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 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

pH 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. At room temperature, pure water is neither acidic nor basic and has a pH of 7. The pH scales is logarithmic and approximate the negative of the base 10 logarithm of the molar concentration (measured in units of moles per litre) of hydrogen ion in a solution. 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.

Works by Ohlsson and Bengtsson (6) 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 (7) 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 increasing 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. 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 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 and were not excessively ripe, free from diseases, Mechanical bruises and rot.

2.2 Processing Method

2.3 Processing of Orange Juice

The modified method of Aurelie *et al.* (5) 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.

Orange fruits
↓
Sorting
↓
Peelings
↓
Washing
↓
Cutting
↓
Spinning
↓
Sieving/Filtration
↓
Pasteurization (85^{0}C for 1 minute to in activate Microorganisms)
↓
Cooling
↓
Orange Juice.

Fig 1: Production flow chart for Orange Juice.

Source: Aurelie *et al.* (5).

2.3.1 Determination of microbiological Analysis of the orange juice.

The method (8) was used to determine the total viable count. The orange juice were seeded with *Escherichia coli* to determine microbial counts with the help of McConkey 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 in 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 inocolums). CFU=Colony Forming Unit………………… (1)

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 ($p \geq 0.05$) (9).

3.0 RESULTS AND DISCUSSION

Effects of chemical preservatives on the growth of *Escherichia coli* in orange juice is presented in 1-6 at different level of temperatures (60^{0}C, 70^{0}C, 75^{0}C and 80^{0}C) and time. As the concentration of the chemical preservatives increased, a remarkable decrease in the bacterial biomass was recorded. This agrees with the findings of (10). In this study it was observed the concentration and combination of preservative alone reduced growth of the microorganism but was unable to prevent growth of the test organism (7). The application of the heat reduced the population of the microorganisms and weakens their ability to germinate. The introduction of heat was vital as the combination of both chemical preservatives and heat reduced growths in 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 (7). At a higher temperatures and higher time there was no significant growth at sample 6 recorded at four minutes at 80^{0}C (4). 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, 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 thereby making it difficult for microbial growth and multiplication than a weakly acidic medium. High growths of the test microorganism maybe due to the following factors, poor handling when carrying out the analysis or it could be that some of the raw materials (oranges) were not free from disease (Mechanical bruises, rot and overripe) Microbial result revealed Sample A & 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 fruit juices due to the acidic nature of the citrus fruit and high chemical preservative which probably inhibit some of the microorganisms.

Table 1: Numbers of Survivors 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

Table 2: Numbers of Survivors 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

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

Table 3: Numbers of Survivors of *E.coli* pH 5.5.4 % 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

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

Table 4: Numbers of Survivors 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

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

Table 5: Numbers of Survivors of *E.coli* pH 4.0 2% Sugar (SAMPLE E)

