EFFECTS OF ANTIMICROBIAL INTERVENTIONS AND BRINING ON MICROBIOLOGICAL QUALITY OF 6 MONTHS' QUICK FROZEN CHICKEN MIXED PORTIONS

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Abstract. This study was to know the effects of abattoir, chlorinated antimicrobial intervention and brining on microbiology of post 6 months' frozen (-10±2 °C) and thawed mixed chicken portions, as well as determine the optimal brining levels. Broilers (5-6 weeks old, n = 180, 1.12-1.25kg carcass weight) processed at 3 different processing plants (n = 60 per abattoir) into chicken portions of breasts, thighs and wings were packaged in 1 kg bags after applying antimicrobial intervention and brining before quick freezing them. After 6 months quick freezing at -10 °C and thawing, standard plate counts (SPC), spore counts and psychrophilic counts (log cfu/g) were evaluated and subjected to ANOVA in a randomized complete block design (RCBD). No coliforms, molds, staphylococci, and yeasts were not detected due to abattoir, antimicrobial and brining interventions. Also no significant SPC, spore count and psychrophilic bacterial count ($P > 0.05$) effects was recorded due to abattoirs. ASC significantly ($P < 0.05$) lowered all the counts compared to Aq. Cl and ClO_2. The 0% brine had highest ($P < 0.05$) counts (3.33, 3.25 and 3.63 logCFU/g) for SPC, spores and psychrophilic bacteria respectively, while 30 and 35% levels had the least ($P < 0.05$) counts (ranges from 1.06 – 1.87 logCFU/g) per sample tested with no significant interaction effects recorded. There is a significant optimal brining levels of 13.27, 35.87 and 34.10 (%) for SPC, spores and psychrophilic counts based on the quadratic regression model.
Keywords: *psychrophilic bacteria, quadratic function, aqueous chlorine, standard plate count, freezing*

Introduction

Food preservation using low temperatures is the most commonly used method for storing fresh foods as well as its distribution. Fresh meat is among the most perishable foods and chill temperatures are mostly used to delay the spoilage process by spoilage bacteria after slaughter, processing cuts and portions, transportation to distributors and the final storage at various retail sites (Xia et al., 2009). The basic aim of cooling techniques for fresh meat preservation is to slow or limit the spoilage rate as temperature below the optimal range can inhibit the microbial growth (Cassens, 1994). The methods of temperature regulation for storage are chilling, freezing and super-chilling which help to inhibit or completely stop bacterial growth (Zhou et al., 2010). However, the growth of psychrophilic group of bacteria, yeasts and molds is not prevented by all levels of refrigeration (Neumeyer et al., 1997) and both enzymatic and non-enzymatic changes will continue at a much slower rate (Van Berkel et al., 2004). During freezing, about 60% of the viable microbial population dies but the remaining population gradually increases during longer frozen storage periods (Rahman, 1999b). Modi et al. (2006) and Sudheer et al. (2011) reported an increase in psychrophilic bacterial count in prolonged freezing storage of spiced chicken.

Most psychrophilic bacteria belong to the microbial genera of both gram positive, such as lactic acid bacteria, and gram-negative bacteria such as *Pseudomonas* spp. and

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Enterobacteriaceae (Holzapfel, 1998). Particular species involved in the spoilage of meat preserved at chill temperature are of *Pseudomonas* spp. (Ercolini et al., 2007), while the microflora of vacuum packaged frozen-preserved meat is characterized mostly by psychotropic lactic acid bacteria (Nychas et al., 1998). It is assumed that antimicrobial intervention and brining before freezing could significantly reduce such spoilage microbes during long frozen preservation periods, however there is paucity of information on spoilage microbial load of chicken portions thawed after quick frozen at -10 °C for 6 months.

The poultry processing plants make use of antimicrobial interventions (chlorine antimicrobials) such as aqueous chlorine, chlorine dioxide and Acidified Sodium Chlorite to abate the effects of spoilage bacteria as the storage period increases (Alonso-Hernando et al., 2013). Also, the poultry processing industry had introduced the method of brining for processed chicken with an objective of improving the flavour, taste and tenderness of chicken through different liquid additive solutions (Venter, 2015). Although, there is paucity of scientific report on the effect of high levels of brining on the portion's microbial load and its effects on the chicken, it had been speculated that increased brining levels above 60% could result in high levels of salts in the products which could affect the consumer's health (Alvarado and McKee, 2007; Ellinger, 1972). However, there is paucity of information on the effects of antimicrobial intervention and brining on the level of standard plate counts (SPC), spore counts and psychrophilic counts (log cfu/g) in 6 months frozen chicken portions, as well as the optimum levels of inclusion to attain the lowest microbial count in frozen chicken portions.

Therefore, this study is aimed at determining the relative effects of antimicrobial intervention and brining on spoilage microbial load in long term (6 months) frozen chicken portions; determine the best chlorinated antimicrobial to be used among the commonly used ones in the market; as well as determine the optimal brining levels that will lead to the lowest spoilage bacterial levels in frozen chicken portions.

Materials and methods

Sources of experimental materials and laboratory analysis

This research was carried out following the ethical approval given by the College of Agriculture and Environmental Sciences (CAES) research ethics review committee of University of South Africa (Ethics ref no: 2016/CAES/066). 180 freshly slaughtered broiler chickens from 3 different abattoirs (60 per abattoir) was used for this experiment. They were processed into chicken portions comprising of breasts, thighs and wings per chicken. These abattoirs were high through-put commercial poultry abattoirs, referred to as sources A, B and C. The standard plate, spore and psychrophilic bacterial count were carried out in a Biotechnology laboratory.

Chicken sample preparation, chlorinated antimicrobial and brining applications at the abattoir

The freshly slaughtered and processed mixed chicken portions from each abattoir was divided equally into 2 portions for antimicrobial intervention and brining. Three types of chlorinated antimicrobial were purchased from suppliers - Chlorine dioxide, Aqueous Chlorine, and Acidified Sodium Chlorite and applied at the rate of 50 mg/g to the chicken portions with brush (aseptically) after sub-dividing the portions into 3 equal

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parts aseptically. Thereafter, they were bagged into 1 kg cellophane bags and labelled for identification, and a total of 12 bags per abattoir were randomly selected for refrigeration, comprising of 4 bags per treatment. A total of 24 bags per plant was randomly selected, with 12 bags each for antimicrobial intervention and brining respectively, replicated 3 and 2 times per treatment accordingly. Chlorine antimicrobials - Aqueous Chlorine (Aq. Cl), Acidified Sodium Chlorite (ASC) and Chlorine Dioxide (ClO_2) all sprayed before packaging while the other 12 bags were assigned to 6 brining levels (0%, 15%, 20%, 25%, 30%, and 35% of 1 kg chicken portion) per treatment before quick freezing them for 6 months at -10 °C and thawing thereafter for standard plate counts (SPC), spore counts and psychrophilic counts (log cfu/g).

The brine solutions were prepared in the abattoirs at the following brining concentration levels of 0%, 15%, 20%, 25%, 30% and 35% of 1 kg chicken portion as treatments (*Table 1*). The brine solutions were prepared separately for each brining level in stainless steel tanks that were automated and transported through a flexible hose to the brine injection unit. The processed portion per abattoir for brining were subsequently divided into 6 parts after weighing out 1 kg each, before brining and packaging in a cellophane bag and identified with an indelible marker. A total of 12 bags comprising of 2 bags per treatment were randomly selected for refrigeration at -10 °C for 6 months.

