10^4$
7 days	Sp	$35x10^3$	Sp	$15x10^4$

ND: Not Detected **Sp:** Spoilage of sample

Table 5. Effect of curing with soy paste on the viability of *E. coli & E. coli O157* inoculated in grilled Bori fish kept at 30 and 4◦C as compared with control.

Storage time (Days)	Viable Counts (cfu/g)			
	Escherichia coli kept at		*Escherichia coli O157* kept at	
	30◦C	4 ◦C	30◦C	4 ◦C
Soy paste no (19)				
Zero time	$34x10^5$	$34x10^5$	$34x10^5$	$34x10^5$
1 day	$40x10^2$	$80x10^5$	$90x10^3$	$80x10^4$
2 days	$4 x10^1$	$65x10^4$	$82x10^1$	$35x10^5$
3 days	Sp	$45x10^4$	Sp	$55x10^5$
7 days	Sp	$35x10^3$	Sp	$35x10^4$
Soy paste no (21)				
Zero time	$84x10^6$	$84x10^6$	$84x10^6$	$84x10^6$
1 day	$40x10^4$	$80x10^5$	$68x10^4$	$77x10^6$
2 days	$54x10^2$	$65x10^5$	$42x10^3$	$55x10^6$
3 days	Sp	$55x10^4$	Sp	$45x10^6$
7 days	Sp	$25x10^3$	Sp	$35x10^5$
Soy paste no (22)				
Zero time	$40x10^5$	$40x10^5$	$40x10^5$	$40x10^5$
1 day	$44x10^4$	$26x10^2$	$60x10^5$	$46x10^3$
2 days	$9x10^1$	$12x10^2$	$6x10^2$	$62x10^2$
3 days	Sp	$35x10^2$	Sp	$5X10^2$
7 days	Sp	$5X10^2$	Sp	$15x10^2$
Soy paste no (23)				
Zero time	$34x10^6$	$34x10^6$	$34x10^6$	$34x10^6$
1 day	$40x10^3$	$80x10^5$	$90x10^4$	$80x10^6$
2 days	$10 x10^1$	$65x10^5$	$42x10^2$	$35x10^5$
3 days	Sp	$45x10^4$	Sp	$55x10^5$
7 days	Sp	$35x10^4$	Sp	$35x10^5$

ND: Not Detected **Sp:** Spoilage of sample

Data of table (4) show the effect of storage temperatures (30 and 4 ◦C) on the viable counts of both microorganism *(E. coli & E. coli O157)* inoculated in grilled Bori fish and cured with soy paste types (no, 10,11,14,15,18). There was some decrease from10^6 to10^4 during all period of experiment in all samples which kept at 4◦C. The counts of *E. coli O157* were found to be more resistant than *E. coli* in treated samples kept at 30◦C. All samples spoiled after 3 days of incubation. It is worth mention to that, marinating of grilled Bori fish with paste lead to a decrease in microbial growth and consequently extended the shelf – life of post cooked fish. The soy past sample (no. 11) scored the most antimicrobial effective against both pathogens at room temperature after 2 days with more effect on *E. coli* only when stored at 4C after 7 days from beginning of the experiment.

Table (5) results show the survival of *E. coli & E. coli O157* inoculated in grilled Bori fish seasoning with soy paste types (19,21,22,23) during storage at different temperatures (30 & 4◦C). The sample (no. 19) revealed a highest effect as antimicrobial activity against two pathogens at room temperature (reached to 40 cfu/g) after 2 days, but cells of *E. coli O157* were more resistant than other strain. From the previous data, it was found that, in all treated samples at 30◦C the number of *E. coli* cells decreased sharply and reached to minimum level (9 $x10^1$ and 6 x 10^2 cfu/g after 2 days for soy paste no.22). According by cells of E. *coli O157* appeared to be more resistant than other strain at the same conditions. Also, when all treated samples kept on refrigerator, the test organism's decreased, in numbers and reached to the lowest level at the end of incubation period.

A according to the previous data related to the behavior of *E. coli & E. coli O157* which inoculated in seasoning grilled fish, based on the inhibition zone values, soy paste is considered unsuitable environment for microbial growth and had inhibiting action. The highest effect of antibacterial activity recorded for soy paste types (no.1, 11, 19 & 22) used in marinated grilled fish in concern to results of inhibition zone. It is known, from experience that soy paste samples differed in storage time are similar in most characteristics such as inhibition zone, color and chemical composition but they differed only according to the materials used in manufacturing. From data of present work, it could be concluded that, soy paste made from barely: soybean and rice: soybean recorded the highest antibacterial effect on pathogens contaminated grilled fish. The storage time for one year revealed higher effect on inhibiting pathogens than two years.

