STUDIES ON THE RECOVERY OF COLD-SHOCKED *Staphylococcus aureus* ISOLATED FROM PROCESSED MARTEDELLA AND PASTRAMI USINF SODIUM PYRUVATE AS GROWTH-PROMOTER

Samia Abdurrahman Abdullah[1] Ahmed Elawad Elfaki, [2*]and S. M. ElSanousi[3]

[1] Sudanese Standards and Metrology Organization
[2] Department of Food Science and Technology, College of Agricultural Studies, Sudan University of Science and Technology, Khartoum North, Sudan
[3] Department of Microbiology, Faculty of Veterinary Science University of Khartoum

المستخلص

الهدف الرئيسي من هذا البحث كان لدراسة معالجة البكتيريا العنقودية الذهبية الموجودة في اللحوم المصنعة والمجروحة بالصدمة الباردة، إستخدمت بيروفات الصوديوم بإضافتها لوسط بيرد باركر لمعالجة البكتيريا المجروحة بالصدمة الباردة. تم جمع ثلاثون عينة من المصانع والبقالات بولاية الخرطوم، وتم عزل البكتيريا المكورة العنقودية الذهبية من اللحم المجمد (المارتديلا – الباسطرما) وتم تعريضها لدرجات حرارة مختلفة (−20ْم ، −30ْم ، −40ْم). أُضيفت بيروفات الصوديوم بتراكيز مختلفة (0.5% ، 1.0% و 1.5%) لوسط بيرد باركر. أظهرت الدراسة أنه عند درجة حرارة −20ْم كانت المعالجة للبكتيريا المجروحة 82.35% ، 85.71% و 75.82% على التوالي وعند درجة حرارة −30ْم كانت المعالجة بنسبة 86.96% ، 68.18% و 0.40% على التوالي. وعند درجة حرارة −40ْم كانت المعالجة بنسبة 24.69% ، 43.48% و 47.62%. من هذا نخلص إلى أن إضافة بيروفات الصوديوم بتركيز (0.5% ، 1.0% و 1.5%) عند درجة حرارة −20ْم وتركيز(0.5%) عند درجة حرارة −30ْم له أثر فعال في استرجاع ومعالجة أعداد كبيرة من البكتيريا العنقودية الذهبية المجروحة بالصدمة الباردة.كذلك نجد ان معدل إنخفاض الإسترجاع والمعالجة للبكتيريا يتناسب طرديا مع معدل إنخفاض درجة الحرارة.

Abstract

The main objective of this research was to study the recovery of cold- shocked injured *Staphylococcus aureus* isolated from contaminated processed meat. Sodium pyruvate was used as a supplement and added to Baird Parker's medium for the recovery of the injured *Staphylococcus aureus* by cold shock. Thirty samples of Martedella and Pastrami were collected from different manufactures

* Corresponding Author: ahmad.elfaki@yahoo.com

and supermarkets at Khartoum state (Omdurman, Bahry). A full loop of *Staphylococcus aureus* was added to a test tube containing peptone water. The suspension was incubated at 37°C for 24hrs. From the serial dilutions 0.1ml was inoculated on Baird Parker's medium prepared with different concentrations of sodium pyruvate(0.5%, 1.0%and1.5%) and incubated at 37 °C for 48 hrs. The same procedure was followed for shocked bacteria at -20°C,-30 °C and -40 °C. The results of the sodium pyruvate concentrations treatments showed that at -20 °C the recovery of the injured bacteria was 82.35%, 85.71%and 75.82%, respectively. While at -30 °C the recovery of the injured bacteria was 86.96%, 68.18%and 0.40%, respectively. Whereas at

-40°C the recovery was 24.69%, 43.48%and 47.62%, respectively. It is concluded that the addition of sodium pyruvate in concentrations (0.5%, 1% and1.5%) to recover shocked bacteria at temperature-20°C and the concentration (0.5%) at temperature-30°C had a beneficial recovery of injured cells of *Staphylococcus aureus* . The recovery rates decrease directly proportional with the temperature decrease.

Keywords: *Staphylococcus aureus* bacteria, sodium pyruvate, cold- shock.

