

## Evaluation of supplements to enhance recovery of thermal induced *Escherichia coli* from fresh chicken meat

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**Abstract:** The recognition of sub lethal stresses on food borne microorganisms and their effect upon growth is very important. *Escherichia coli* was injured by heating at 57° c. Surviving cells from fresh chicken meat were recovered on nonselective tryptic soy agar supplemented with compounds that degrade hydrogen peroxide or block its formation. Various concentrations of the following compounds were tested: yeast extract, sodium pyruvate, n-propyl gallate. Sodium pyruvate and yeast extract, ferrous sulfate and potassium permanganate. Sodium pyruvate and yeast extract added to tryptic soya agar medium, significantly increased ( $P \leq 0.05$ ) the recovering of injured cells. The rest of compound had variable effects on recovery of heat stressed cells but they weren't as efficiency as needed. It is therefore recommended that sodium pyruvate (0.5%) plus yeast extract (0.5%) to be used to supplement in the repair detection procedure.

**Kew words:** *Escherichia coli*, hydrogen peroxide, sodium pyruvate, tryptic soya agar, yeast extract.

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### I. Introduction

Physiologically injured bacteria can result from exposure to heat, freezing, desiccation, or chemicals. Injury may be revealed by a loss of cellular membrane integrity, sensitivity to sodium chloride or bile salts, and degradation of rRNA, (Wesche et al, 2009). Injured cells of pathogenic bacteria may heal repair and regain the ability to cause illness when conditions are favorable for growth (Ukuku et al, 2010). However, selective media used to detect microorganisms in foods samples often do not support the repair of injured cells. Some agents, such as bile salts and crystal violet, may inhibit repair of injured cells, (Hara-Kudo et al, 2000), and as a result of that, injured bacteria may not be detected during routine laboratory analysis. Consumption of insufficiently cooked chicken meat containing *Escherichia coli* has resulted in illness ranging from mild bloody diarrhea to severe and life-threatening hemolytic-uremic syndrome, (Qiu, et al, 2007). Direct-plating media for detecting healthy and injured cells of *E. coli* in raw and processed foods have yet to be developed, although methods for accurate biochemical and immunological properties of *E. coli* isolated from foods have been described. (Gannon et al, 1991 and March and Ratnam. 1989). For the detection of injured bacteria, resuscitation in an enrichment broth is often required. The time required for repair is critical, because multiplication of other microorganisms may occur before the injured cells of the target organism can recover. Several growth media have been shown to be adequate for selective enumeration of *E. coli*, (March and Ratnam. 1989). The purpose of this study was to determine the proficiency of supplements to enhance the recovery of *Escherichia coli* from heat-treated chicken meat on solid agar media.

### II. Materias And Method

*E.coli* isolate was obtained from Biology Department/ College of Science /Baghdad University. Bacteria were grown on tryptic soy agar (TSA) slant and stored at 4°c. Bacteria were activated in non selective tryptic soya broth contained 0.3% yeast extract and incubated at 37°c for 24 hours. Grown cells were transferred to 90 ml of tryptic soy broth (TSB) and incubated at 37°c for 12 h. The absorbance at 1.0 for the exponential phase culture of *E.coli* grown in TSB was fixed using Pye unicum spectrophotometer at 650 nm (Allawi et al, 2012). The cells were centrifuged in refrigerated centrifuge at 7000 x g for 12 minutes and suspended in sterile distilled water to an optical density at 650 nm of 1.0. 25 grams of fresh chicken meat were contaminated with 225 ml of *E. coli* culture grown in TSB to fix a concentration of 10<sup>7</sup> cfu/ml of fresh chicken meat. Treated chicken meat samples were subjected to stress by heating at 57°c for 0,5,10 ,15 and 20 min. Cells were enumerated before and after being stressed by pour plating with tryptic soy agar supplemented with various concentrations of compounds that either have an antioxidant function or degrade hydrogen peroxide (Table 1).

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Sodium pyruvate was added to TSB before being autoclaved (Allawi et al, 2012). Solutions of n-propyl gallate, ferrous sulfate, and potassium permanganate were filter sterilized and added to the media with different concentrations after cooling to 45°C. All plates were incubated for 24 h at 37°C. Relative recovery efficiency was calculated from the colony counts obtained with the supplemented and unsupplemented media.

