Original Research Article

Effect of pH and Sugar level on Heat Resistance of *Escherichia Coli* in Sweet Orange Juice (*Citrius Sinensis*).

Abstract

The effect of pH and sugar levels on the microbiological properties of sweet orange juice was evaluated. Microbial analysis of the treated Orange juice (*Citrus Sinensis*) were determined using standard method. The standard method of was used. The microbial load of the produce reduced as the concentration of the derived preservatives increased. Both pH and sugar level used had inhibitive effect on the test organism. The result revealed that the use of pH and sugar level as hurdles should be encouraged in processing food products.

Key word: pH, Sugar, Hurdle Technology, Orange Juice.

1.0 INTRODUCTION

is a scale used to specify how acidic or basic a solution is. Acidic solutions have lower pH, while basic solutions have a higher pH.. The pH measurement is used in a wide variety of applications: agriculture, waste water, treatment, industrial processes, environmental monitoring and in research and development. It is the negative of the base 10 logarithm of the activity of the hydrogen ion [1, 2].

Sugar is the generic name for sweet tasting soluble carbohydrate, many of which are used in food. The various types of sugar are derived from different sources. Simple sugars are called monosaccharide and include glucose [dextrose], fructose and galactose. 'Table sugar" or granulated sugar refers to sucrose a disaccharides of glucose and fructose. In the body, sucrose is hydrolysed into fructose and glucose. Sugar are found in the tissue of most plant but sucrose is especially concentrated in sugar cane and sugar beet, making them ideal for efficient commercial extraction to make refined sugar [3].

The microbial safety of orange juice is based on a combination of several empirically applied preservative hurdles, and more recently on knowing how to employ hurdle technology. Deliberate and intelligent application of hurdle technology allows a gentle but efficient preservation of food is advancing worldwide. Hurdles are applicable not only to microbiological quality, but also other quality aspect of foods, although this area of knowledge has been much less explored than the microbiological aspects [4].

Orange juice refers to the juice of oranges. It is made by extraction from fresh fruits by desiccation and subsequent reconstitution of dried juice or by concentration of the juice and subsequent addition of water to the

concentrate [5]. Orange comes in several varieties including blood range, navel oranges, valencia oranges, clementine and tangerine.

Gargia-Garcia et al *et al.* (6) investigated the effect of hurdle technology applied to pricky pear beverages for inhibiting *S. Cerevisiae* and *EscherichiaColi.* Their findings reveals that the addition of Sodium benzoate and Potassium sorbate had a signesgistic effect on the organisms which is desirable to maintain pricky pear beverages for 21 days/25^{0}C. Further works by Ohlsson and Bengtsson [7] on vegetable fermentation indicated that the desired product quality and microbial stability were achieved by a combination of factors such as salt and acidifications. According to ohlsson and Bengtsson [8] hurdle technology provides a framework for combining a number of milder preservation techniques to achieve an enhanced level of products safety and stability and that hurdle technology is increasingly used for food design in industrialized and developing countries for optimizing fruits juices. Hurdle technology is the process of employing the intelligent combination of different hurdles or preservation techniques to achieve multi-target, mild but reliable preservation effects Velugoti [9] and Rahman [10]. The aim of this work was to determine the heat resistance of *Escherichia coli* in Orange juices as influenced by pH and Sugar level.

2.0 MATERIALS AND METHODS.

2.1 Source of Raw Material.

Citric acid (Foodchem brand) used was obtained from the Department of Food Science and Technology, Federal University of Agriculture, Makurdi, Nigeria. Sugar and Oranges was obtained from Railway Market, Makurdi. Graphs 1-6 reflects the logarithms of E.coli survivors at respective time and temperature 1-4mins and 60^{0}C-80^{0}C respectively. The D value or decimal reduction time is the time (or dose) required at a given condition or set of condition to achieve a log reduction of 90 % (1 log) of relevant microorganisms. The D-Values for this study are reflected in the graph 7-12 below.

2.2 Processing Method

2.3 Processing of Orange Juice

The modified method of Aurelie *et al.* [11] was used for orange juice production as shown in fig 1. The oranges were sorted by hand, cooled, and peeled with knife. It was then washed with water and the juice was extracted using the juice extractor and filtered using a Muslin Cloth.

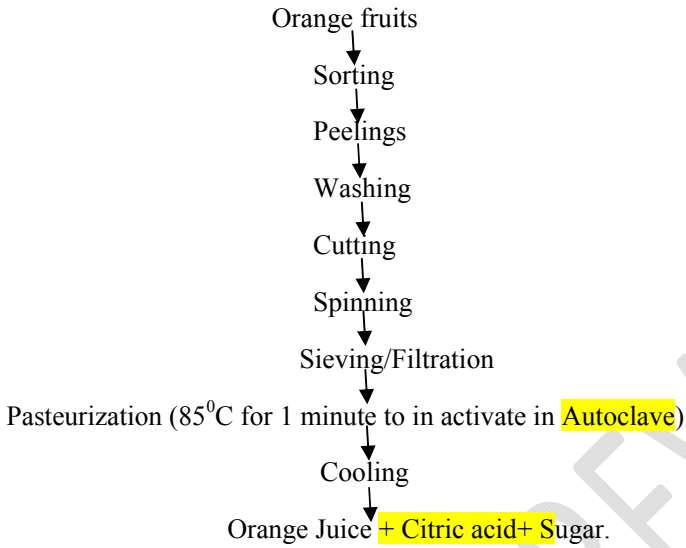

Fig 1: Production flow chart for Orange Juice.

Source: Aurelie *et al.* [11].

2.3.1 Microbiological Analysis of the orange juice.

The method of Prescott [12] was used to determine the total viable count. The orange juice was seeded with *Escherichia coli* to determine microbial counts with the help of nutrient agar. A wire loop was used to extract the microorganisms into a test tube containing 10ml peptone water which was immediately covered with cotton wool. The samples were kept for 24hours, at this time the microorganisms were evenly distributed among the peptone water. Pour plate method was used. 3ml of the diluents was pour plated into Petri-dishes and the number of colonies counted using the formula. TVC (CU/g) = (Number of colonies x original concentration)/ (Dilution factor x volume of inoculums). CFU=Colony Forming Unit

2.4 Statistical analysis.

Data obtained were subjected to Analysis of Variance (ANOVA) followed by Duncan's new multiple range test (DNMRT) to compare treatment means. Statistical significance was accepted at [$p \geq 0.05$] Steele and Torrie. [13].