Introduction

Meat is an important human diet throughout the world. It contains many of the nutrients that are required by man to carry out his own body function. Therefore, it is necessary to insure that meat for human consumption is wholesome, safe and of high keeping quality (Nickerson and Louis, 1982).

Meat is highly perishable food and is an excellent medium for growth of wide types of microorganisms. Some of which induce spoilage while others cause disease and death (Thornton, 1969). Meat processing differentiates between those processes that enter into the preservation and manufacture of the meat products, and those that alter the form of the fresh meat in preparation for consumption (Pearson and Gillett, 1999). Sun drying and natural freezing initiated the bases of meat preservation world- wide. The major contamination of meat during processing is from skin or hides (Robert, *et al*., 1980). Bacteria sense the change in temperature mainly at level of cell membrane, nucleic acid and ribosomes (Phadtare, 2000). Most of our knowledge about the cold shock response of bacteria is obtained from experiments in which cells are fast cold shocked and left at low temperatures until they recover. In the environment, however, cells are exposed to changes in temperature on a regular diurnal or seasonal basis, as well as on less predictable time scales. Temperature fluctuations can dramatically affect the physiology of bacterial cultures (Morozov, 2007)

El Sanousi (1975) was the first to solve the riddle of the phoenix phenomenon and termed it "cold-shock". He proved beyond doubt that the cold-shock phenomenon, previously, wrongly termed phoenix phenomenon ,that happened in Gram –negative aerobic bacteria, can also affect Gram-positive anaerobic bacteria i.e. *Clostridium perfringens per se*, He also studied, for the first time the sensitivity of the different phases of growth to cold-shock and reported that the temperature of the diluent was responsible for this rapid and extensive loss of viability which could be prevented by using diluents equilibrated at the same temperature as that at which cells were grown. Kraft (1992) recorded that the responses of organisms to freezing vary considerably; some are killed and some survive and may remain viable to different degrees during frozen storage and after thawing. Injured cells are those cells which can form colonies on enriched medium, but not on stressing media (Clark and Ordal, 1969).The addition of sodium pyruvate to the selective media was more effective and employed as important way to recover injured cells. (Layla, 1987). The aim of this research was to study the recovery of cold -shocked *Staphylococcus aureus* isolated from processed Martedella and Pastrami using sodium pyruvate as growth-promoter.

Materials and Methods

Bacteria: The bacteria used was *Staphylococcus aureus* isolated from thirty samples of Martedella and Pastrami collected, from different factories and supermarkets in Khartoum state(Omdurman-Bahry) and identified according to methods of Harrigan and Mc Cance(1998) and ISO6888-1,(1999).

Media: Baird Parker's medium, Confirmation medium :(Brain-heart infusion broth, rabbit plasma), Selective enrichment medium (peptone water – OXOID/2013) and non – selective medium nutrient agar (Scharlau/2013) were used. Recovery media: Selective medium of Baird Parker's (OXOID/2013) and sodium pyruvate with different concentrations i.e. (0.5%, 1%and1.5%) were used for the enumeration of *Staphylococcus aureus*

The procedures:

Firstly before shock: - Different concentrations of sodium pyruvate (0.5%, 1%and1.5%) were added aseptically to Baird Parker's medium after sterilization and cooling to 45 – 50°C. The medium was poured onto sterile Petri dishes about 18-20 ml in each and allowed to solidity. A full loop of *Staphylococcus aureus* was added to a test tube containing peptone water. The suspension was incubated at 37°C for 24h.One ml of the suspension was added to 9 ml normal saline and serial dilutions of 10-fold were prepared .From the serial dilutions10^5and10^6 0.1ml was inoculated on Baird Parker's medium prepared with different concentrations of the sodium pyruvate (0.5%, 1%and1.5%) and incubated at 37 °C for 48 hrs.

Secondly after shock:- Serial dilutions as mentioned above10^5and10^6 were shocked for one hr at -20°C.From above serial dilutions of the shocked cell 0.1 ml was inculcated on Baird Parker's medium prepared with different concentrations (0.5%, 1%and1.5%) of sodium pyruvate and incubated at 37 °C for 48 hrs. The same procedure was followed for shocked bacteria at -30 °C and -40 °C.