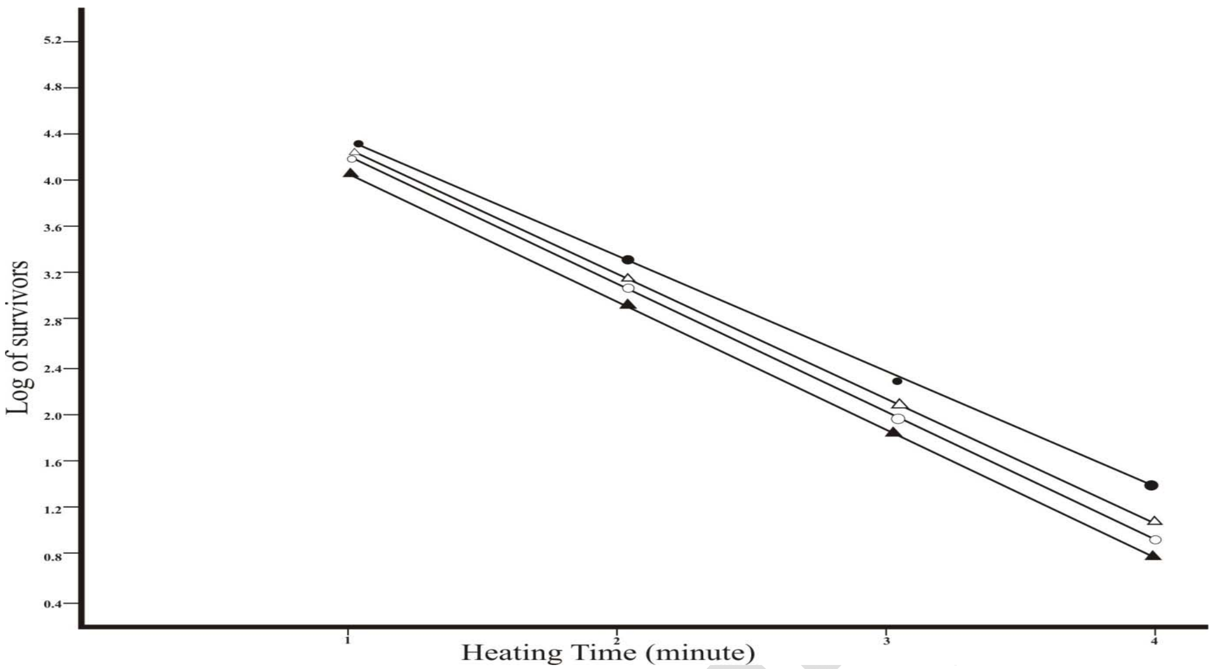
Heating Time (mins)	*E.coli* survivors (logcfu/ml) Temperatures (^{0}C) 60	70	75	80
0	$3.5X10^{3a}$	$3\ .5X10^{3a}$	$3\ .5X10^{3a}$	$3\ .5X10^{3a}$
1	$3.10X10^{3b}$	$1.990X10^{3b}$	$1.310X10^{3b}$	$6.20\ X10^{2b}$
2	$3.11X10^{2b}$	$1.99X10^{2b}$	$1.24X10^{2c}$	$62.2X10^{1c}$
3	$3.1X10^{1c}$	$2.0X10^{1c}$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

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

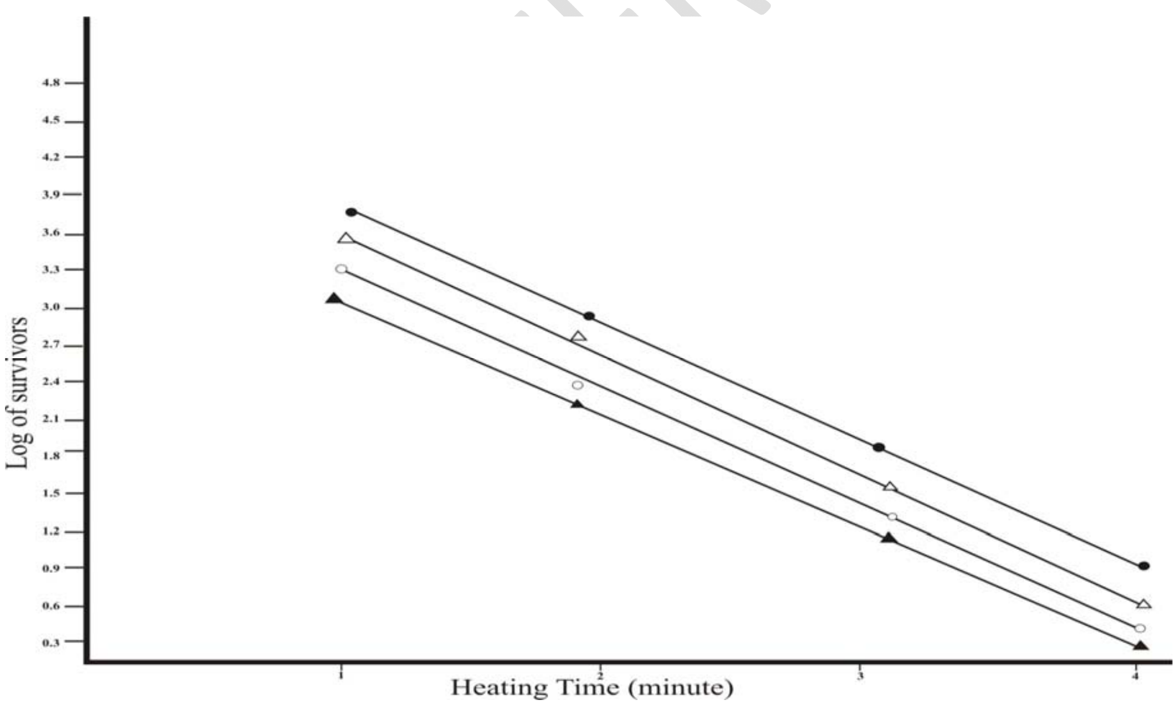
Table 6: Numbers of Survivors of *E.coli* pH 4.0, 4% Sugar (SAMPLE F)