3.0 RESULTS AND DISCUSSION

Effects of chemical preservatives on the growth of *Escherichia coli* in orange juice are presented in table 1-6 at different level of temperatures [60^{0}C, 70^{0}C, 75^{0}C and 80^{0}C] time [1.4 mins] in water bath respectively. As the concentration of the chemical preservatives increased, a remarkable decrease in the bacterial biomass was

recorded. This agrees with the findings of [6]. In this study it was observed that concentration and combination of preservative alone reduced growth of the microorganism but was unable to prevent growth of the test organism (14). The application of the heat reduced the population of the microorganisms and weakend their ability to germinate. The introduction heat was vital as the combination of both chemical preservatives of pH 4.0, 5.5% and 0,2, 4% sugar level respectively and heating for 1-4 mins in water bath reduced growths of the orange juice. The heat may have affected the DNA while the hostile environment, which include the presence of chemical preservatives, as another hurdle was difficult for the organism to overcome as reported by [14]. At a higher temperatures and higher time there was no significant growth at sample 6 recorded at four minutes at 80^0C [4] as presented in figure six [6]. The growths generally in a strong acidic medium of pH 4.0 were less than growth in a weakly acidic medium of pH 5.5, this is because microorganisms survive less in strong acidic medium and possibly due to the fact that citrus fruits are acidic plus the high sugar content of about 20-25% present naturally plus the 4% and 2% sugar added which bind the water in the orange juice together. Making it difficult for microbial growth and multiplication than a weakly acidic medium.

Microbial result revealed Sample A and B have the highest growth, growth in sample C were not too different from sample D, but less compare to sample D, low counts were obtained in Sample E and F respectively which indicates low level of microorganisms in orange juices due to the acidic nature of the citrus fruit and high chemical preservative which probably inhibit some of the microbes.

Table 1: Microbial count of *E.coli* pH 5.5 and 0 % Sugar in Orange juice [Sample A].

Heating Time (mins).	*E.coli* Survivors (LogCfu/mL) Temperatures (^{0}C)			
	60	70	75	80
0	$1.9X10^{5a}$	$1.9X10^{5a}$	$1.9X\ 10^{5a}$	$1.9X10^{5a}$
1	$1.9X10^{4b}$	$11.1X104^{b}$	$1.00X10^{4\ b}$	$9.90X10^{3b}$
2	$1.9X10^{3b}$	$1.112X10^{3b}$	$1.004X10^{3c}$	$9.91X10^{2c}$
3	$1.9X102^{b}$	$1.05X10^{2c}$	$1.04X10^{2\ c}$	$99.4X10^{2d}$
4	$18.4X10^{1c}$	$11.0X10^{1c}$	$0.9X10^{1d}$	$9.3X10^{0d}$
LSD	8.26	8.14	7.80	6.34

Means with same superscript down the column are not significantly (P≥0.05) different

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117 **Table 2:** Microbial count of *E.coli.* pH 5.5 and 2 % sugar [SAMPLE B]

Heating Time (mins)	*E. Coli* survivors (Logcfu/ml) Temperatures (^{0}C)			
	60	70	75	80
0	$5.80X10^{4a}$	$5.80X10^{4a}$	$5.80X10^{4a}$	$5.80X10^{4a}$
1	$8.810X10^{3b}$	$4.04X10^{3b}$	$4.04X10^{3b}$	$1488.1X10^{1b}$
2	$8.81X10^{2b}$	$4.39x10^{2c}$	$190.1x10^{1c}$	$148.1x10^{1c}$
3	$88.4x10^{1c}$	$4.4x10^{1c}$	$1.9x10^{1d}$	$14.5x10^{1d}$
4	$9.0x10^{0c}$	$4.2x10^{0d}$	$2.0x10^{0d}$	$1.2x10^{0d}$
LSD	7.12	6.91	5.54	5.04

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119 Means with same superscript down the column are not significantly (P≥0.05) different

120 **Table 3:** Microbial count of *E.coli* pH 5.5. and 4 % SUGAR [SAMPLE C]

Heating Time(mins)	*E.Coli* Survivors (Logcfu/ml) Temperatures(0^C)			
	60	70	75	80
0	$4.06X10^{4a}$	$4.06X\ 10^{4a}$	$4.06X10^{4a}$	$4.06\ X10^{4a}$
1	$4.20X10^{3b}$	$3.50X10^{3b}$	$1.9x10^{3\ b}$	$1.009x10^{3b}$
2	$4.2X10^{1}$b	$3.51X10^{2\ c}$	$1.89X10^{2c}$	$1.01X10^{2c}$
3	$4.1X10^{1c}$	$3.3X10^{1d}$	$18.8X10^{1d}$	$9.9X10^{1d}$
4	$4.0x10^{0}$c	$3.2x10^{0}$d	$1.9x10^{0d}$	$1.0x10^{0e}$
LSD	5.19	4.91	4.45	4.11

121 Means with same superscript down the column are not significantly (P≥0.05) different

122 **Table 4:** Microbial count of *E.coli* pH 4.0 and 0 % Sugar. [SAMPLE D]

Heating Time (mins)	*E.Coli* Survivors (Logcfu/ml) Temperatures (^{0}C)			
	60	70	75	80
0	$4.2X10^{4a}$	$4.2\ X10^{4\ a}$	$4.2X10^{4\ a}$	$4.2\ X10^{4a}$
1	$6.04X10^{3b}$	$3.5X10^{3\ b}$	$1.901X10^{3b}$	$1.70X10^{3b}$
2	$6.03X10^{2b}$	$3.52X10^{2c}$	$1.91X10^{2c}$	$1.72X10^{2c}$

3	$6.1X10^{1c}$	$3.5X10^{1d}$	$1.9X10^{1c}$	$1.8x10^{1d}$
4	$6.0X10^{0c}$	$3.4X10^{0\,d}$	$2.0X10^{0d}$	$1.8X10^{0d}$
LSD	5.28	5.01	4.91	4.13