Table 1. *Graded levels of brine mixes applied to each replicate of 1 kg chicken portion*

Ingredients	**Brining (%)***					
	0	**15**	**20**	**25**	**30**	**35**
Salt (g)	0	5.5	7.3	9.1	10.9	12.7
Sugar (g)	0	2.6	3.5	4.4	5.3	6.2
Water (L)	0	24.2	34.9	43.7	52.4	61.1

*Chicken to brine ratio = 80:20 in percentage of 1 kg

After 6 months of refrigeration, cold water thawing procedure at 26 °C was applied to thaw the samples. All of the frozen samples were firmly tied and placed in a leak-proof plastic bag, before submerging in a sterile large bowl filled with water and allowed to thaw for 24 h. The samples were then removed aseptically and sampled for standard plate counts (SPC), spore counts and psychrophilic counts (log CFU/g) in the laboratory which were recorded and analyzed for mean effects.

Standard plate counts (SPC), spore counts and psychrophilic counts (log cfu/g)

The procedure of Woteki (1998) was used to determine the microbiological quality by aseptically collected (60 ± 0.1 g) of the sample (either the breast, wing or thigh randomly) from each replicate. The total plate count was conducted using the procedure of (AOAC, 1995). Psychrophilic bacterial count was carried out according to the procedure of (APHA, 2001). The counting was achieved using the same procedure as in Total Plate count; 1 ml of the series dilutions, then the plates were incubated at 5-7 °C for 10 days. The blended samples were then tested for standard plate counts (SPC), bacterial spores, coliform, staphylococci and yeast and molds by pour-plate method as per APHA (2001) procedures (*Fig. 1*).

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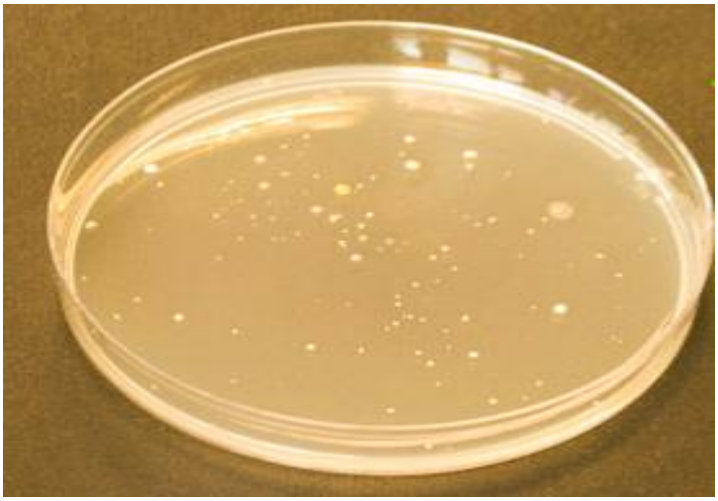

Figure 1. *A petri dish showing a sample colony of psychrophilic bacteria from chicken portions rinsates using the procedure of APHA (2001)*

Statistical analysis

The effects of source, antimicrobial intervention and brining levels on microbiological quality of chicken were analyzed with the general linear model (GLM) procedures of the (SPSS, 2017). The statistical model was:

$$Y_{ijk} = \mu + T_i + B_j + C_k + (TB)_{ij} + (TC)_{ik} + \Sigma_{ijk} \quad \text{(Eq.1)}$$

where Y_{ijk} = the overall observation (Bacterial load); μ = population means; T_i = effect of abattoir source; B_j = effect of antimicrobials; C_k = effect of brining levels; $(TB)_{ij}$ = interaction effect of abattoir source and antimicrobials; $(TC)_{ik}$ = interaction effects of abattoir source and brining and Σ_{ijk} = residual effect.

A breakdown of the experimental design is as shown below:
Treatment 1 = Abattoirs effects only
Treatment 2 = Antimicrobial effects only
Treatment 3 = Brining effects only
Treatment 4 = Abattoirs and antimicrobial agents.
Treatment 5 = Abattoirs and brining levels

Where there was a significant F-test ($P < 0.05$), the Duncan New Multiple Range test method was used to separate the means according to SPSS (2017).

The related responses in Psychrophilic bacterial count to antimicrobial and brining levels were modeled using the quadratic regression model below:

$$Y = a + b_1x + b_2x^2 \quad \text{(Eq.2)}$$

where Y = microbiological load; a = intercepts on Y axis; b = co-efficient of the quadratic equation; x = brining levels in percentages and $b_1/2b_2$ = x value for optimal or minimal responses. The quadratic model was fitted to the experimental data by means of the regression using curve estimation procedure of SPSS (2017). The quadratic model was used because it gave the best fit (Okoro and Mbajiorgu, 2017).

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Results

The application of chlorine antimicrobials had a significant effect ($P < 0.05$) on the chicken portions from within abattoirs used in the study (*Table 2*), they however did not differ between different abattoirs within treatments. Although the values of samples treated with acidified sodium chlorite were consistently lower in all the samples from different abattoirs than those treated with aqueous chlorine and chlorine dioxide, no statistical significant differences were recorded between them. However, between the three chlorine antimicrobials, the ASC consistently recorded a significantly lowest SPC, spores and psychrophilic bacterial count while Aq.Cl recorded the highest ($P < 0.05$) But there were no significant difference between values of spore counts generated from abattoir C. This could be due to all three antimicrobials being made from chlorine, resulting in their effectiveness appearing to be the same in terms of reducing standard plate counts (SPC), spore counts and psychrophilic counts.

Table 2. *Effects of chlorine antimicrobials on standard plate count, spores and psychrophilic bacteria count (logCFU/g) of frozen chicken from 3 commercial chicken abattoirs sampled*

Microbial* counts (logCFU/g)	Abattoirs	Chlorine antimicrobials (50 mg/g)				
		ASC	Aq. Cl.	ClO_2	SEM	P-Value
SPC	A	1.33^{c}	2.50^{b}	2.98^{a}	0.189	0.030
	B	1.33^{c}	2.67^{b}	2.92^{a}	0.221	0.029
	C	1.17^{c}	2.92^{b}	3.11^{a}	0.367	0.045
Spores	A	1.36^{c}	2.72^{b}	3.18^{a}	0.379	0.050
	B	1.37^{c}	3.07^{b}	3.22^{a}	0.221	0.052
	C	2.76	3.02	3.21	0.367	0.560
Psychrophiles	A	1.37^{c}	2.60^{b}	2.98^{a}	0.389	0.030
	B	1.51^{c}	2.57^{b}	2.92^{a}	0.321	0.034
	C	1.42^{c}	2.52^{b}	3.09^{a}	0.467	0.025

^{a,b,c}Means in the same row and column not sharing a common superscript are significantly different ($P < 0.05$) SEM = standard error of mean, P- value = probability value, Aq. CI = aqueous chlorine, ASC = acidified sodium chlorite, CIO_2 = chlorine dioxide

The effect of the brining levels on standard plate count, spores and psychrophilic bacterial count was the same across all three major abattoirs (*Table 3*). However, at 0% brine, the microbiological quality was significantly higher ($P < 0.05$) than any other levels in all three abattoirs. Furthermore, the results have indicated no significant difference of SPC, spores and psychrophilic bacterial count between 15, 20 and 25% across all samples from the three abattoirs. The brining levels between 30 and 35% also has no significant differences ($P < 0.05$) among them for the microbial loads.

The levels of brining and chlorine antimicrobials affected the levels of standard plate count, spores and psychrophilic bacterial load in the frozen chicken portions (*Table 4*). The control samples (0% brine) had significantly higher ($P < 0.05$) bacterial load than all samples injected with various brining levels. At 20% and 25% brining levels however, there was no difference ($P > 0.05$) in bacterial count except for SPC where 20% is significantly higher than 25%.