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

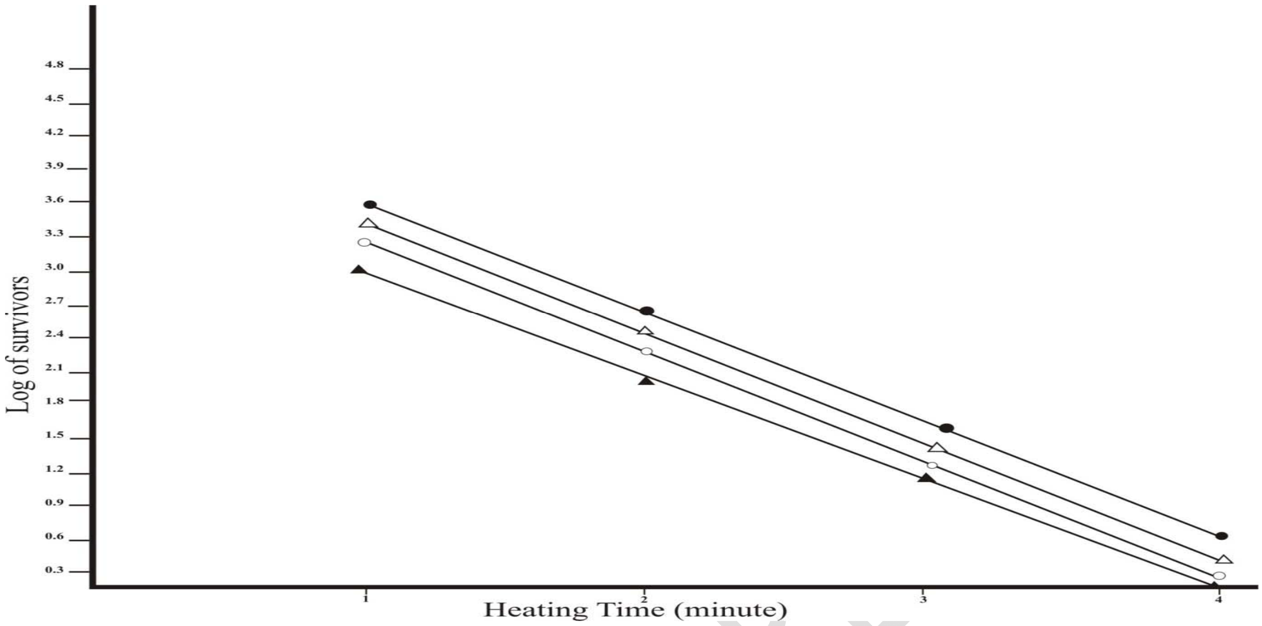
Means with same superscript down the column are not significantly (P≥0.05) different



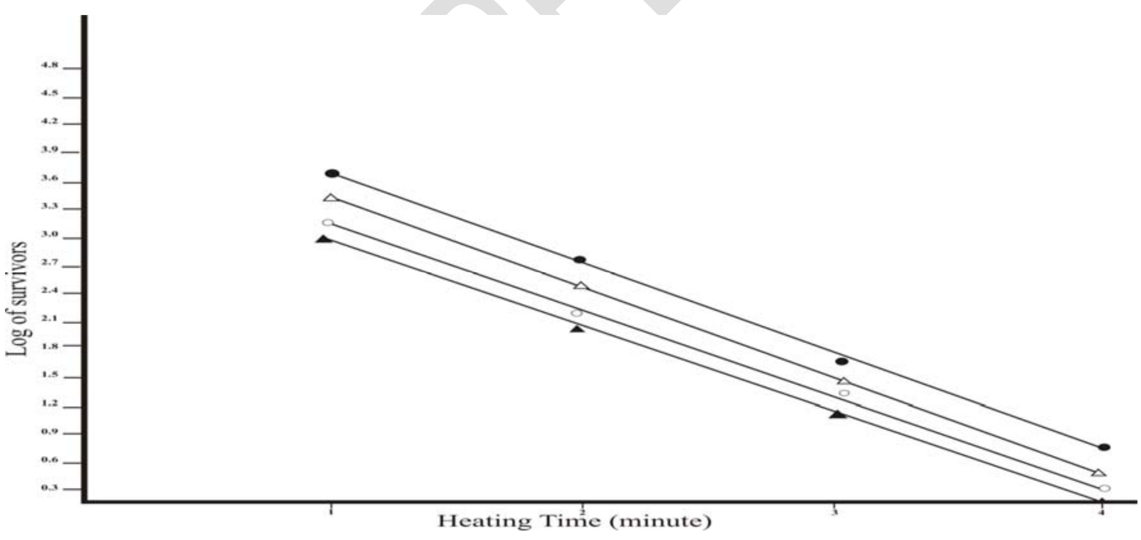
Graph 1: Log of *E. Coli* Survivors against Heating Time (mins) in Orange juice of pH 5.5 and 0% sugar at 60 (•), 70 (Δ), 75 (0) and 80 (▲)^{0}C respectively.