123 Means with same superscript down the column are not significantly (P≥0.05) different

124 **Table 5**: Microbial count of *E.coli* pH 4.0 and 2% Sugar [SAMPLE E]

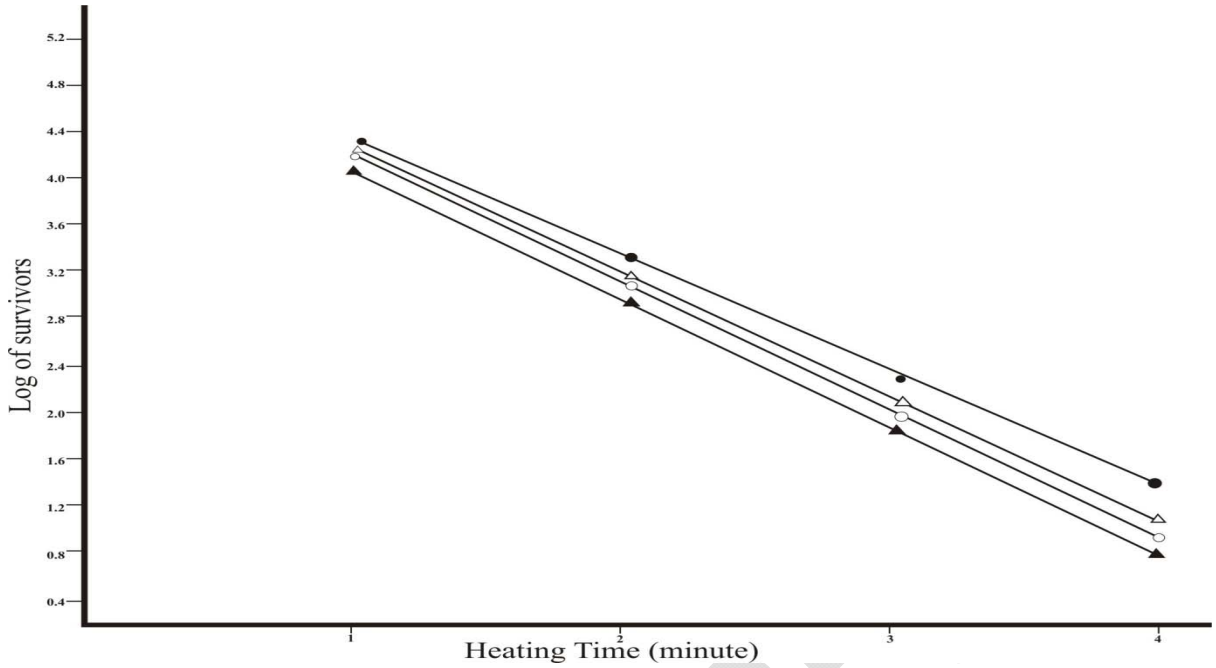
Heating Time (mins)	*E.coli* survivors (logcfu/ml) Temperatures (^{0}C)			
	60	70	75	80
0	$3.5X10^{3a}$	$3\ .5X10^{3a}$	$3\ .5X10^{3a}$	$3\ .5X10^{3a}$
1	$3.10X10^{3b}$	$1.990X10^{3b}$	$1.310X10^{3b}$	$6.20\ X10^{2b}$
2	$3.11X10^{2b}$	$1.99X10^{2b}$	$1.24X10^{2c}$	$62.2X10^{1c}$
3	$3.1X10^{1c}$	$2.0X10^{1c}$	$12.4X10^{1d}$	$4.9X10^{0d}$
4	$3.0X10^{0d}$	$1.9X10^{0d}$	$1.0X10^{0e}$	–
LSD	3.14	2.05	2.05	1.45

125 Means with same superscript down the column are not significantly (P≥0.05) different

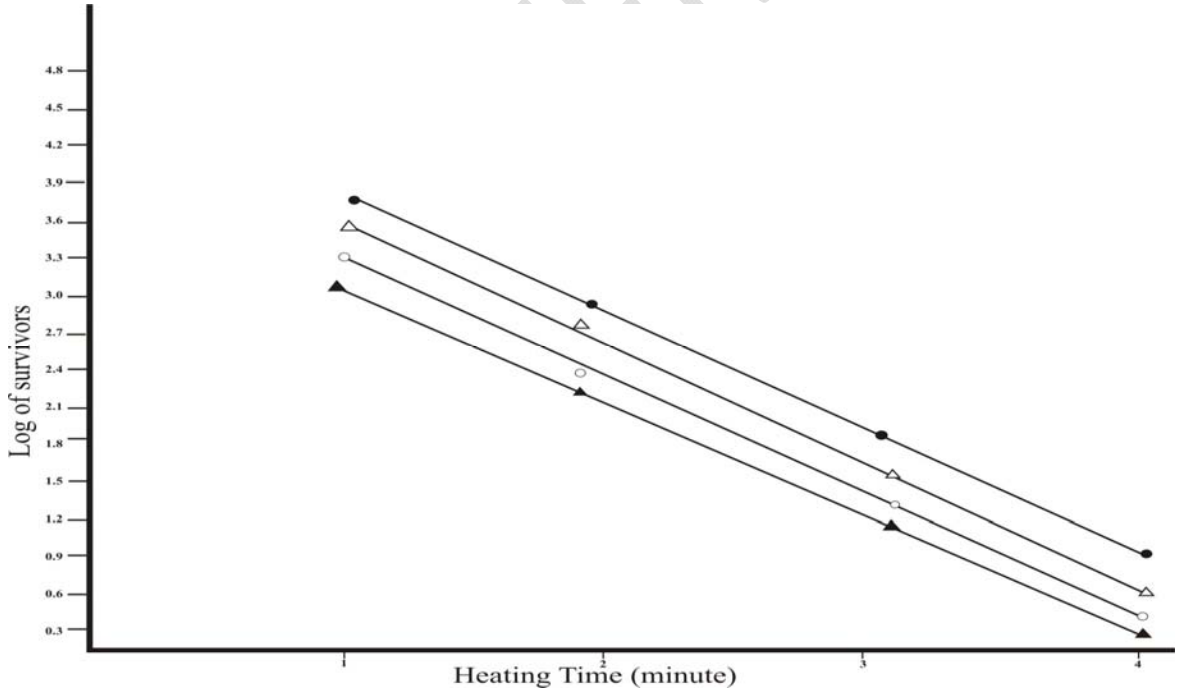
126 **Table 6**: Microbial count of *E.coli* pH 4.0 and 4% Sugar [SAMPLE F]