Application of quadratic regression model to identify optimal brining levels to achieve the least SPC, spore and psychrophilic bacterial count resulted in a significant

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($P < 0.05$) optimum levels (*Table 5*). The effect of percentage brining on average SPC, spore and psychrophilic bacteria count had optimum quadratic values of 20.67 + 20.578 brining – 7.986 brining^2, 80.142 – 31.793 brining + 2.178 brining^2 and 31.77 + 5.909 brining - 3.748 brining^2 respectively. The optimal brining levels were 13.27, 35.87 and 34.10 for SPC, spore and psychrophilic counts respectively. These are the optimal brining level to give the lowest microbial count in 6 months frozen (-10 ± 2 °C) chicken portions. The co-efficient of determination (R^2) which is very high, implies that the optimum brining levels have significant influence in reducing microbial load.

The result of the optimization function indicates that brining has a direct effect on the amount of microbial counts that develop during long term refrigeration; hence, the best brining level of inclusion to achieve the fewest number of bacterial loads is determined (*Figs. 2* and *3*).

Table 3. *Effects of brining levels on standard plate count, spores and Psychrophilic bacteria count (logCFU/g) of frozen chicken from 3 commercial chicken abattoirs sampled*

Microbial* counts (logCFU/g)	Abattoirs	Brining levels (%)							
		0	15	20	25	30	35	SEM	P-value
SPC	A	3.23^{a}	2.67^{b}	2.27^{bc}	2.11^{c}	1.92^{d}	1.33^{d}	0.352	0.001
	B	3.00^{a}	2.33^{b}	2.19^{c}	2.09^{c}	1.89^{d}	1.67^{d}	0.584	0.001
	C	3.13^{a}	1.97^{b}	2.33^{bc}	2.12^{c}	1.87^{d}	1.17^{d}	0.419	0.001
Spores	A	3.18^{a}	2.47^{b}	2.28^{bc}	2.10^{c}	1.89^{d}	1.23^{d}	0.552	0.021
	B	3.25^{a}	2.43^{b}	2.27^{c}	2.11^{c}	1.88^{d}	1.56^{d}	0.444	0.035
	C	3.30^{a}	2.52^{b}	2.25^{bc}	2.08^{c}	1.90^{d}	1.47^{d}	0.319	0.033
Psychrophiles	A	3.93^{a}	3.17^{b}	2.87^{bc}	2.21^{c}	1.52^{d}	1.13^{d}	0.252	0.041
	B	3.60^{a}	3.33^{b}	2.79^{c}	2.14^{c}	1.69^{d}	1.07^{d}	0.384	0.044
	C	4.13^{a}	2.97^{b}	2.43^{bc}	2.02^{c}	1.47^{d}	1.11^{d}	0.219	0.026

^{a,b,c}Means in the same row and column not sharing a common superscript are significantly different ($P < 0.05$). SEM = standard error of mean, P-value = probability value, CLO_2 = chlorine dioxide, Aq. CI = aqueous chlorine, ASC = acidified sodium chlorite

Table 4*. Effects of antimicrobial intervention and brining on microbial counts of mixed chicken portions frozen (-10 °C ±2) for 6 months*

Microbial* counts (logCFU/g)	Antimicrobial intervention* (50mg/g)			Brining (%)						SEM	P-value
	ASC	Aq. Cl.	ClO_2	0	15	20	25	30	35		
SPC	1.17^{c}	2.52^{b}	3.21^{a}	3.33^{a}	2.87^{b}	2.63^{b}	2.32^{c}	1.76^{d}	1.57^{d}	0.267	0.023
Spores	1.47^{c}	3.01^{b}	3.42^{a}	3.25^{a}	2.43^{b}	2.12^{c}	2.11^{c}	1.87^{d}	1.56^{d}	0.412	0.044
Psychrophilic	1.39^{c}	2.30^{b}	3.08^{a}	3.63^{a}	3.32^{b}	2.40^{c}	2.13^{c}	1.70^{d}	1.06^{d}	0.321	0.001

^{a,b,c}Means in the same row not sharing a common superscript are significantly different ($P < 0.05$). SEM = standard error of mean, P-value = probability value, Aq. CI = aqueous chlorine, ASC = acidified sodium chlorite, CIO_2 = chlorine dioxide, *SPC = standard plate count, ASC = acidified sodium chlorite, Aq. CI = aqueous chlorine, CIO_2 = chlorine dioxide

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Table 5. *The optimal brining levels on standard plate count, spores and psychrophilic bacteria count (logCFU/g) using quadratic regression model*

Microbial* counts (logCFU/g)	R^2 values	Optimal Y levels of bacteria	Optimal brining levels (%)	P - Value
SPC	0.988	1.289	13.27	0.001
Spore	0.980	7.299	35.87	0.003
Psychrophile	0.938	0.788	34.10	0.016

SPC = standard plate count, R^2 = co-efficient of determination, P-value = probability value

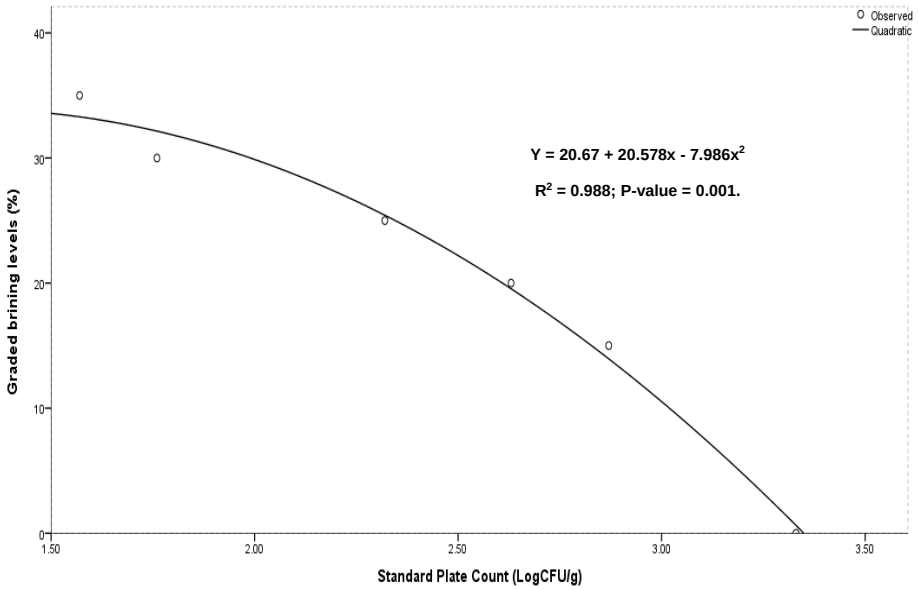

Figure 2. *Quadratic function showing the optimum brining levels of quick frozen chicken for standard plate count*