**Table 1. Source, type, concentration and function of supplements**

Supplement to TSB	Concentration	Function	Time added relative to sterilization	Source
Yeast extract	0.25, 0.5%	Support TSB	Before	Oxide ltd
Sodium pyruvate	0.10, 0.25, 0.5%	Degrade H <sub>2</sub> O <sub>2</sub> Ukuku ,2008(13)	Before	Sigma chemical co
N.Propyl gallate	0.05, 0.1, 0.5%	Anti oxidize Gurtler and Kornacki .2009 (5)	Before	Sigma chemical co
Ferrous sulfate	0.05, 0.1, 0.5%	Scavenge O <sub>2</sub> Allawi et al, 2012 (1)	After	Sigma chemical co
Potassium permanganate	10, 100 mg	Degrade H <sub>2</sub> O <sub>2</sub> Gurtler and Kornacki .2009 (5)	After	Baker Chemical co

### Enumeration of cells

Serial dilutions were made in peptone saline water as needed. Cells were enumerated before and after being heat stressed by pour plating with tryptic soy agar medium.

### Injury repair studies

Stressed cells were serially diluted and plated on 15 ml of media. The plates were incubated at 37°C for 24 hrs, and the colonies were enumerated. Further incubation did not increase the colony count significantly.

### Microbiological analysis

The Percentage of bacterial inhibition was determined according to the following equation: (Blackburn and McCarthy., 2000)

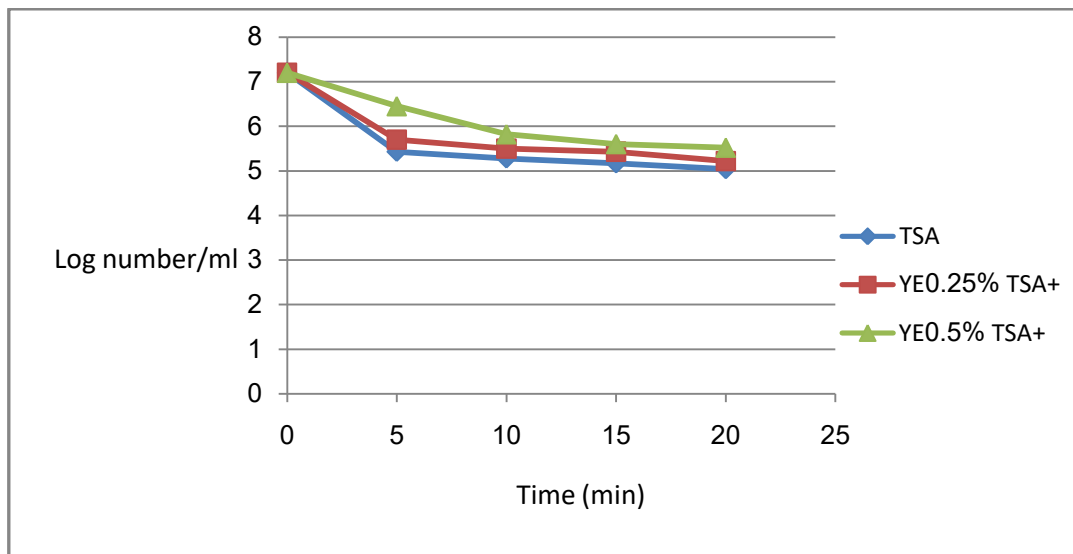
$$\% \text{Inhibition} = \frac{\text{counts in unsupplemented TSA} - \text{counts in supplemented TSA} \times 100}{\text{counts in unsupplemented TSA}}$$

### Statistical Analysis

Bacterial populations were converted to log<sub>10</sub> cfu ml±1 and analyzed statistically by the Statistical Analysis System (SAS) program (SAS. 2004). Significant differences (P≤0.05) between media in the recovery of *Escherichia coli* were determined by the least significant differences test.