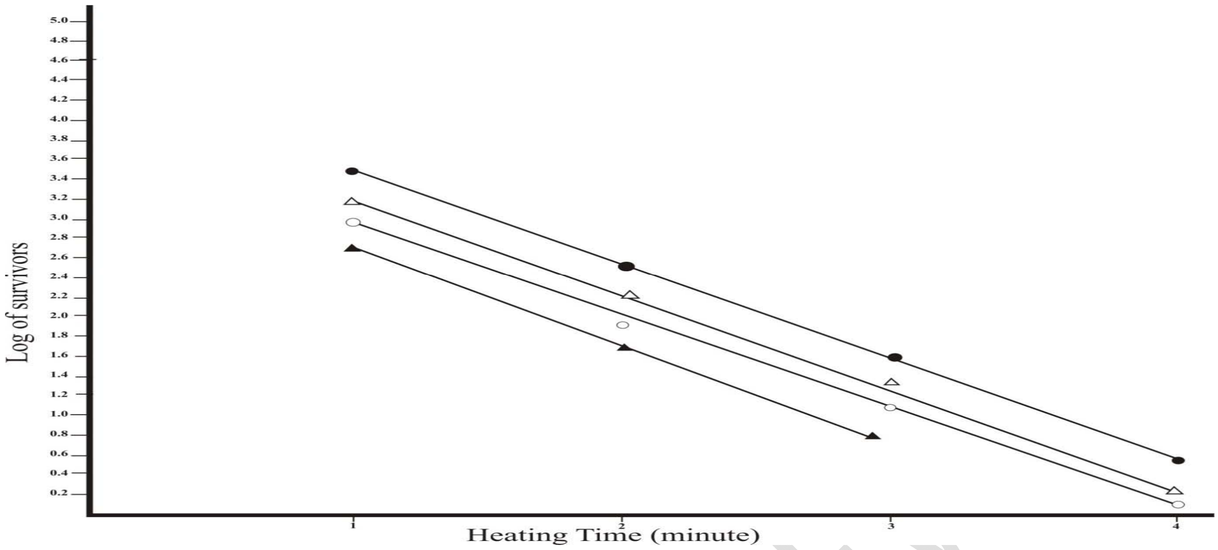
Graph 2: Log of *E.Coli* Survivors against Heating Time (Mins) in Orange juice of pH 5.5 and 2% sugar at 60 (•), 70 (Δ), 75 (0) and 80 (▲) ^{0}C respectively.