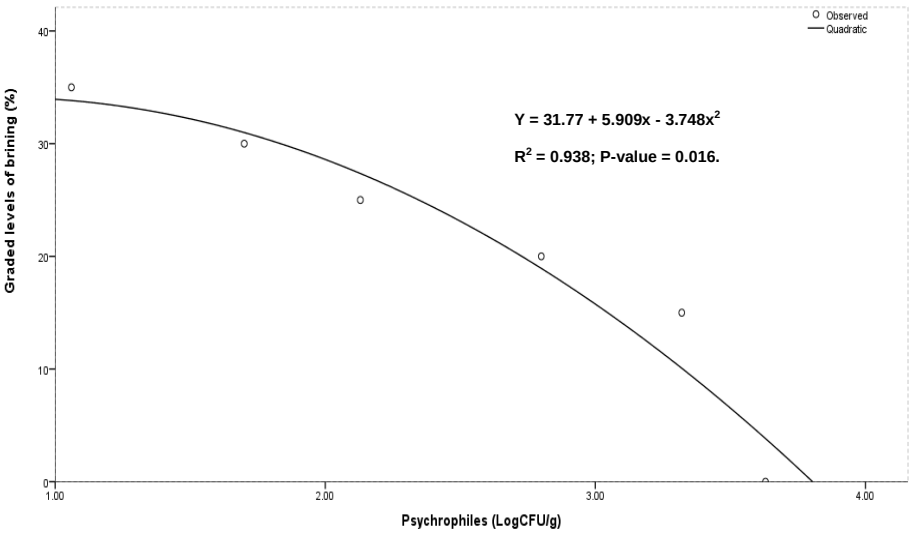

Figure 3. *Quadratic function showing the optimum brining levels of quick frozen chicken for psychrophilic bacterial count*

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Discussion

Antimicrobial intervention and brining of chicken portions are important in preservation and storage of meat as Perez-Chabela and Mateo-Oyague (2004) reported that pathogenic microorganisms are commonly isolated from thawed frozen raw meat with larvae of Taenia spp. and Trichinella spiralis killed after 1-3 weeks of refrigeration at -18 °C or after ultra-rapid freezing at -29 °C of raw meat. Also Lowry and Gill (1984) reported that moulds grew at temperatures lower than -10 °C with Knøchel et al. (2007) also reporting the development of spoilage microbes in non-marinated chicken which increases rapidly when refrigerated. Chlorine is a major antimicrobial intervention agent and its widely used in poultry processing industry being effective against a whole range of microorganism in poultry processing (Purnell et al., 2014) and is regarded as the most effective antimicrobial to control bacterial, viral and protozoan pathogens (Duan et al., 2017; Purnell et al., 2014). However, it is reported that the efficacy of the antimicrobials is determined by the level of attachment of bacterial infection on the chicken skin. This factor might have a slight contribution to the results as the samples were sprayed directly on each sample before the portions were packed. It is reported that chlorine dioxide, when used under commercial operations, has the potential to reduce up to 2- 3 logCFU/g in microbial levels in frozen poultry (Tamblyn and Conner, 1997). The present study indicates that all the abattoirs were not affected by the effects of the antimicrobials when they were compared. However, among the antimicrobials, there was a significant difference in terms of the microbial load of the samples tested with acidified sodium chlorite having the least load while chlorine dioxide having the most load. Although all three chosen antimicrobials originated from chlorine as a base, acidified sodium that reduced more bacteria than all other antimicrobials is in acidic format, a form which is very convenient to lactic acid bacteria that is capable of causing great reduction of microbial load in white meat (Oyarzabal, 2008; Vlahova-Vangelova and Dragoev, 2014). This is also in agreement with the findings of Purnell et al. (2014) showing that acidified sodium chloride could be the most effective antimicrobial to be used in the poultry processing industry.

Brining of chicken slows formation of colony for spoilage bacteria and the bacterial development remains below 1.03 logCFU/g (Knøchel et al., 2007; Vlahova-Vangelova and Dragoev, 2014) for a period of 3 months. Different amounts of brine required for use in chicken preservation has been reported by different authors. Alvarado and McKee (2007) reported that salt within brine was meant to reduce rate of deterioration by reducing the growth of microorganisms in the chicken with higher concentration of salt proven to inhibit the microbial spoilage in white meat. This study has shown that higher level of brining consistently reduces the levels of microbial organisms causing spoilage. Although 5% brine is reported for best quality of meat products when assessing the chicken from sensory properties (Venter, 2015); it did not consider levels of spoilage microbes effect on the meat due to longer periods of storage. This study has shown that at lower levels of inclusion of brine (0-10%), the level of spoilage microbes is still within safe limits after 6 months of refrigeration, implying that higher levels might not be necessary. But as the period of refrigeration increases beyond 6 months, the spoilage bacteria level could increase, hence The United States Food and Drug Administration (USFDA) recommended a storage period of 9 months for chicken and turkey parts (FDA, 2017). Meanwhile, Venter (2015) reported the prohibition of abattoirs from exceeding 15% level of brining by the country's regulatory body resulting in a suggested 20% brining levels by the abattoirs as the most accommodative

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amount for inclusion when considering longer preservation periods. This study however suggests an inclusion of up to 34.10% brining level to optimally remove psychrophilic microbes, and 13.27 for optimal eradication of standard plate count of microbes. Brining at levels up to 60% may reduce the quality of the chicken through dilution of nutrients such as proteins, fat and energy (Tamblyn and Conner, 1997; Whitman and Marshall, 1971). Farber and Idziak (1984) had reported high moisture content in the 60% brining level treated samples resulting in lower salt which has been diluted by the moisture. However, Farber and Idziak (1984) reported that the highest injection level of 60% was tenderer than lower inclusion levels.

The primary preservation agents of brine are salt and sugar, which do not only prevent spoilage of the chicken but serve to inhibit growth of pathogens and spoilage bacteria when applied correctly (Pichpol, 2009; Purnell et al., 2014). These preservative agents have antimicrobial mechanisms which capacitate them to disrupt microbial enzyme activity and weaken their DNA structure. Sugar's preservative mechanism is to accelerate accumulation of antimicrobial compounds from the growth of spoilage microorganisms. This includes conversion of sugar molecules to organic acids by lactic acid bacteria (Vlahova-Vangelova and Dragoev, 2014). Moreover, Vlahova-Vangelova and Dragoev (2014) had reported that development of spoilage bacteria depend on the presence of lactic acid bacteria solutions not sugars. This may explain why the greatest reduction of spoilage microbes is found between 30-35% of brining level in this study.

Conclusion

Generally, this study has shown that usage of antimicrobial intervention and brining could achieve the same level of microbial reduction, hence either of the two could be used when preserving chicken portions. An antimicrobial intervention of acidified sodium chlorite could be used in the preservation of chicken portions, while brining up to the level of 13.27% for reduced standard plate count and 34.10% for optimum psychrophilic bacterial count reduction could be important. Therefore, ASC could be used interchangeably while brining, as it is easily accessible in the market and relatively cheap, making it a better preservative commercially for chicken preservation than other antimicrobials.