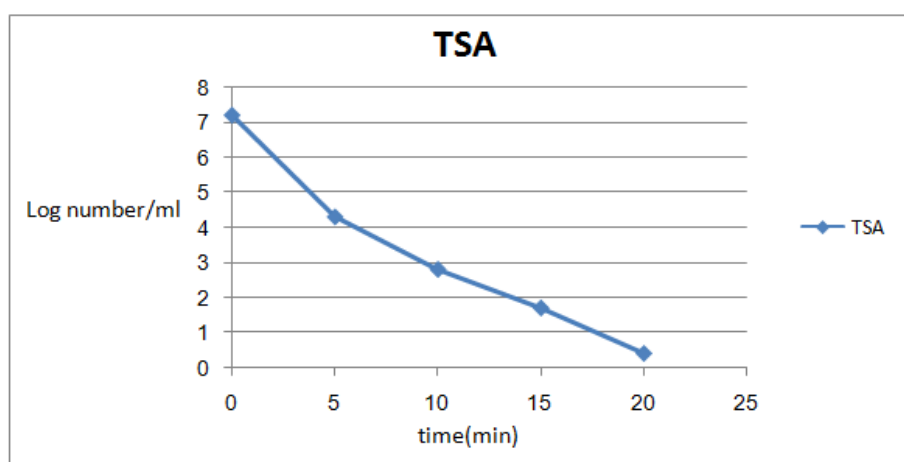
## III. Results and Discussion

Although the frequency of outbreaks of food-borne illness epidemiologically linked to foods containing *E. coli* has increased during the last decade, the efficacy of detection and isolation methods is still inadequate. For selective enumeration, Sorbitol MacConkey agar (SMA) supplemented with 4-methylumbelliferyl-b-D-glucuronide (MUG) is the recommended medium (Conner and Hall. 1994), however, noted that MSMA failed to resuscitate a portion of the viable cells of *E. coli* in frozen chicken and heated roasted beef, respectively. The number of surviving cells of *E. coli* decreased when *E. coli* was grown in TSB, then heated for 0, 5, 10, 15 and 20 minutes (zero time as control) at 57°C and plated on TSA. The difference in counts was 1.8, 1.92, 2.03 and 2.16 log cycles compared with the control at zero time of heating. Survival curves for *E. coli* cells in fresh chicken heated in non selective TSB at 57°C for 5, 10, 15 and 20, min and subsequently plated on TSA are shown in Fig 1. *E. coli* cells in fresh meat chicken stressed by heating were enumerated by pour plating simultaneously with and without supplemented TSA.