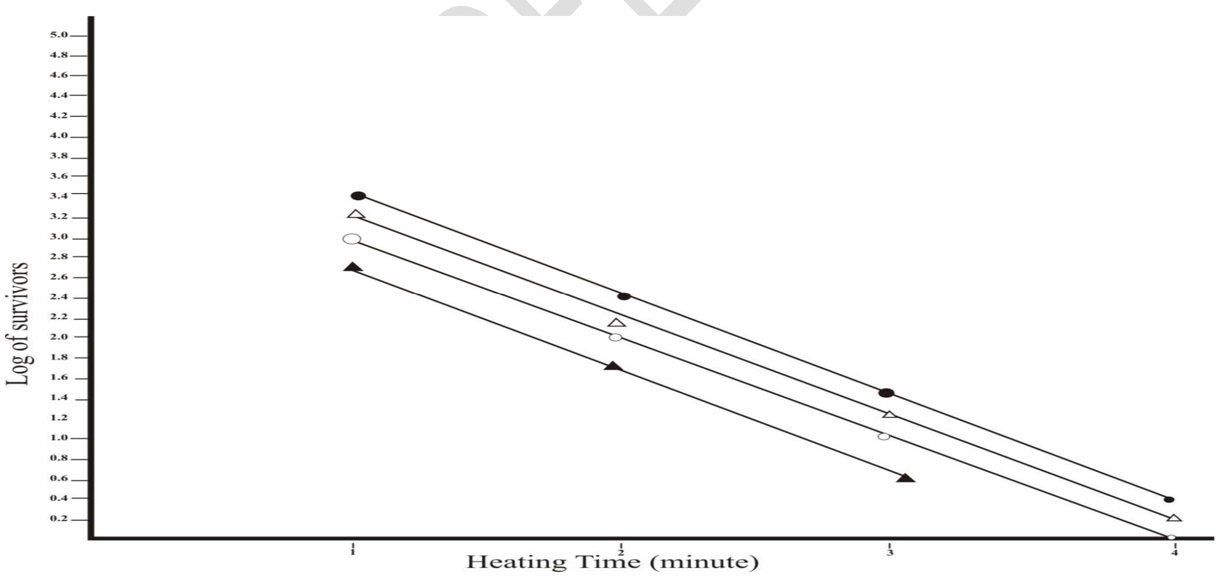
Graph 3: Log of *E.coli* Survivors against heating Time (mins) in Orange juice of pH 5.5 and 4% sugar at 60 (•), 70 (Δ), 75 (0) and 80 (▲) ^{0}C respectively.



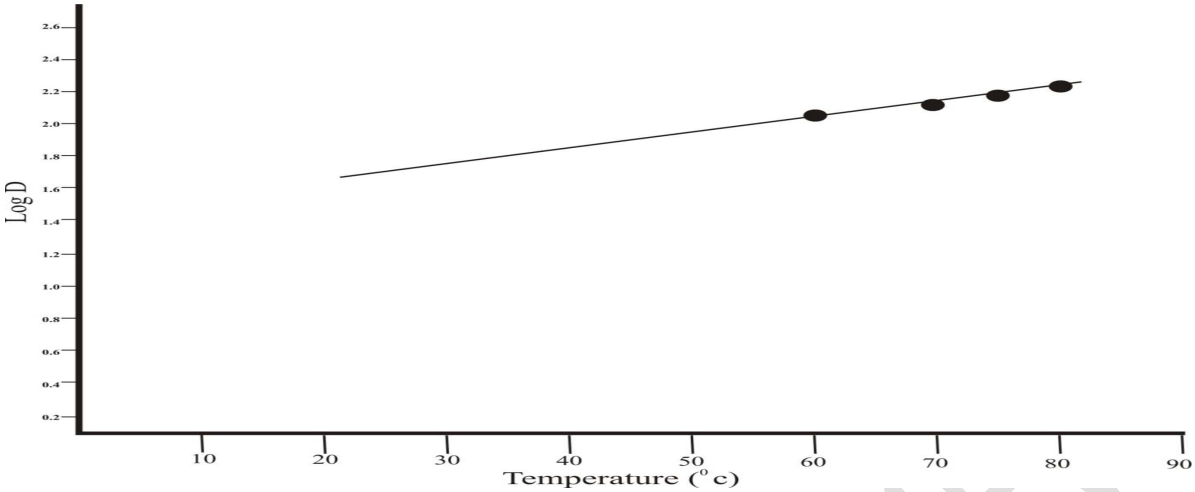
Graph 4: Log of *E.coli* Survivors against heating time (mins) in Orange juice of pH 4.0 and 0% sugar at 60 (•), 70 (Δ), 75 (0) and 80 (▲)^{0}C respectively.