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APPENDIX

OUTPUTS FROM SPSS2017

ANOVA for independent variable: chlorine antimicrobials and abattoirs for SPC, spore count and psychrophilic bacterial count

SPC

Descriptive

ABT	ATM2	N	Mean	Std. dev.	Std. error	95% confidence interval for mean		Min.	Max
						Lower bound	Upper bound		
A	1.00	12	1.33072	3.12856	.189034	1.1789	1.1545	1.1789	1.4545
A	2.00	12	2.5012.	4.31611	.224595	1.1743	1.6590	2.1743	2.6590
A	3.00	12	2.98324	4.26046	.367299	1.4597	1.8736	2.4597	3.2396
	Pr.	.87	.0301	3.76552	.073	1.933	.004	.9822	.3236
	Total	36	2.45500	.00268	.66711	1.3957	1.1043	2.00	3.00
B	1.00	12	1.33072	2.12856	.19034	1.3789	1.1545	1.1789	1.545
B	2.00	12	2.6712.	3.10611	.324595	1.7743	1.6590	2.1743	2.5590
B	3.00	12	2.92445	3.30046	.465299	1.3797	1.8736	2.4597	3.0391
	Pr.	.87	.02901	.34552	.303	1.633	.005	.9812	.13226
	Total	36	2.3510	4.1268	.86711	1.2157	1.2043	1.40	2.780
C	1.00	12	1.17204	2.89856	.179034	1.3289	1.2345	1.2739	1.2545
C	2.00	12	2.9212.	3.71611	.52595	1.3443	1.4290	2.2243	2.1190
C	3.00	12	3.11324	3.62046	.26299	1.1957	1.3436	2.8507	3.2766
	Pr.	.87	.045201	.76552	.003	.933	.054	.9822	.2136
	Total	36	2.65500	4.00268	.66711	1.3057	1.2043	2.100	3.00

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	ANOVA					
	ATM2	**Sum of squares**	**df**	**Mean square**	**F**	**Sig.**
	Between groups	48.500	17	24.250	1.562	.025
	Within groups	512.250	35	15.523		
	Total	560.750	52			

1 = ASC, 2 = AQ.CL, 3 = CLO2, ATM = Antimicrobial intervention, ABT = Abattoir

SPORES

Descriptive									
ABT	**ATM2**	**N**	**Mean**	**Std. dev.**	**Std. error**	**95% confidence interval for mean**		**Min.**	**Max**
						Lower bound	**Upper bound**		
A	1.00	12	1.37233	3.12856	.191034	1.2789	1.6545	1.2789	1.2545
A	2.00	12	2.7212.	4.21611	.324595	1.5543	1.8910	2.5743	2.4590
A	3.00	12	3.18324	4.23046	.417299	1.4976	1.9936	2.8597	3.1396
	Pr.	.87	.0501	3.36552	.093	1.7303	.024	.5822	.4246
	Total	36	2.67500	.03268	.46711	1.4157	1.2043	1.90	2.170
B	1.00	12	1.37202	2.33856	.29034	1.1789	1.2545	1.1289	1.745
B	2.00	12	3.0712.	3.00611	.34595	1.2743	1.4390	2.1243	2.990
B	3.00	12	3.22445	3.29046	.40299	1.4797	1.9736	2.9197	3.4391
	Pr.	.87	.0521	.324552	.3503	1.833	.2005	.9212	.12226
	Total	36	2.3510	4.1268	.86711	1.2157	1.2043	1.40	3.780
C	1.00	12	2.7604	2.89856	.179034	1.3289	1.2345	1.2739	1.2545
C	2.00	12	3.0212.	3.71611	.52595	1.3443	1.4290	2.2243	2.1190
C	3.00	12	3.21324	3.62046	.26299	1.1957	1.3436	2.8507	3.2766
	Pr.	.87	.045201	.76552	.003	.933	.054	.9822	.2136
	Total	36	2.15500	4.00268	.66711	1.3057	1.2043	2.100	3.00

	ANOVA					
	ATM2	**Sum of squares**	**df**	**Mean square**	**F**	**Sig.**
	Between groups	55.300	17	124.250	1.62	.015
	Within groups	432.870	35	418.523		
	Total	487.170	52			

PSYCHROPHILES

	Descriptive								
ABT	**ATM2**	**N**	**Mean**	**Std. dev.**	**Std. error**	**95% confidence interval for mean**		**Min.**	**Max**
						Lower bound	**Upper bound**		
A	1.00	12	1.37211	3.02856	.129034	1.8189	1.1445	1.1389	1.2345
A	2.00	12	2.6012.	4.11611	.204595	1.6243	1.3590	2.1543	2.4490
A	3.00	12	2.98024	4.46046	.37299	1.3497	1.4736	2.3397	3.4396

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	Pr.	.87	.0301	3.36552	.083	1.833	.124	.9722	.3636
	Total	36	2.45500	.01268	.44711	1.3457	1.223	2.30	3.20
B	1.00	12	1.51072	2.02856	1.19034	1.2989	1.2545	1.1389	1.445
B	2.00	12	2.5712.	3.30611	.624595	1.3343	1.4890	2.2743	2.4190
B	3.00	12	2.9215	3.10046	.565299	1.6597	1.5636	2.6597	3.0791
	Pr.	.87	.03009	.31552	.5303	1.233	.105	.9512	.13226
	Total	36	2.3510	4.1268	.86711	1.2157	1.2043	1.40	2.380
C	1.00	12	1.42204	2.59856	.179034	1.3289	1.2345	1.2739	1.2545
C	2.00	12	2.5212.	3.91611	.52595	1.3443	1.4290	2.2243	2.1190
C	3.00	12	3.09124	2.62046	.26299	1.1957	1.3436	2.8507	3.2766
	Pr.	.87	.045201	.76552	.003	.933	.054	.9822	.2136
	Total	36	2.65500	4.20268	.66711	1.3057	1.2043	2.150	3.33

	ANOVA					
	ATM2	**Sum of squares**	**df**	**Mean square**	**F**	**Sig.**
	Between groups	79.100	17	44.250	1.62	.005
	Within groups	603.015	35	85.513		
	Total	682.110	52			

Post hoc tests

Homogeneous Subsets

SPC

ATM2
Duncan[a]

ABT	**ATM2**	**N**	**Subset for alpha = 0.05**		
			1	**2**	**3**
A	3.00	12	2.98167		
A	2.00	12		2.50327	
A	1.00	12			1.33067
	Sig.	.025			
B	3.00	12	2.92017		
B	2.00	12		2.67027	
B	1.00	12			1.33017
	Sig.	.025			
C	3.00	12	3.11167		
C	2.00	12		2.92027	
C	1.00	12			1.36721
	Sig.	.025			

Means for groups in homogeneous subsets are displayed
[a]Uses harmonic mean sample size = 12.000

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ANOVA for dependent variable: SPC, spore count and psychrophilic bacterial count on brining levels in different abattoirs

SPC

	Descriptives								
ATM	**PSC1**	**N**	**Mean**	**Std. dev.**	**Std. error**	**95% confidence interval for mean**		**Min.**	**Max.**
						Lower bound	**Upper bound**		
A	.00	6	3.2333	.75277	.30732	.5432	1.7661	2.0433	3.6233
A	15.00	6	2.6667	.75277	.30732	.5565	1.4331	1.3767	2.9567
A	20.00	6	2.2733	.81650	.33333	.0986	1.8932	2.1765	2.1902
A	25.00	6	2.1133	1.4719	.60093	.7821	.98821	1.8886	2.3781
A	30.00	6	1.9166	.51640	.21082	.6786	1.7699	1.9127	2.8086
A	35.00	6	1.3336	.75227	.30732	.5698	1.9883	1.0767	1.9567
	Pr	.87	.00121	1.1912	.61911	1.987	1.8990	.04521	.1765
	Total	36	2.0166	3.7148	.35321	1.007	1.7236	1.2310	3.231
B	.00	6	3.0013	.63277	.32782	.4332	1.6961	2.3343	3.7633
B	15.00	6	2.3334	.72277	.31332	.6715	1.7731	14467	2.5467
B	20.00	6	2.1902	.91250	.41333	.4216	1.5932	2.2765	2.7702
B	25.00	6	2.0913	1.5719	.71093	.3891	1.7821	1.6667	2.4481
B	30.00	6	1.8916	.51640	.43182	.5092	1.6699	1.5127	2.7686
B	35.00	6	1.6713	.55177	.35732	.5923	1.7983	1.3767	1.5867
	Pr	.87	.00111	1.1912	.51914	.7637	1.8954	.07521	.1965
	Total	36	2.0166	3.3348	.58371	.4007	1.5636	1.4210	3.7631
C	.00	6	3.1333	.43237	.29732	.4332	1.6543	2.0433	3.9933
C	15.00	6	1.9667	.76271	.31402	.6665	1.5441	1.5767	2.7867
C	20.00	6	2.3333	.71650	.41933	.8986	1.9344	2.0765	2.5502
C	25.00	6	2.1123	.94719	.61593	.8621	1.4821	1.6986	2.6581
C	30.00	6	1.8726	.41620	.33482	.7686	1.5455	1.4427	2.3486
C	35.00	6	1.1726	.65237	.45732	.7598	1.7881	1.6767	1.9067
	Pr	.87	.00111	1.2912	.44321	.6987	1.7691	.06521	.1865
	Total	36	2.0166	3.1148	.4192	.6707	1.8236	1.410	3.731