Data in this study agree with that obtained by Hurst and Hughes (1983) who reported that *Staphylococcus aureus*, present in chicken meat slurry supplemented with soy sauce and kept at 37 ^{O}C had been died off. Results related to the behavior of *E. coli* in soy sauce agree with the findings of Masuda *et al.*, (1998) who indicated that the cell numbers of *E. coli* O157:H7 decreased to undetectable level (<30 cfu/ml) within 9 days of incubation in soy sauce at 30^{O}C. Moreover, Yokoigawa *et al,.* (1999) proved that incubation of *E. coli* strains (*O157:H7* and non – pathogenic) in the presence of 12.5% soy sauce allowed the growth of *E. coli (O157:H7),* but the viable cell numbers of non – pathogenic *E. coli* strain reduced.

Calicioglu *et al.,* (2002) achieved inactivation of acid - adapted and non-acid - adapted *Escherichia coli O157:H7* in beef jerky (treated with 86 % soy sauce) during drying process, and reported the destruction of acid and non acid - adapted *Listeria monocytogenes* inoculated in beef jerky during drying process. They found also that the cured beef jerky during processing and the low water activity of the dried product

provide antimicrobial effects against possible post processing contamination with *Listeria monocytogenes, E. coli O157:H* and *Salmonella.*

Onda *et al.,* (2003) explained the causes of antibacterial effect, which drift from miso and other fermented products and isolated lactic acid bacteria produce bacteriocine, and lower pH < 4. For this reasons the pathogens (Enterotoxigenic *E. coli*) could not grow in the acidic environment. Also, Abu-zaid, (2005) agreed with our finding (Tables 2-5) and explained that cells of *Escherichia coli,* and *Staphylococcus aureus,* completely died off in commercial soy sauce after 48 hours of incubation at 30 ^{o}C. Antibacterial activity of peanut soy sauce was stronger than that of wheat or rice soy sauce against *E. coli* and *B. cereus* cells at 30 ^{o}C. At 4 ^{o}C there was no difference in their activity. *Pseudomonas aeruginosa followed by Escherichia* species among all the test organisms had the highest inhibition values with all types of soy sauce (inhibition zone was 25-29 mm). The marinating of all slices (meat products) led to a decrease in microbial growth (from 10^4 to10^2 cfu/g), and consequently led to extension the shelf - life of post frying contamination.

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تأثيرتتبيل السمك البوري بعد الشى بعجائن الصويا المتخمرة علي حيوية خلايا الإيشريشيا كولاي والإيشريشياكولاي (*O157*)

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كان هذا البحث يهدف إلى دراسة تأثير تتبيل أنواع متعددة من عجائن الصويا المتخمرة علي سلوك كل من الايشريشيا كولاي و ايشريشياكولاي (O157) بتلقيح كل منهما على حده في السمك البوري بعد الشي (على أفتراض أنه قد حدث تلوث بهما أثناء الشى) ثم تخزينه علي درجة حرارة الغرفة (٣٠ م°) والثلاجة (٤ م°).

أوضحت النتائج أن العينة الكنترول A (سمك بوري مشوى ملقح ولكن غير متبل) أظهرت ارتفاع واضح في أعداد خلايا كلا من *E. coli* and *E. coli 0157* حتى يصلا للحد الأقصي (٩٩ x ١٠ ^{٩} و ٨٢ x ١٠ ^{٩} خلية/ جم) علي التوالي بعد يومين عند التخزين في حرارة الغرفة يلى ذلك الفساد الكامل. ولكن العينة الكنترول B (سمك بورى مشوى غير ملقح بميكروب ولكن متبل) كانت خالية من أي عدد من البكتريا خلال يوم واحد تخزين على درجة حرارة الغرفة وثلاثة أيام على درجة حرارة الثلاجة .

أظهرت كل العينات المخزنة علي درجة حرارة الغرفة إنخفاض في أعداد خلايا E. coli الملقحة حتى وصلت إلى أقل حد بعد يومين (٩, ، ٨,٤ ، ٩,٩ x ١٠ ^{٢} خلية / جم) علي التوالي مع العينات ١، ٣، ٤ يليها فساد واضح بعد ثلاثة أيام من التخزين بينما خلايا E. coli 0157 المخزنة في نفس درجة الحرارة أظهرت عدد أعلي من الخلايا السابقة . و قد حدث فيها إنخفاض بطئ في كلا من خلايا *E. coli* and *E. coli 0157* و دائما كانت الأعداد فى حالة *E. coli 0157* هى الأكبر حتى نهاية فترة التحضين (٧ أيام / ٤ م°).

كانت العينات رقم ١١ و رقم ١٩ أو رقم ٢٢ هى الأعلى في التأثير المثبط لكلا الخلايا المرضية حتى يومين من الحفظ على درجة حرارة الغرفة بينما أعداد *E. coli* تقل بدرجة أعلي من خلايا *E. coli 0157* الأكثر ثباتاً عند الحفظ على درجة حرارة الثلاجة بينما درجة الحرارة المنخفضة لكل العينات تقلل من أعداد كلا من خلايا *E. coli* و *E. coli 0157* إلي أن تصل لأقل عدد في نهاية فترة التخزين.

يتضح من النتائج السابقة بصفة عامة أن التأثير المثبط لكل أنواع عجائن الصويا المتخمرة أعلي لخلايا *E. coli* عن خلايا *E. coli 0157*.