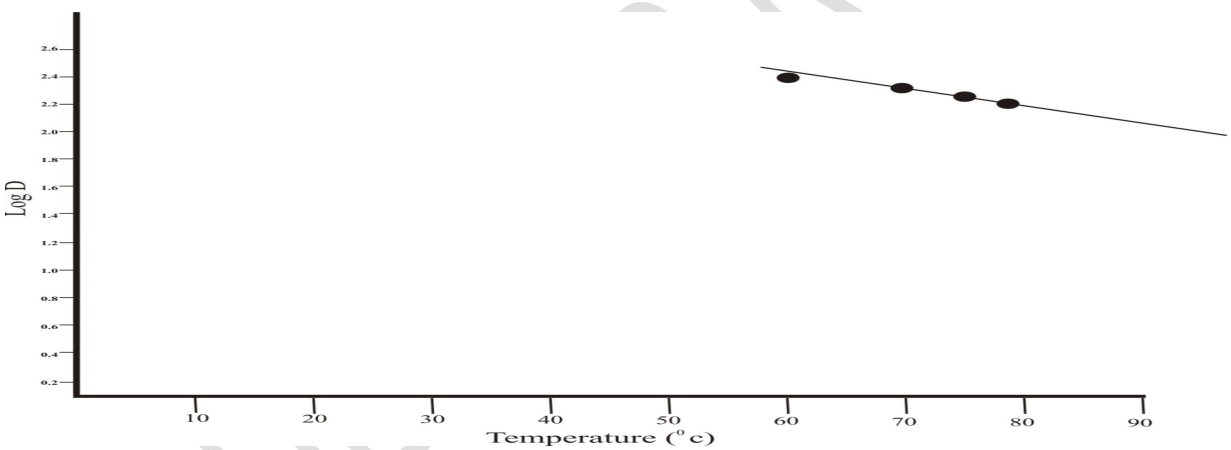
Graph 5: Log of *E. Coli* Survivors against Heating Time (Mins) in Orange juice of pH 4.0 and 2 % Sugar at 60 (•), 70 (Δ), 75 (0) and 80 (▲)^{0}C respectively.



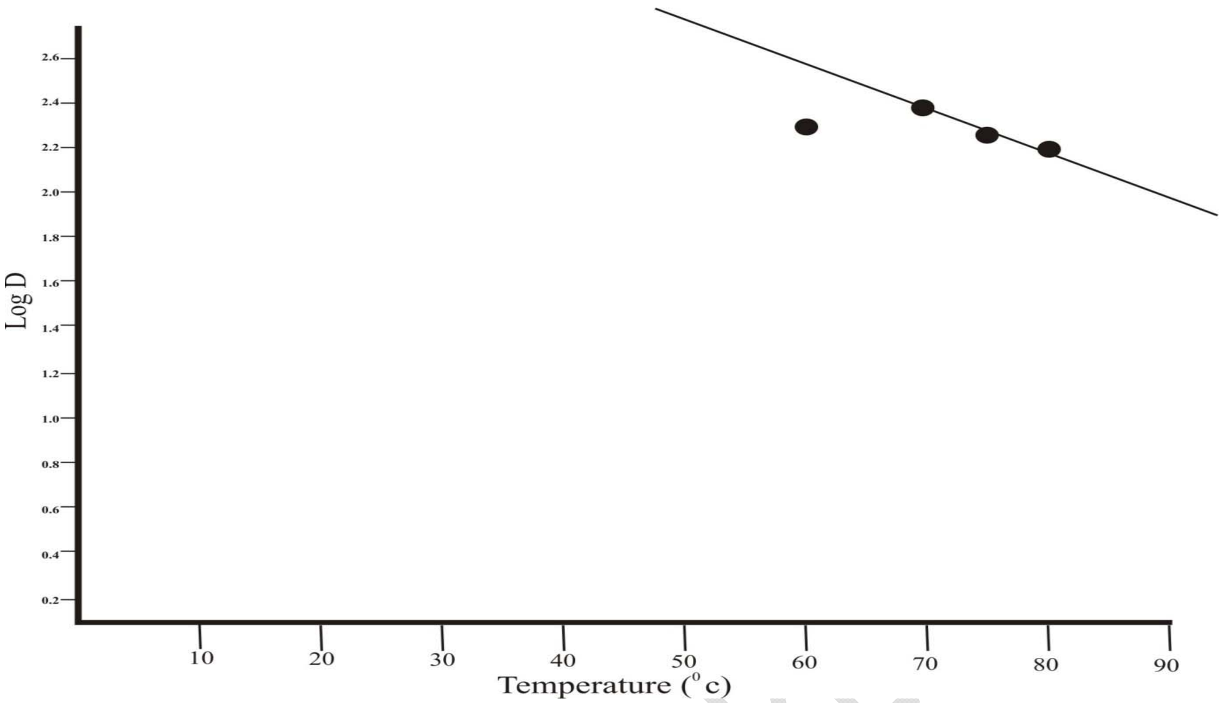
Graph 6: Log of *E.Coli* Survivors against Heating Time (Mins) in Orange juice of pH 4.0 and 4% sugar at 60 (•), 70 (Δ), 75 (0) and 80 (▲)^{0}C respectively.