ANOVA					
PSC1	**Sum of squares**	**df**	**Mean square**	**F**	**Sig.**
Between groups	459.000	11	91.800	10.5420	.000
Within groups	524.000	30	.800		
Total	983.000	41			

Spores count

Descriptives								
ATM	**PSC1**	**N**	**Mean**	**Std. dev.**	**Std. error**	**95% confidence interval for mean**	**Min.**	**Max.**

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						Lower bound	Upper bound		
A	.00	6	3.1833	.75277	.30732	.5432	1.7661	2.0433	3.6233
A	15.00	6	2.4737	.75277	.30732	.5565	1.4331	1.3767	2.9567
A	20.00	6	2.2833	.81650	.33333	.0986	1.8932	2.1765	2.1902
A	25.00	6	2.1033	1.4719	.60093	.7821	.98821	1.8886	2.3781
A	30.00	6	1.8916	.51640	.21082	.6786	1.7699	1.9127	2.8086
A	35.00	6	1.2336	.75227	.30732	.5698	1.9883	1.0767	1.9567
	Pr	.87	.02121	1.1912	.61911	1.987	1.8990	.04521	.1765
	Total	36	2.6236	3.7148	.55211	1.007	1.7236	1.2310	3.231
B	.00	6	3.2513	.63277	.32782	.4332	1.6961	2.3343	3.7633
B	15.00	6	2.4334	.72277	.31332	.6715	1.7731	14467	2.5467
B	20.00	6	2.2702	.91250	.41333	.4216	1.5932	2.2765	2.7702
B	25.00	6	2.1113	1.5719	.71093	.3891	1.7821	1.6667	2.4481
B	30.00	6	1.8816	.51640	.43182	.5092	1.6699	1.5127	2.7686
B	35.00	6	1.5613	.55177	.35732	.5923	1.7983	1.3767	1.5867
	Pr	.87	.03521	1.1912	.51914	.7637	1.8954	.07521	.1965
	Total	36	2.3166	3.3348	.44441	.4007	1.5636	1.4210	3.7631
C	.00	6	3.3033	.43237	.29732	.4332	1.6543	2.0433	3.9933
C	15.00	6	2.5227	.76271	.31402	.6665	1.5441	1.5767	2.7867
C	20.00	6	2.2533	.71650	.41933	.8986	1.9344	2.0765	2.5502
C	25.00	6	2.0823	.94719	.61593	.8621	1.4821	1.6986	2.6581
C	30.00	6	1.9026	.41620	.33482	.7686	1.5455	1.4427	2.3486
C	35.00	6	1.4726	.65237	.45732	.7598	1.7881	1.6767	1.9067
	Pr	.87	.03312	1.2912	.44321	.6987	1.7691	.06521	.1865
	Total	36	2.0106	3.1148	.4192	.6707	1.8236	1.410	3.731

ANOVA

PSC1	Sum of squares	df	Mean square	F	Sig.
Between groups	513.139	11	102.628	136.837	.000
Within groups	22.500	30	.750		
Total	535.639	41			

Psychrophilic

Descriptives

ATM	PSC1	N	Mean	Std. dev.	Std. error	95% confidence interval for mean		Min.	Max.
						Lower bound	Upper bound		
A	.00	6	3.9333	.75277	.30732	.5432	1.7661	2.0433	3.6233
A	15.00	6	3.1667	.75277	.30732	.5565	1.4331	1.3767	2.9567
A	20.00	6	2.8733	.81650	.33333	.0986	1.8932	2.1765	2.1902
A	25.00	6	2.2133	1.4719	.60093	.7821	.98821	1.8886	2.3781
A	30.00	6	1.5216	.51640	.21082	.6786	1.7699	1.9127	2.8086
A	35.00	6	1.1336	.75227	.30732	.5698	1.9883	1.0767	1.9567

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	Pr	.87	.04121	1.1912	.61911	1.987	1.8990	.04521	.1765
	Total	36	2.3157	3.7148	.25213	1.007	1.7236	1.2310	3.231
B	.00	6	3.6013	.63277	.32782	.4332	1.6961	2.3343	3.7633
B	15.00	6	3.3334	.72277	.31332	.6715	1.7731	14467	2.5467
B	20.00	6	2.7902	.91250	.41333	.4216	1.5932	2.2765	2.7702
B	25.00	6	2.1413	1.5719	.71093	.3891	1.7821	1.6667	2.4481
B	30.00	6	1.6916	.51640	.43182	.5092	1.6699	1.5127	2.7686
B	35.00	6	1.0713	.55177	.35732	.5923	1.7983	1.3767	1.5867
	Pr	.87	.04411	1.1912	.51914	.7637	1.8954	.07521	.1965
	Total	36	2.0166	3.3348	.38371	.4007	1.5636	1.4210	3.7631
C	.00	6	4.1333	.43237	.29732	.4332	1.6543	2.0433	3.9933
C	15.00	6	2.9667	.76271	.31402	.6665	1.5441	1.5767	2.7867
C	20.00	6	2.4333	.71650	.41933	.8986	1.9344	2.0765	2.5502
C	25.00	6	2.0213	.94719	.61593	.8621	1.4821	1.6986	2.6581
C	30.00	6	1.4726	.41620	.33482	.7686	1.5455	1.4427	2.3486
C	35.00	6	1.1126	.65237	.45732	.7598	1.7881	1.6767	1.9067
	Pr	.87	.02611	1.2912	.44321	.6987	1.7691	.06521	.1865
	Total	36	2.0166	3.1148	.2192	.6707	1.8236	1.410	3.731

ANOVA

PSC1	Sum of squares	df	Mean	F	Sig.
Between groups	28.389	11	14.194	1.146	.003
Within groups	408.583	33	12.381		
Total	436.972	41			

Post hoc tests

Homogeneous subsets

SPC

BRINE1
Duncan[a]

ATM	BRINE1	N	Subset for alpha = 0.05			
			1	2	3	4
A	35.00	6	1.33333			
A	30.00	6	1.92167			
A	25.00	6		2.11333		
A	20.00	6			2.27333	
A	15.00	6			2.67333	
A	.00	6				3.23167
	Sig.		.341	.341	.117	.112
B	35.00	6	1.67333			
B	30.00	6	1.89167			
B	25.00	6		2.09333		
B	20.00	6		2.19333		

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B	15.00	6			2.3316	
B	.00	6				3.0017
	Sig.		.341	.341	.117	.112
C	35.00	6	1.17333			
C	30.00	6	1.87167			
C	25.00	6		2.12333		
C	20.00	6		2.33333		
C	15.00	6			1.97333	
C	.00	6				3.13167
	Sig.		.341	.341	.117	.112