Heating Time (mins).	*E.coli* Survivors (logcfu/ml) Temperatures (^{0}C)			
	60	70	75	80
0	$2.70X10^{4a}$	$2.70\ X10^{4a}$	$2.70\ X10^{4a}$	$2.70\ X10^{4a}$
1	$2.710X10^{3}$ b	$1.90X10^{b}$	$1.90X10^{b}$	$4.49X10^{2b}$
2	$2.69X10^{2c}$	$1.70X10^{2b}$	$120.1X10^{2c}$	$44.4X10^{1c}$
3	$2.7X10^{1c}$	$16.4X10^{1c}$	$11.9X10^{1d}$	$3.4X10^{0d}$
4	$2.3x10^{0d}$	$1.6X10^{0d}$	$1.0X10^{0e}$	_
LSD	2.19	1.42	1.05	0.49

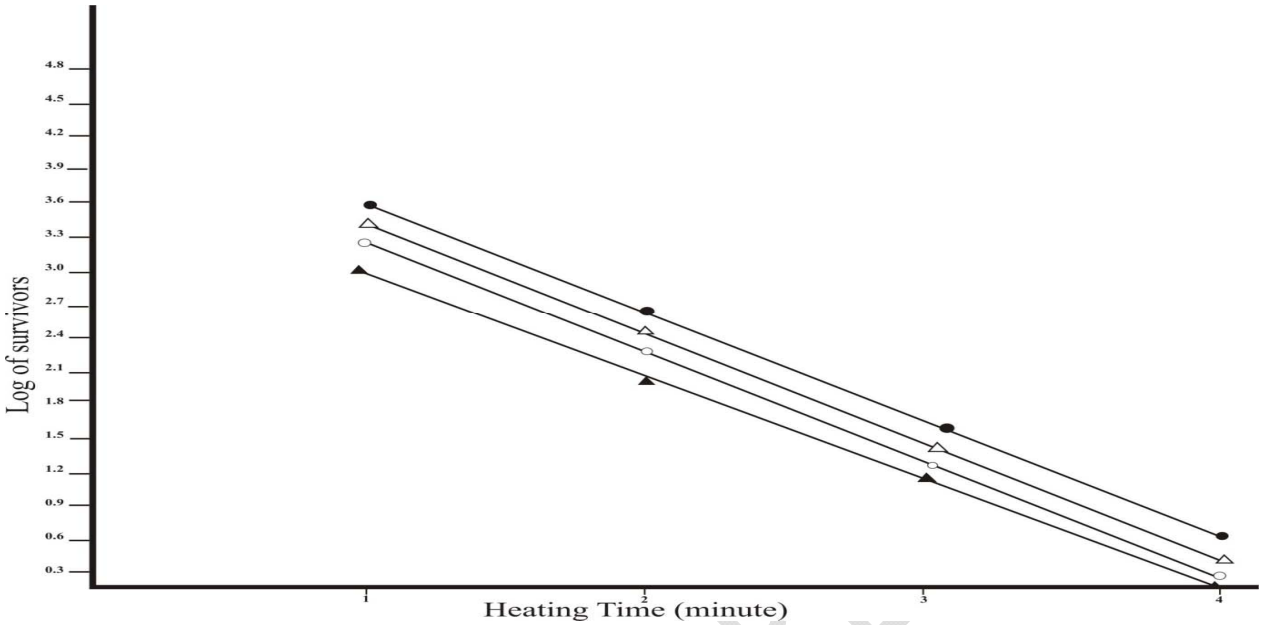
127 Means with same superscript down the column are not significantly (P≥0.05) different



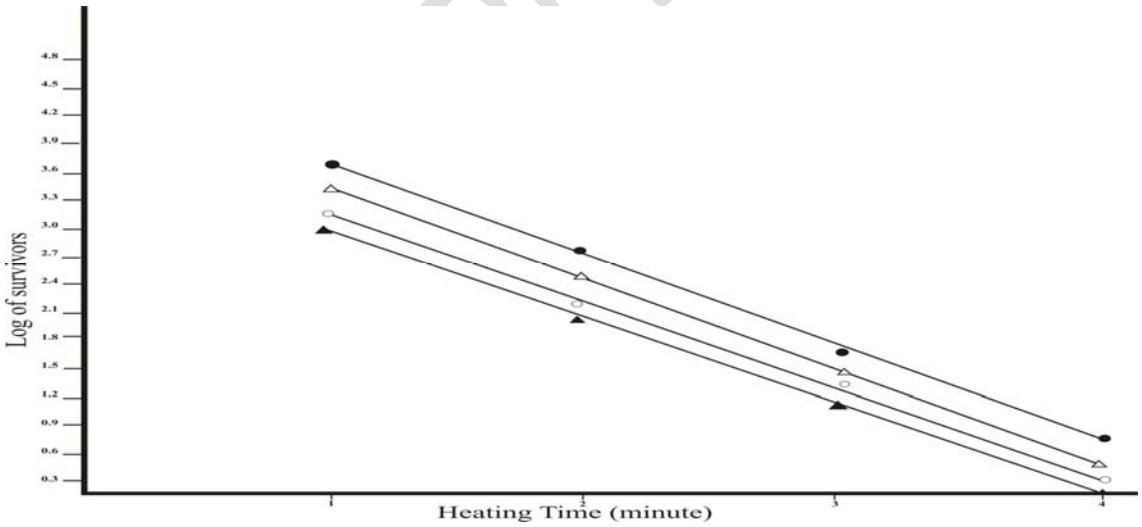
Graph 1: Log of *E. Coli* Survivors against Heating Time (mins) in Orange juice of pH 5.5 and 0% sugar at 60 (•), 70 (Δ), 75 (0) and 80 (▲)^{0}C respectively.