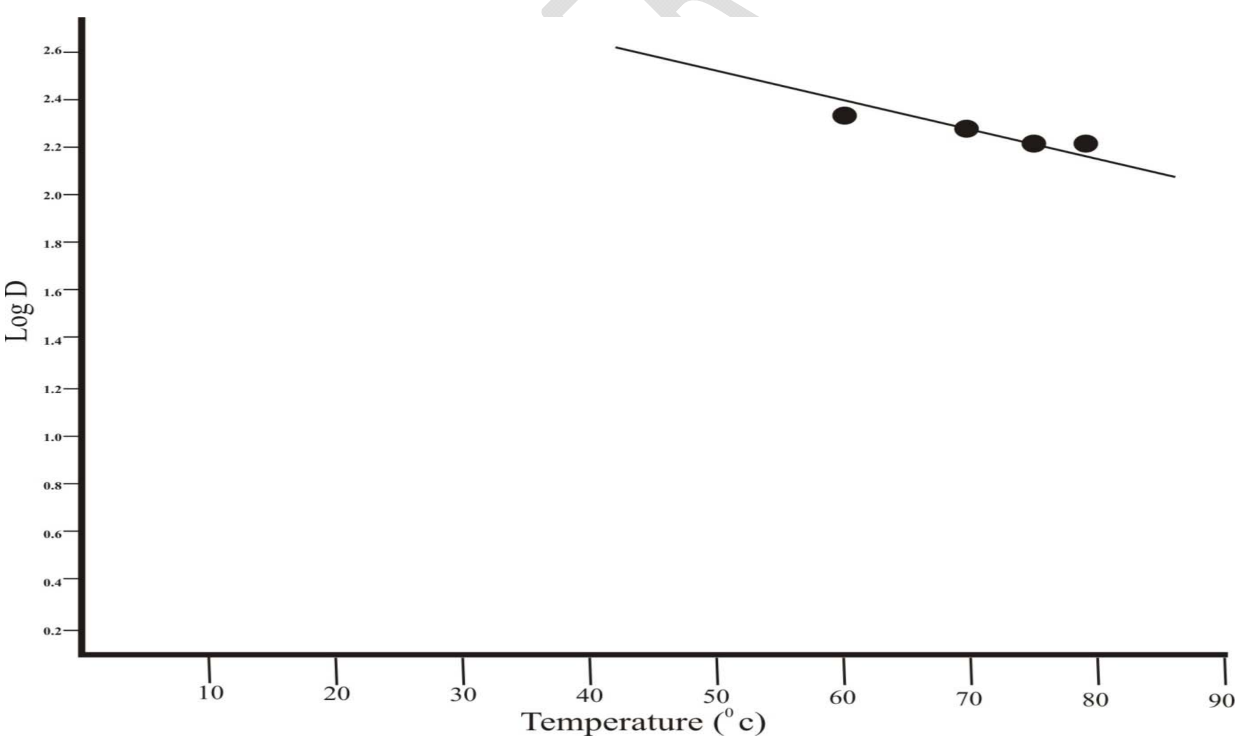
Graph 7: Log D of *E.Coli* Survivors against Temperature in Orange juice of pH 5.5 and 0% Sugar (Sample A)



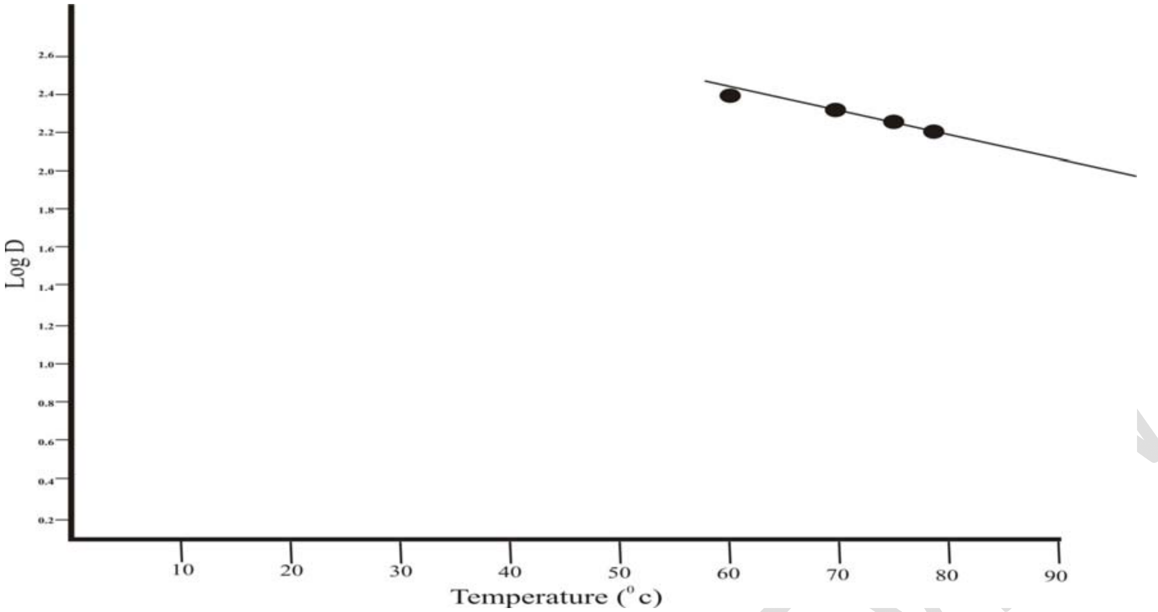
Graph 8: Log D of *E.Coli* Survivors against Temperature in Orange juice of pH 5.5 and 2% Sugar (Sample B)