Means for groups in homogeneous subsets are displayed
[a]Uses harmonic mean sample size = 6.000

ANOVA for dependent variable: SPC, spore count and psychrotrophic bacterial load for antimicrobial intervention and brining

Antimicrobials intervention

Descriptives

ABT	ATM2	N	Mean	Std. dev.	Std. error	95% confidence interval for mean		Min.	Max.
						Lower bound	Upper bound		
SPC	1.00	12	1.1733	.93361	.84686	1.4694	2.1973	.063	1.00
SPC	2.00	12	2.4720	.77793	1.09059	2.0996	2.9004	1.50	2.50
SPC	3.00	12	2.3048	.77692	1.09030	1.1836	2.9831	2.50	2.50
	Pr.	.87	.023	.33729	.26711	.4457	.6674	.987	.0887
	Total	36	1.772	1.53340	.58890	4.2767	14.6678	7.00	21.00
Spores	1.00	12	1.4733	.45361	.23446	1.3294	2.9273	1.00	3.00
Spores	2.00	12	3.0107	.87293	1.5932	2.1236	3.1004	.605	2.70
Spores	3.00	12	3.4203	.76692	1.0370	1.2656	2.8651	1.09	3.50
	Pr.	.87	.044	1.8762	.4121	.6652	.8875	.876	.987
	Total	36	2.2072	1.61120	.36790	3.4267	7.6678	2.340	8.201
PSY	1.00	12	1.3933	.77461	.67186	1.3344	3.1373	.090	3.00
PSY	2.00	12	2.3040	.59193	.99059	1.4316	2.8704	1.00	1.52
PSY	3.00	12	3.0831	.68222	.43030	1.2836	1.6831	2.50	1.30
	Pr.	.87	.001	1.2331	.3211	.9982	.5542	.4333	.3244
	Total	36	2.0612	1.3140	1.0090	3.5437	6.3378	4.030	5.00

ANOVA

	ATM2	Sum of s	df	Mean	F	Sig.
	Between groups	28.389	8	14.194	1.146	.003
	Within groups	408.583	26	12.381		
	Total	436.972	34			

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Post hoc tests

Homogeneous subsets

ATM2
Duncan[a]

Microbe	ATM2	N	Subset for alpha = 0.05		
			1	2	3
	3.00	12	3.21		
SPC	2.00	12		2.25	
	1.00	12			1.17333
	Sig.		.003		
	3.00	12	3.42		
	2.00	12		3.01	
Spores	1.00	12			1.47
	Sig.		.003		
	3.00	12	3.08		
	2.00	12		2.30	
PSY	1.00	12			1.39
	Sig.		.003		

Means for groups in homogeneous subsets are displayed
[a]Uses harmonic mean sample size = 12.000

BRINING

Descriptives

ABT	Brine	N	Mean	Std. dev.	Std. error	95% confidence interval for mean		Min.	Max.
Microbes						Lower bound	Upper bound		
SPC	.00	6	3.33333	.93361	.84686	1.4694	2.1973	.063	1.00
SPC	15.00	6	2.87267	.77793	1.09059	2.0996	2.9004	1.50	2.50
SPC	20.00	6	2.63167	.77692	1.09030	1.1836	2.9831	2.50	2.50
SPC	25.00	6	2.32223	.66792	.44592	2.0882	2.6551	0.87	3.12
SPC	30.00	6	1.76333	1.2331	.67552	1.7763	2.0898	1.45	3.08
SPC	35.00	6	1.57333	.98872	.43552	0.8778	1.6652	2.45	2.99
	Pr.	.87	.02311	.33729	.26711	.4457	.6674	.987	.0887
	Total	36	11.1944	1.53340	.58890	4.2767	14.6678	7.00	21.00
Spore	.00	6	3.25012	.45361	.23446	1.3294	2.9273	1.00	3.00
Spore	15.00	6	2.43771	.87293	1.5932	2.1236	3.1004	.605	2.70
Spore	20.00	6	2.12111	.76692	1.0370	1.2656	2.8651	1.09	3.50
Spore	25.00	6	2.11066	.93361	.84686	1.4694	2.1973	.063	1.00
Spore	30.00	6	1.87122	.77793	1.09059	2.0996	2.9004	1.50	2.50
Spore	35.00	6	1.56233	.77692	1.09030	1.1836	2.9831	2.50	2.50
	Pr.	.87	0.044	1.2331	.3211	.9982	.5542	.4333	.3244
	Total	36	11.6772	1.3140	1.0090	3.5437	6.3378	4.030	5.00
PSC	.00	6	3.63122	.77461	.67186	1.3344	3.1373	.090	3.00
PSC	15.00	6	3.32122	.59193	.99059	1.4316	2.8704	1.00	1.52

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PSC	20.00	6	2.79341	.68222	.43030	1.2836	1.6831	2.50	1.30
PSC	25.00	6	2.13021	.45361	.23446	1.3294	2.9273	1.00	3.00
PSC	30.00	6	1.69872	.87293	1.5932	2.1236	3.1004	.605	2.70
PSC	35.00	6	1.05782	.76692	1.0370	1.2656	2.8651	1.09	3.50
	Pr.	.87	0.001	1.8762	.4121	.6652	.8875	.876	.987
	Total	36	12.7662	1.61120	.36790	3.4267	7.6678	2.340	8.201

ANOVA

BRINE

	Sum of s	df	Mean	F	Sig.
Between groups	28.389	8	14.194	1.146	.003
Within groups	408.583	26	12.381		
Total	436.972	34			

Post hoc tests

Homogeneous subsets

BRINE1

Duncan[a]

Microbes	BRINE	N	Subset for alpha = 0.05			
			1	2	3	4
SPC	35.00	6	1.57333			
SPC	30.00	6	1.76333			
SPC	25.00	6		2.32333		
SPC	20.00	6			2.6311	
SPC	15.00	6			2.8711	
SPC	.00	6				3.3333
	Sig.		.325	.106	.325	1.000
Spores	35.00	6	1.56112			
Spores	30.00	6	1.87122			
Spores	25.00	6		2.11322		
Spores	20.00	6		2.11876		
Spores	15.00	6			2.42981	
Spores	.00	6				3.6321
	Sig.		.145	.056	.325	1.000
PSC	35.00	6	1.06211			
PSC	30.00	6	1.70212			
PSC	25.00	6		2.13321		
PSC	20.00	6		2.40121		
PSC	15.00	6			3.32112	
PSC	.00	6				3.63112
	Sig.		.325	.106	.325	1.000

Means for groups in homogeneous subsets are displayed
aUses harmonic mean sample size = 6.000