Graph 2: Log of *E.Coli* Survivors against Heating Time (Mins) in Orange juice of pH 5.5 and 2% sugar at 60 (•), 70 (Δ), 75 (0) and 80 (▲) ^{0}C respectively.



Graph 3: Log of *E.coli* Survivors against heating Time (mins) in Orange juice of pH 5.5 and 4% sugar at 60 (•), 70 (Δ), 75 (0) and 80 (▲) ^{0}C respectively.



Graph 4: Log of *E.coli* Survivors against heating time (mins) in Orange juice of pH 4.0 and 0% sugar at 60 (•), 70 (Δ), 75 (0) and 80 (▲)^{0}C respectively.

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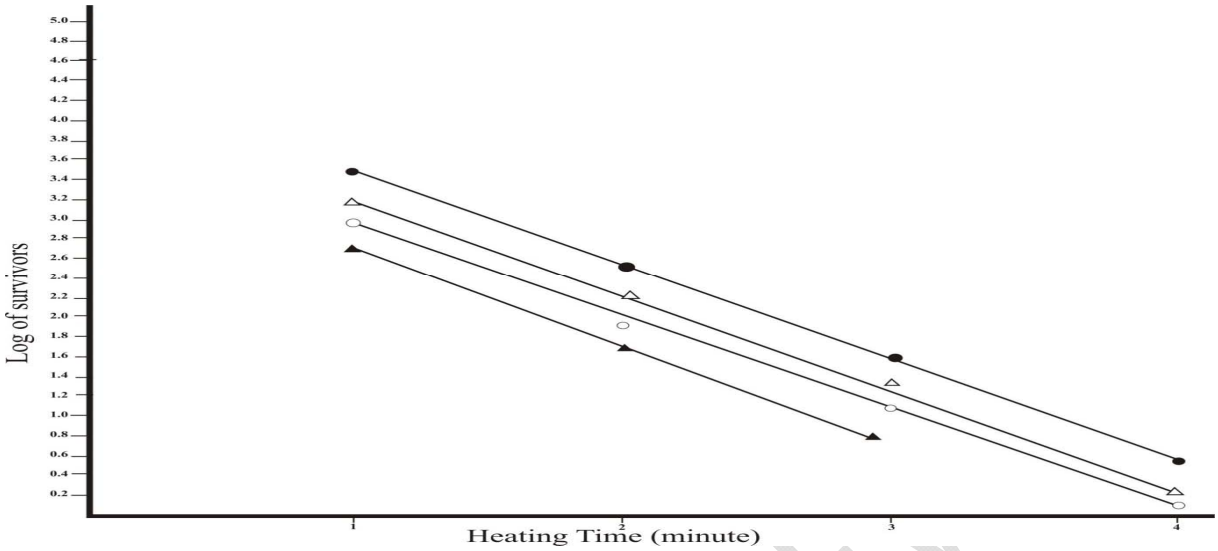

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145 Graph 5: Log of *E. Coli* Survivors against Heating Time (Mins) in Orange juice of pH 4.0 and 2 % Sugar at 60

146 (•), 70 (Δ), 75 (0) and 80 (▲)^{0}C respectively.

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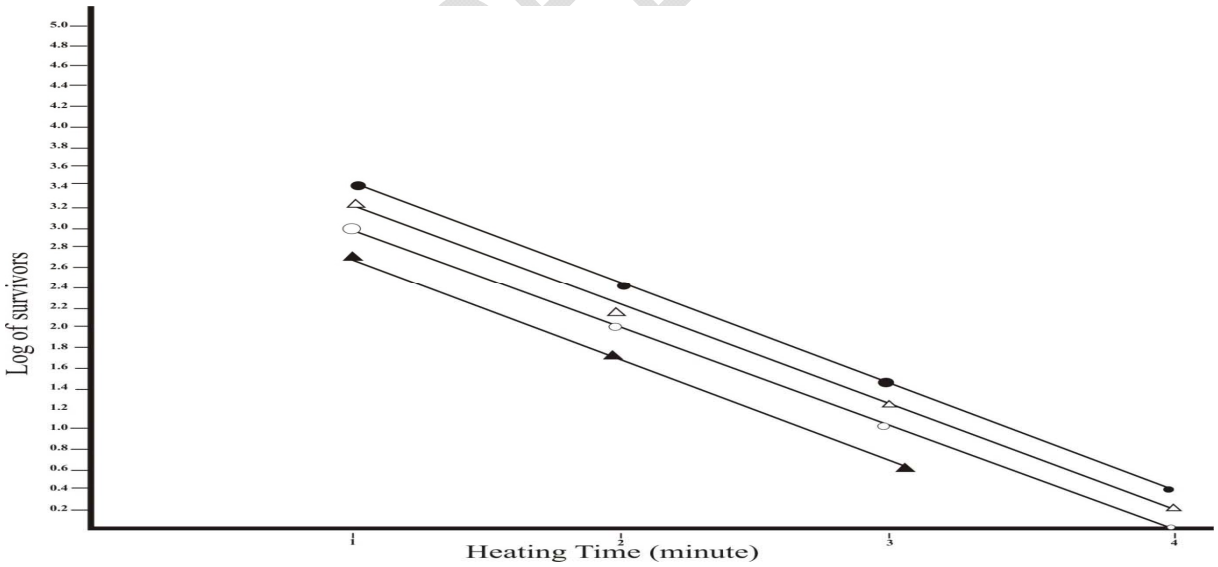

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149 Graph 6: Log of *E.Coli* Survivors against Heating Time (Mins) in Orange juice of pH 4.0 and 4% sugar at 60

150 (•), 70 (Δ), 75 (0) and 80 (▲)^{0}C respectively.