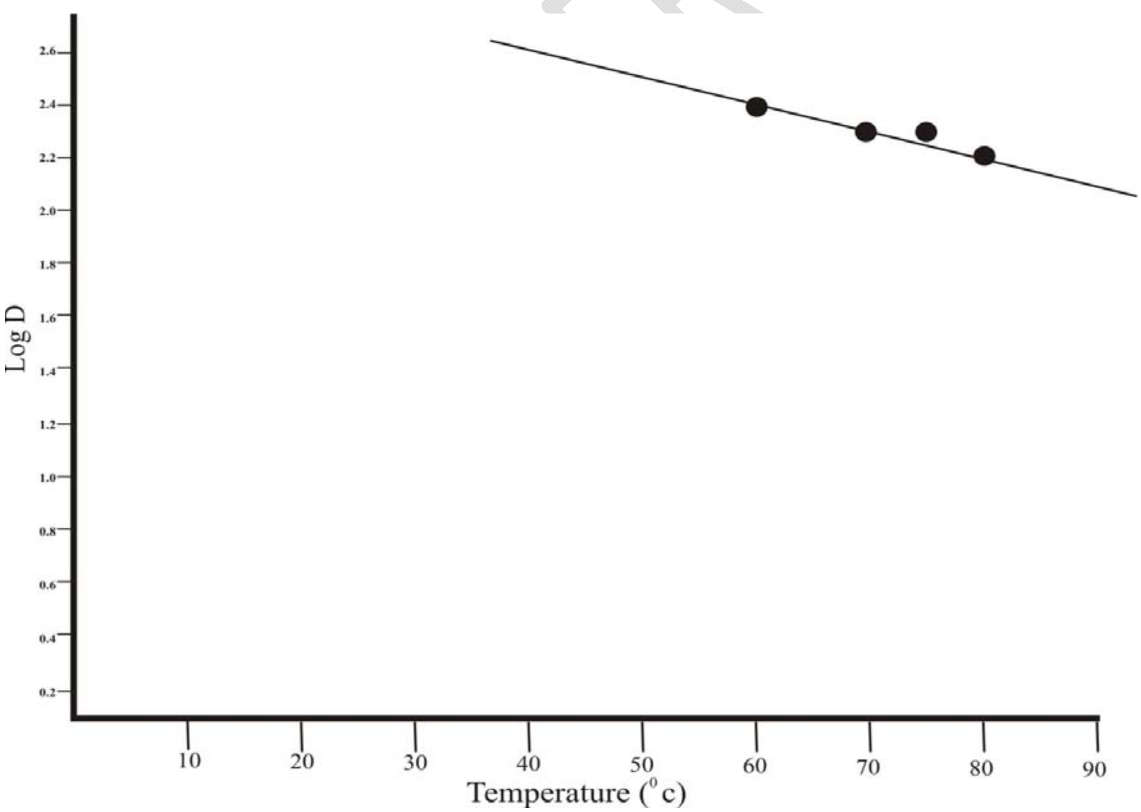
Graph 9: Log D of *E.Coli* Survivors against Temperature in Orange juice of pH 5.5 and 4% Sugar Sample C.



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 showed 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, 2.0 and 4% sugar levels respectively.

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