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Optimization outputs

Curve fit

Quadratic for SPC

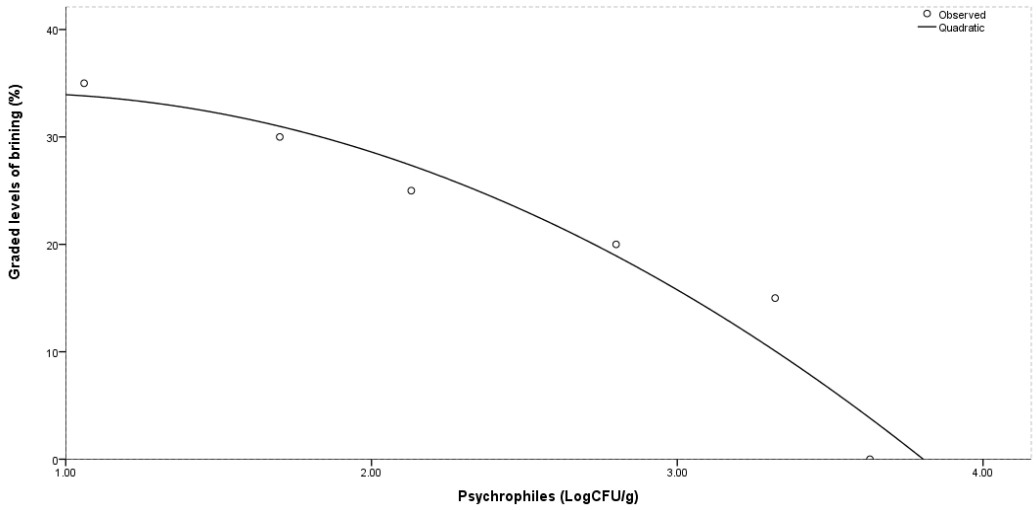
Model summary			
R	**R**	**Adjusted R**	**Std. error of the estimate**
.968	.938	.896	3.999

The independent variable is psychrophiles

ANOVA					
	Sum of s	**df**	**Mean**	**F**	**Sig.**
Regression	722.869	2	361.434	22.606	.016
Residual	47.964	3	15.988		
Total	770.833	5			

The independent variable is psychrophiles

Coefficients					
	Unstandardized coefficients		**Standardized coefficients**	**t**	**Sig.**
	B	**Std. Error**	**Beta**		
Psychrophiles	5.909	12.090	.470	.489	.659
Psychrophiles ** 2	-3.748	2.518	-1.430	-1.488	.233
(Constant)	31.774	13.013		2.442	.092




```
* Curve Estimation.
TSET NEWVAR=NONE.
CURVEFIT
 /VARIABLES=Levels WITH spores
 /CONSTANT
 /MODEL=QUADRATIC
 /PRINT ANOVA
 /PLOT FIT.
```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Curve fit

Quadratic for spores

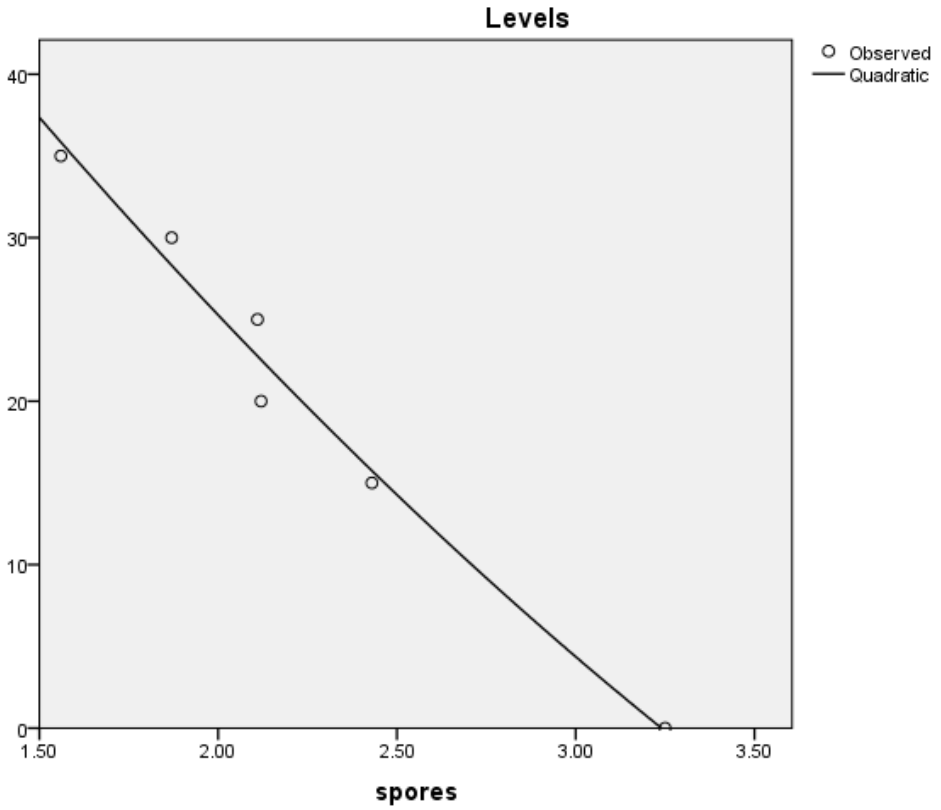
Model Summary			
R	R	Adjusted R	Std. Error of the Estimate
.990	.980	.966	2.281

The independent variable is spores

ANOVA					
	Sum of s	**df**	**Mean**	**F**	**Sig.**
Regression	755.221	2	377.611	72.560	.003
Residual	15.612	3	5.204		
Total	770.833	5			

The independent variable is spores

Coefficients					
	Unstandardized coefficients		**Standardized coefficients**	**t**	**Sig.**
	B	**Std. error**	**Beta**		
spores	-31.793	15.683	-1.487	-2.027	.136
spores ** 2	2.178	3.185	.501	.684	.543
(Constant)	80.142	18.453		4.343	.023



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```
* Curve Estimation.
TSET NEWVAR=NONE.
CURVEFIT
 /VARIABLES=Levels WITH SPC
 /CONSTANT
 /MODEL=QUADRATIC
 /PRINT ANOVA
 /PLOT FIT.
```

Curve fit

Quadratic for psychrophilic

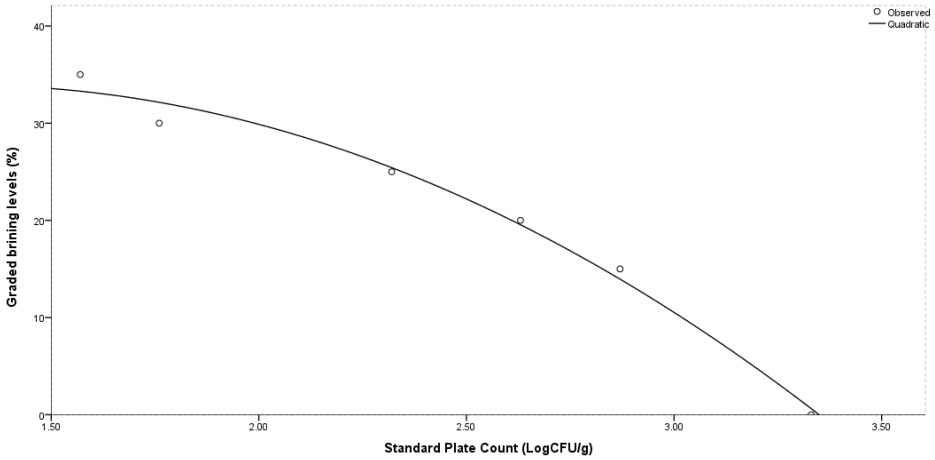
Model summary			
R	**R**	**Adjusted R**	**Std. error of the estimate**
.994	.988	.980	1.773

The independent variable is SPC

ANOVA					
	Sum of s	**df**	**Mean**	**F**	**Sig.**
Regression	761.401	2	380.700	121.082	.001
Residual	9.432	3	3.144		
Total	770.833	5			

The independent variable is SPC

Coefficients					
	Unstandardized coefficients		**Standardized coefficients**	**t**	**Sig.**
	B	**Std. Error**	**Beta**		
SPC	20.578	11.093	1.110	1.855	.161
SPC ** 2	-7.986	2.286	-2.090	-3.494	.040
(Constant)	20.670	12.794		1.616	.205



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