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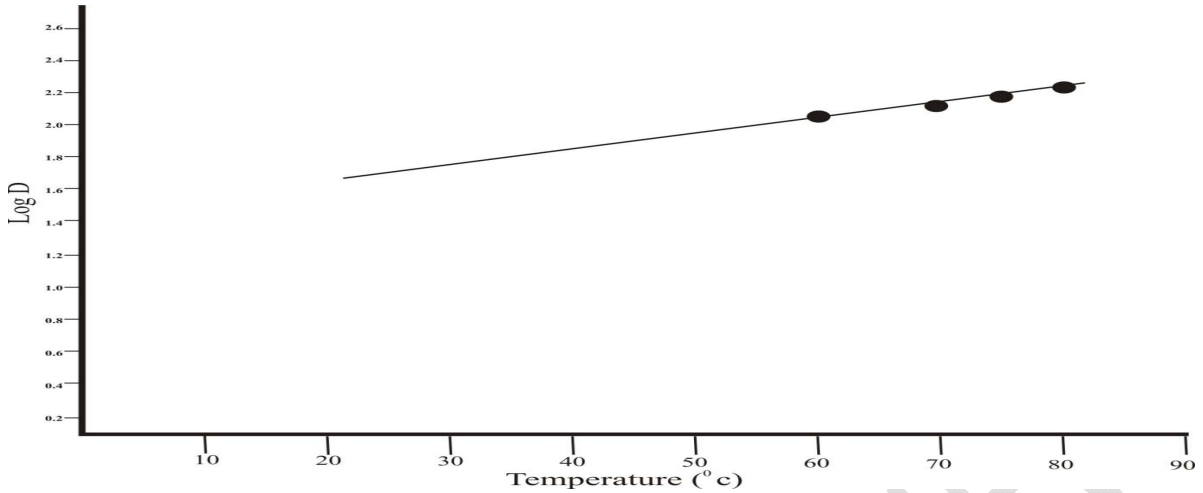
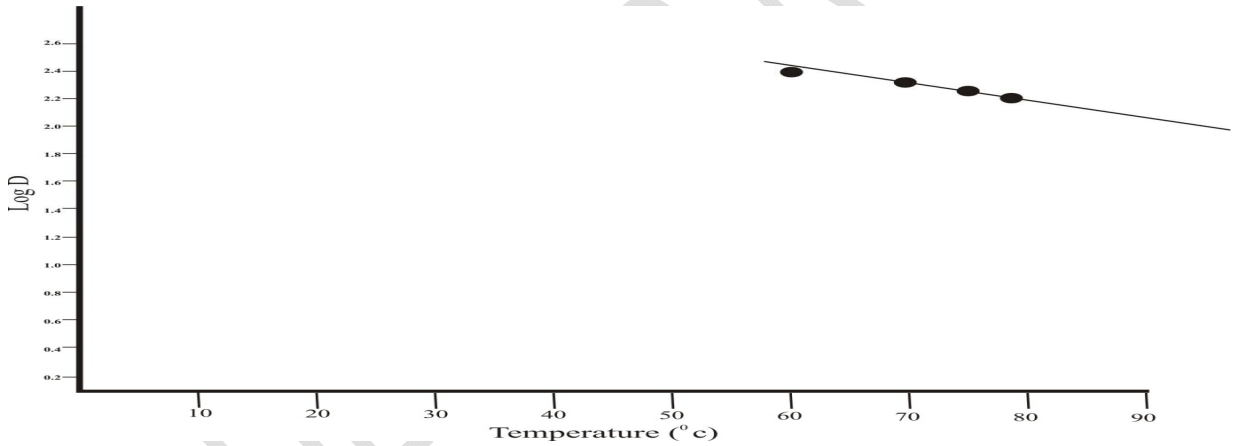
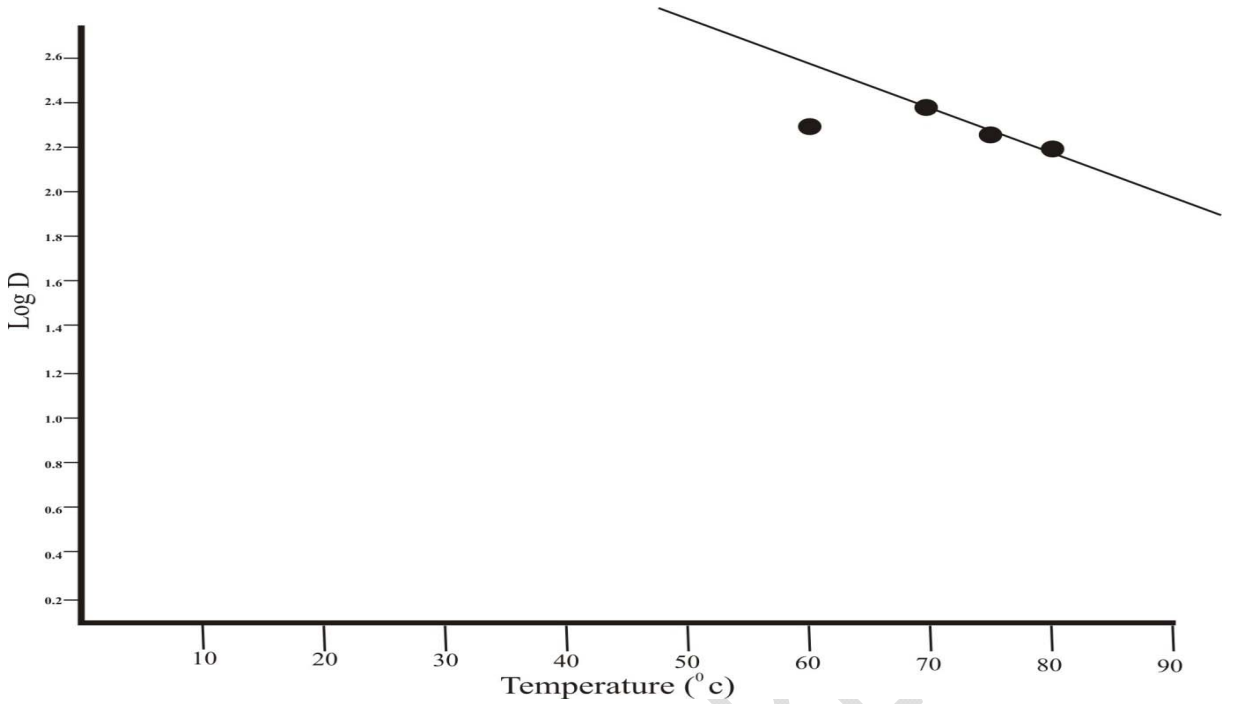