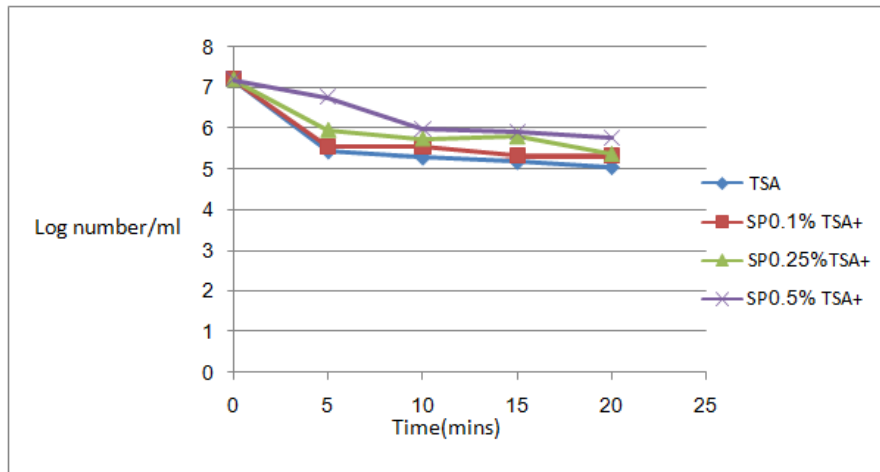


**Fig .1:** Influence of heat on the enumeration of viable cells of *E. coli* in fresh chicken meat

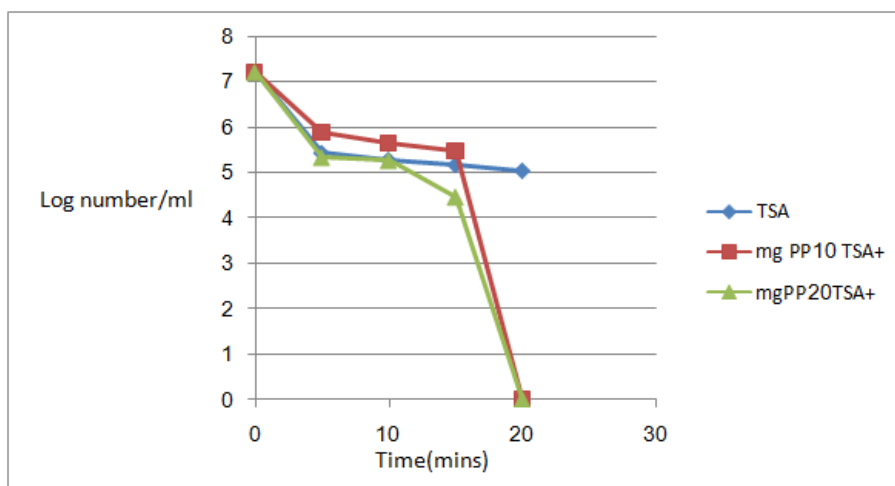
Heat treatment may have caused structural injury, involving damage to the outer membrane of heated cells; as a result of this the peroxide which would be excluded by normal cells can cross the membrane and interact with components within the cytoplasm, (Blackburn and McCarthy, 2000). As shown from results that there were no significant recovery effect by using supplemented TSA with 0.25% and 0.5% yeast extract or 0.1, 0.25 and 0.5% sodium pyruvate or 10 and 100 mg of potassium permanganate respectively( Kobayashi et al. 2005), **Fig 2, 3, 4**, in spite of yeast extract being to be as a nitrogen source, and assumed to be the main factor of bacterial cell wall recovery of heat stressed bacteria, supporting TSA and the effect of sodium pyruvate and potassium permanganate suppose as an enhancer of the recovery of heat stressed cells by degrading a poisonous hydrogen peroxide formed during bacterial bioactivity(Wu, V.C.H. 2008) **Fig 7**, and that might be for its ability to Scavenges O<sub>2</sub> (Allawi et al, 2012). N.propyl gallate wasn't active in recovering heat stressed cells at low concentrations (**Fig 6**) and it was poisonous in high concentration at 0.5% due to its high antioxidative ability (Gurtler, and Kornacki .2009). The synergy between yeast extract and sodium pyruvate, **Fig 5**, had a perfect result in term of recovering heat stressed *E.coli*, especially at 0.5% of yeast extract and sodium pyruvate supplemented to TSA (Ukuku et al, 2008 and Perni et al,2007 ), Addition of sodium pyruvate plus yeast extract supported greater resuscitation than unsupplemented tryptic soy agar (TSA), supplementing with N-propyl gallate, KMnO<sub>4</sub>, ferrous sulfate, yeast extract and sodium pyruvate separately. Thus this treatment was recommended to enhance the recovery of heat stressed *E. coli* fresh chicken meat among all treatments used for that purpose. So it might be applied this modified medium to recovery heat injured gram negative bacteria in different food samples, otherwise food poisoning could occurs afterwards.



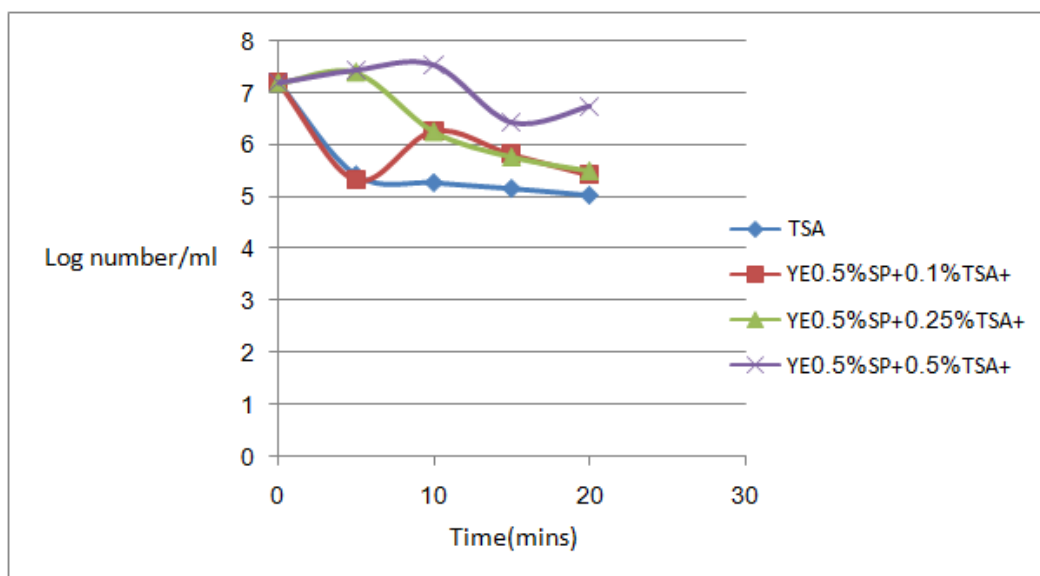
**Fig 2:** Effect of yeast extract supplemented to tryptic soy agar on the enumeration of viable cells of *E. coli* in fresh chicken meat