Results

The effect of addition of sodium pyruvate on growth of *Staphylococcus aureus* on Baird Parker's medium before and after shocking at -20 °C showed that at -20 °C the recovery of the injured bacteria was 82.35%, 85.71%and 75.82%, respectively as shown in Table (1).

Table (1): The effect of addition of sodium pyruvate on growth of *Staphylococcus aureus* on Baird Parker's medium, before and after cold shock at -20 ^{O}C

Bacterial count treatment (cfu/g)	Baird Parker's medium	Baird Parker's medium +Sodium pyruvate concentration		
		0.5%	1%	1.5%
Before cold shock	8.70×10^6	6.80×10^6	6.30×10^6	9.10×10^6
After cold shock	5.60×10^6	5.60×10^6	5.40×10^6	6.90×10^6
Reduction in viable bacterial count (%)	35.64	17.65	14.30	24.18
Recovery (%)	64.37	82.35	85.71	75.82

The effect of addition of sodium pyruvate on growth of *Staphylococcus aureus* on Baird Parker's medium before and after shocking at -30 °C revealed that at -30 °C the recovery of the injured bacteria was 86.96%, 68.18%and 0.40%, respectively as shown in Table (2).

Table (2): The effect of addition of sodium pyruvate on growth of *Staphylococcus aureus* on Baird Parker's medium, before and after cold shock at -30 ^{O}C

Bacterial count treatment (cfu/g)	Baird Parker's medium	Baird Parker's medium +Sodium pyruvate concentration		
		0.5%	1%	1.5%
Before cold shock	3.60×10^6	4.60×10^6	4.40×10^6	5.00×10^6
After cold shock	1.0×10^5	4.00×10^5	3.00×10^5	2.00×10^5
Reduction in viable bacterial count (%)	75.24	13.05	32.81	0.60
Recovery (%)	24.78	86.96	68.18	0.40

The effect of addition of sodium pyruvate on growth of *Staphylococcus aureus* on Baird Parker's medium before and after shocking at -40 °C substantiated that at -40 °C the recovery of the injured bacteria was 24.69%, 43.48%and 47.62%, respectively as shown in Table (3).

Table (3): The effect of addition of sodium pyruvate on growth of *Staphylococcus aureus* on Baird Parker's medium, before and after cold shock at -40 ^{O}C

Bacterial count treatment (cfu/g)	Baird Parker's medium	Baird Parker's medium +Sodium pyruvate concentration		
		0.5%	1%	1.5%
Before cold shock	6.10×10^6	8.10×10^6	6.90×10^6	6.30×10^6
After cold shock	3.00×10^5	2.00×10^5	3.00×10^5	3.00×10^5
Reduction in viable bacterial count (%)	50.82	75.33	56.52	52.39
Recovery (%)	49.18	24.69	43.48	47.62

Discussion

The magnitude of the cold-shock response is dependent on both the rate of temperature decrease and the magnitude of change in relation to thermal tolerance limits (Van den Burg *et al.* , 2005). Bacteria sense the change in temperature mainly at level of cell membrane, nucleic acid and ribosomes (Phadtare, *et. al* 2000), Bacteria express a well-defined set of proteins after a rapid decrease in temperature, which differ from those expressed under heat shock conditions. Cold shock proteins may include helicases, nucleases, and ribosome-associated components that interact with DNA and RNA. Processes such as cold signal perception, membrane adaptation, and the modification of the translation apparatus are involved (Weber and Marahiel, 2003) .The mechanisms of sodium pyruvate on cells is to give energy and the additions of compounds such as catalase or pyruvate to the media increases the chance of recovering stressed- bacteria (McDonald *et al.*, 1983). Our results of bacterial loss after shocking at-20°C, -30 °C and -40 °C for one hr simulate those of Busta (1973) who reported that a large number of bacteria is susceptible to the effect of cold shock which leads to the injury of these bacteria, also the rate of death and injury in bacteria were greatly influenced by the composition of the suspending media during freezing storage (Ray, 1989).