Graph 7: Log D of *E.Coli* Survivors against Temperature in Orange juice of pH 5.5 and 0% Sugar (Sample A)



Graph 8: Log D of *E.Coli* Survivors against Temperature in Orange juice of pH 5.5 and 2% Sugar (Sample B)



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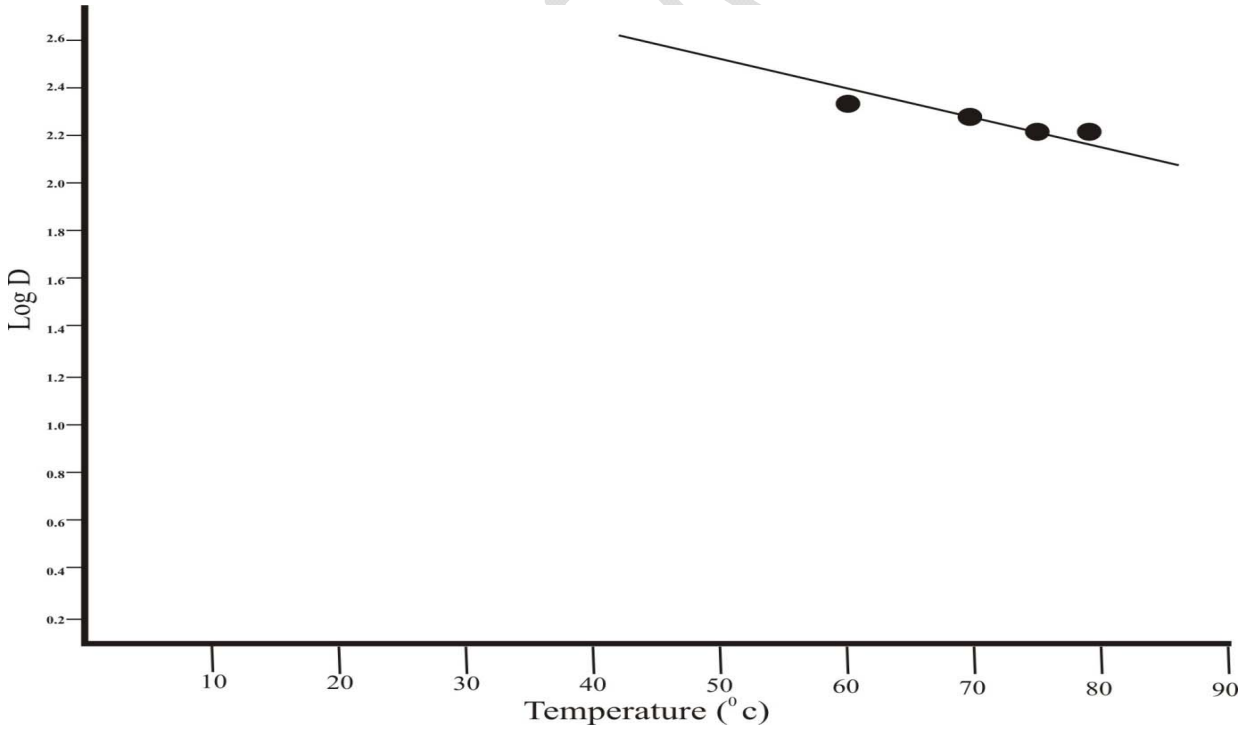
159 **Graph 9**: Log D of *E.Coli* Survivors against Temperature in Orange juice of pH 5.5 and 4% Sugar Sample C.