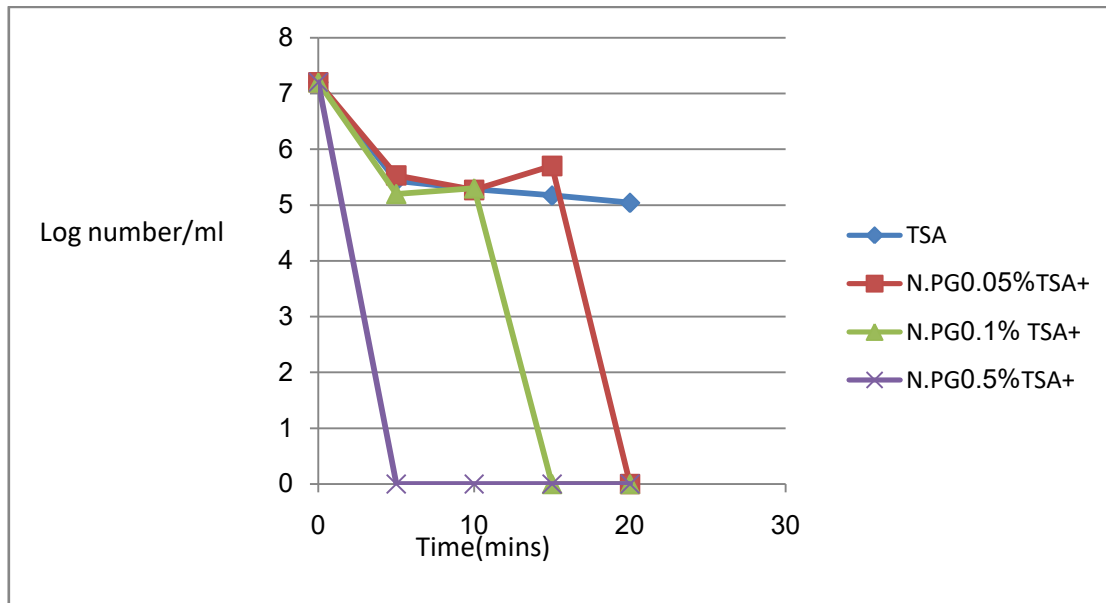
**Fig 3:** Influence of sodium pyruvate supplemented to tryptic soy agar on the enumeration of viable cells of *E. coli* in fresh chicken meat .



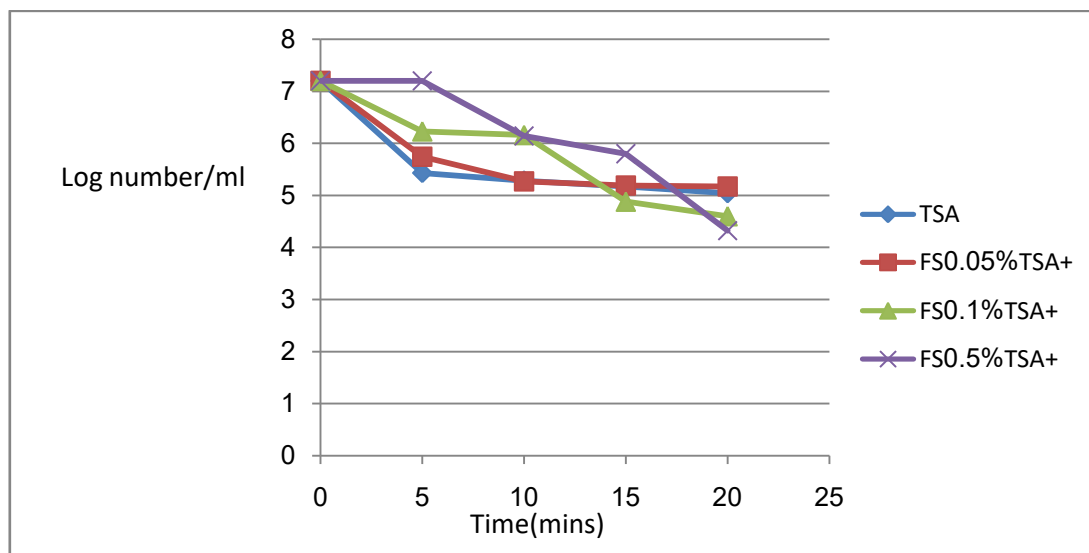
**Fig4:** Effect of potassium permanganate supplemented to tryptic soya agar on the enumeration of viable cells of *E. coli* in fresh chicken meat.



**Fig 5:** Effect of supplements to tryptic soy agar on the enumeration of viable cells of *E. coli* in fresh chicken meat



**Fig 6:** Effect of n-propyl gallate supplemented to tryptic soy agar on the enumeration of viable cells of *E. coli* in fresh chicken meat



**Fig 7:** Effect of ferrous sulfate supplemented to tryptic soya agar on the enumeration of viable cells of *E. coli* in fresh chicken meat

#### IV. Conclusion

This research has established the effect of some agents that either acts as an antioxidant or degrading hydrogen peroxide formed. The results yeast extract and sodium pyruvate assay revealed that this synergetic had the highest recovery rate on heat injured *Escherichia coli*.

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