These findings are in agreement with those obtained by Baird Parker and Danvenport (1965) who reported that incorporation of pyruvate to selective media enhanced the recovery of *Staphylococcus aureus* .Also Martin *et.al* (1976) noted improved recovery of injured cells of *Staphylococcus aureus, Pseudomonas fluorescence, Salmonella typhimurium and E. coli* when the selective media of these microorganisms were supplemented with either catalase or sodium pyruvate .Our results substantiated those of Layla (1987) who studied the effect of the curing agents on the injured cells of *E.coli, Staphylococcus aureus , C. Perfringens and Ps. aeruginosa* she proved that these agents act in

similar manner as the selective agents and they have an adverse effect on the injured cells .

Conclusion

The addition of sodium pyruvate in concentrations of 0.5%, 1% and1.5% on bacteria shocked at temperature-20 ^{O}C and the concentration of 0.5% on bacteria shocked at temperature-30 ^{O}C had a recovery effect on the injured cells of *Staphylococcus aureus*

References

Baird Parker, A.C. and Davenport E **(1965)** the effect of recovery medium on the isolation of *Staphylococcus aureus* after heat treatment and after storage of frozen or dried cells .*J .appl*. *Bacteriol* , 28:390- 402.

Busta,F.F**(1973).**Practical implication of injured microorganisms in food .j. milk food technal. 39:138-145.

Clark, C .W. and Ordal, Z.J. **(1969).** Thermal injury and recovery of *Salmonella typhimurium* and its effect on enumeration procedures APPI. Microbial; 18:332-336.

Elsanousi, S.M**. (1975).**Oxidation reduction potential of *Clostridium Perfringens*: Ph.D. Theis Bristol University, England.

Harrigan, W. F. and McCance, M. E. **(1998).** Laboratory methods in food and Dairy Microbiology. Academic Press Inc. London.

ISO 6888-1**(1999)** Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of coagulase –positive staphylococci (*Staphylococcus aureus* and other spices)-Part 1: Technique including conformation of colonies

Kraft, A.A **(1992).**Refrigeration and freezing in psychotropic bacteria in food. CRC Press, inc.London.241.

Layla **(1987).**Studies on the properties of cold shock- bacteria isolated from processed meat. M. V. Sc. thesis, Faculty of Veterinary Science, U of K.

Martin, S. E.F lowers, R.S. and Ordal, Z.J. **(1976)** .Catalase, its effect on microbial enumeration .Appl.Environ.Microbial.32: 731-734.

Mc Donlad, L.C., Cameron, R.H. and Ray**. (1983).**Enhanced recovery of injured Escherichia Coli by compounds that degarde hydrogen peroxide or blocks its formation.Appl.Environ.Microbiol.45: 360-365.

Morozov II, Petin VG **.(2007)** : The influence of cyclic heating and cooling on Escherichia coli survival. Microbiology 76:172-176. PubMed Abstract

Nickerson, J.T.R. and Louis .J.R. **(1982).**Elementary Food Science, second edition states of America Eastern Graphics, I.n.c.

Person.A.M.and Gillett.T.A **(1999).** Processed meat 3rd.ed, achapman and hall food science book, an Aspen publication, Aspen publisher, Inc. Gaithersburg, Maryland.

Phadtare ,S., Alsina , J. ,and Inouye, M.(**2000).**Cold shock response and cold shock proteins.Curr.Opin.Microbiol.2:175-180.

Phadtare, S., Yamanaka, K, and Inouye**,** M **(2000).** The Cold Shock Response. In: The Bacterial Stress Responses G. Storz, and R. Hengge-Aronis, ed. ASM Press, Washington DC. P. 33-45

Ray, B. **(1989) .**Fundamental food microbiology, 3rd edition CRC press, Inc .London .pp: 109.

Roberts, T. A., Baird parker, A. C. and Tompkin, R. B. **(1980).**International Commission On Microbiological Specification for Foods *Salmonella.*217_264 in Microorganism in Foods 5.Characteristics of Microbial Pathogens. Published by Blackie Academic& professional, London.

Thornton, H. **(1969).**Text book of meat inspection, 5thed.Bailliere Tindal, London.

Van den Burg, E. H., Peeters, R. R., Verhoye, M., Meek, J., Flik, G. and Van der Linden,A. **(2005)**. Brain responses to ambient temperature fluctuations in fish: reduction of blood volume and initiation of a whole-body stress response.Journal of Neuro-physiology 93, 2849–2855.

Weber., M H; Marahiel MA **(2003).** Bacterial cold shock responses. Science Progress 86: 9–75