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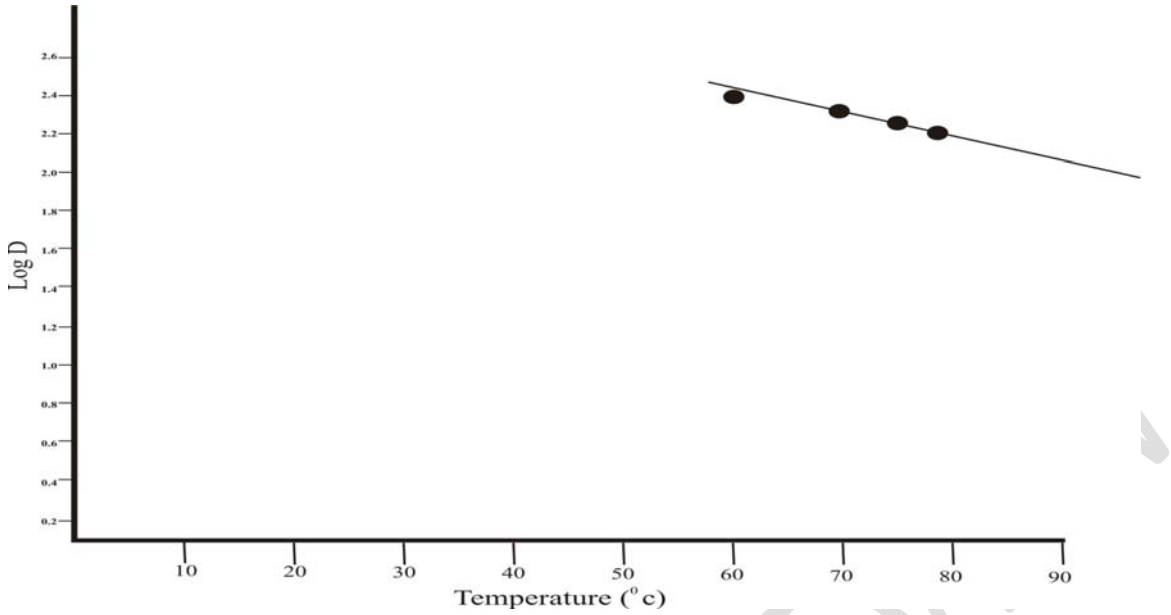
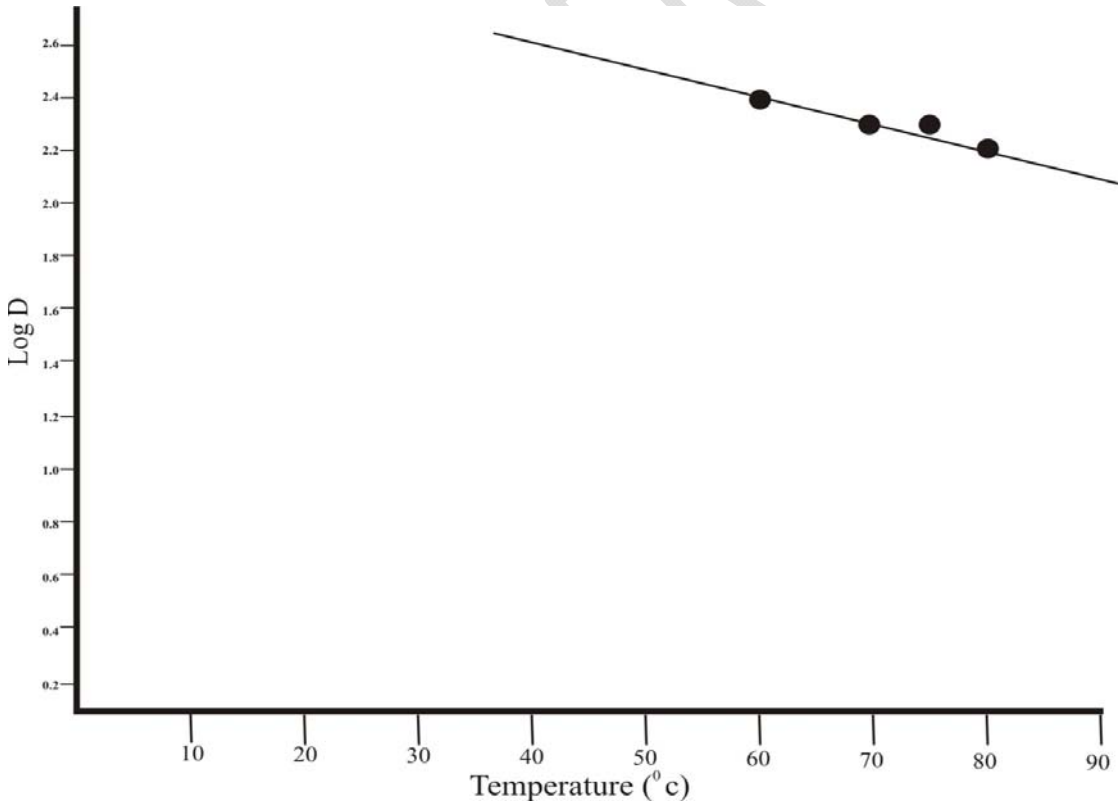
161 **Graph 10:** Log D of *E.Coli* survivors against temperature in Orange juice of pH 4.0 and 0% sugar (Sample D).



Graph 11: Log D of *E.Coli* survivors against temperature in Orange juice of pH 4.0 and 2% sugar Sample E.



Graph 12: Log D of *E.Coli* Survivors against Temperature in Orange juice of pH 4.0 and 4% Sugar Sample F.

4.0 CONCLUSION.

The work has shown that there was drastic inhibition of the test micro-organism by the application of chemical preservatives and heat treatment. There were fewer growths in the orange juice samples when chemical preservatives were used at higher temperature. The bacteria growths of the treated samples were significantly affected by the hurdle treatment when compared to the control. This led to a significant reduction in the bacterial load. It is recommended that a single hurdle should not be used in the preservation of orange juice. Hurdle application improves greatly the microbial stability and safety of orange juice thus consumer safety. Commercial processors of orange juice are encouraged to apply these hurdles at a pH 4.0 and 4% sugar levels respectively.

REFERENCES

(1) Lim, K.F. Negative pH does Exist. Journal of Chemical Education. 2006; 83 (10): 1465-1469.

(2) (2)Mysers, R.J. One hundred years of pH. Journal of Chemical Education. 2010; 87 (1): 30-32.

(3) (3)World Health Organization.Guideline on sugar intake for adults and children. Geneva. 2005.

(4) (4)Leistner, L. Combined method for food preservation. In:Rahman MS (ed). Handbook of food preservation. CRC. Boca Raon, FL., 2007 pp 867-894.

(5) (5)U.S. Food and Drug Administration Inspection guides-sterilizing Symbols (D, Z AND F). www. Fda.gov. Retrived 2018;-09-28.

(6)Garcia-Garcia, R., Avelianeda, E., Tejada-Ortigoza, V and Martin-Belloso A. Hurdle technology applied in prickly pear beverages for inhibiting Saccharomyces Cerevisiae and Escherichia Coli. Letters in Applied Microbiology, 2015;60, 558-564.

(7) Ohlsson, T. and Bengston. The hurdle concept of Minimal processing technology in the food industry, Crc Press, New York. 2013; Pp. 175-195.

(8) Ohlsson, T. and Bengston, N. The hurdle concept of minimally processed foods 2012; (3):17-25

(9).Velugoti, P.R., Bohra, L.K., Juneja, V.K., Huang, L., Wasseling, A.L., Subbiah, J. and Thippareddi, H. Dynamic model for predicting growth of Salmonella spp. in ground sterile pork. Food Microboil 2011; 28:796-803.

(10) Rahman, M.S. Applications of macro-micro region concept in the state diagram and critical temperature concepts in determining the food stability. Food Chem 2012; 132:1679-1685.

(11). Aurelie, C.R., Veronique, B., Julien, C. Nutritional Composition of Orange juice: A comparative study between French commercial and homemade Juices. Food and Nutrition Sciences, 2016; 7, 252-261.

(12). Prescott, L.M., Harely, J.P., Klein, O.A. Microbial nutrition, types of media. In: microbiology,6th edition, Mc Graw Hill publishers, New York. 2005; 95-105.

(13). Steele, R.G.D. and Torrie, J.H. Principles and procedures of statistics. 2nd Ed. McGraw hill book co.Inc.New York. 1980

(14). Carrasco, E.del Rosal, S., Racero, J.C. and Gimeno, R.M. A review on growth/no growth Salmonella models. Food Research Int 2012;47:90-99.