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Effect Of Different Salt Concentration On The Microbiological Quality Of Fresh Meat Sold In The Okigwe Local Abattoir, Imo State, Nigeria.

Okoronkwo, C. U[1]. , Udensi, E. A[2]., and Nwaigwe G. A[3].
Department Of Food Microbiology, Abia State University, Uturu Abia State Nigeria

Abstract: *The effects of different salt concentration on the Microbiological quality of meat were studied in order to fashion out the ideal concentration that will reduce or retard Microbial spoilage of meat and become cheaper than high cost preservative. Ten (10) samples of fresh meat of the same weight (10cm) length, 1cm in diameter, and 2cm in width) were purchased from the Okigwe abattoir and labelled from salt concentration 2-16, and with the following percentages = 2%, 4%, 6%, 8%, 9.8%, 10%, 12%, 14% and 16% respectively. The above percentages were subjected to microbial analysis. The results obtained show that Acetobacter, Vibro Spp., Micrococci spp., and Bacillus spp., were identified with 2% salt concentration: Acetobacter, Vibro spp., Micrococci spp., and Clostridium spp., were present in sample with 4% salt concentration, Staphylococci spp. and Clostridium spp. were predominant in all samples analyzed. The results revealed that microbial growth retarded immensely with an increase of salt concentration and vise versa*
Keywords*: Salt, concentration, fresh meat, microbiological*

I. Introduction

Effective means of preservation is a fundamental requirement of any modern meat distribution, marketing and storage system. The development of efficient methods of preservation has therefore engaged the attention of scientist in Nigeria in recent time (Ikeme, 1990)

Food preservation can be defined as any method of treating food to prolong its shelf life. The healthy inner flesh of meats has been reported to contain few or no microorganism, although they have been found in lymph nodes, bone marrow, and even the flesh (CDCP, 1998). *Staphylococci*, *Streptococci*, *Clostridium* and *Salmonella* have been isolated from the lymph nodes of red meat animals. In order to reduce the activities of microorganisms, the preservation of meats usually is accomplished by a combination of preservative methods. The fact that meat is a very good culture media makes the preservation of meat more difficult than other food products (Sofos et al., 1999). Unless cooling is prompt and after slaughter, meat may undergo undesirable changes in appearance and flavor and may support the growth of microorganism, before been processed in some way for its preservation.

Originally, curing of meat was for the purpose of preserving by salting without refrigeration but most cured meats of the present day have other ingredient added and are refrigerated (Zhao, et al., 2001)

The curing agents permitted today are sodium chloride, sugar, sodium nitrate and vinegar, but only the first three are commonly used. Reports have shown that sodium chloride or common salt is used primarily as a preservative and flavoring agent, sugar adds flavour and also serves as an energy source for nitrate reducing bacteria in the curing solution, sodium nitrate is a color fixation and a bacteriostatic agent in an acid solution (Alonge, 1991). The principle of curing meat is that the salt combines with meat protein to lower the water activity (a_w) value of the cured meats. Four (4) methods by which curing agents are added have been reported which are the dry cure in which the dry ingredients are rubbed into the meat, the injection cure in which a concentrated solution of the ingredients is injected by needle into the meat, the pickle cure in which the meat are immersed in the solution of the ingredients and the direct addition method in which the ingredient will be added directly on the ground meat.

Fresh meat is a nutrient rich substrate that supports the growth of microorganism and is highly susceptible to microbial contamination from a wide variety of physical, microbial, chemical and radiological agents (Soyiri, et al., 2008). Raw retail meats have been identified as potential vehicles for transmitting food born diseases and hence the need for increased implementation of hazard analysis critical control point (HACCP) and consumer safety education effort (Zhao, et al., 2001).

Refrigeration and freezing of meat have been used to preserve meat during shipment over long distance or for holding until times of storage. Considerable quantities of meat now are frozen in home freezers. This method of preservation is not widely used in Nigeria and other undeveloped countries due to inadequate power supply and the cost of alternative power (Hinton, 2000).

The option in Nigeria is to locate a local abattoir near the market where meat will be sold immediately after slaughter. The meat is usually at a temperature of between 30 and 32^0c. Enzymic and microbial degradation under such conditions are rapid, storage and distribution where necessary are carried out under such similar abuse. The food industry incorporates sodium chloride in preservation, processing and manufacturing operations for vegetables, poultry, fish and meat. A number of food preservative techniques have been used for many years, including drying, freezing, canning, filtration, enzymatic treatment, high energy irradiation and chemical such as organic acids, liquid smoke, salt, pumping brine into the tissues of meat(Doyle and Roman, 1982).

There are numerous public health concern about the sanitation of meat processed and sold in Okigwe, Imo state Nigeria. The main challenge to the meat industry is the unhygienic conditions of meat processing which predisposes it to contamination with microorganisms.

The contaminating organisms can facilitate spoilage of valuable meat product rendering it unattractive and tasteless or making it a source of food borne disease (Sofos, 1994).

The objective of this work is to evaluate the effect of different salt concentration on the microbiological quality of fresh meat sold in Okigwe, Imo state, Nigeria.

II. Materials And Methods

Sample Collection:

The raw meat sample was collected from local abattoir in Okigwe Local Government Area of Abia State Nigeria. The salt (NaCl) were purchased in the Eke Okigwe main market

2.1 Sample Preparation

The meat (i.e. boneless beef) were cut into strand first along the muscle fibre and then across the muscle fibre. Uniform size of strips measuring 2cm wide, less than 1cm in thickness and 10cm long, weighing 200grams were collected.

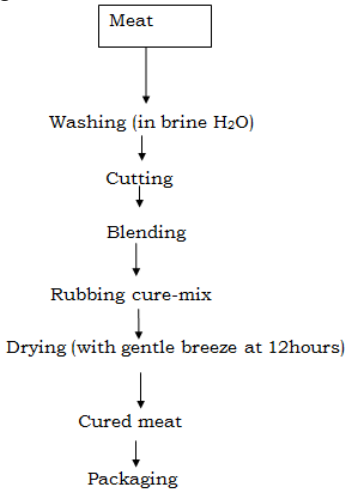
A blend of cure mix were produced by mixing 100g of sodium chloride with 0.5g of salt petry and 0.3g of anti-mycotic agent, 4.0g of the cure mix was used to cure a slice (i.e. 200g) of meat sample. Half of the sample measurement of cured-mix (4.0g) was rubbed completely on slice of meat. The slice was then covered, handled again after 6-7days with the rest of the cure mix. This was done for 30-60minutes every 24hours, the above step was carried out for the other strips of meat with 8.0g, 12.0g, 16.0g, 19.0g, 20.0g, 24.0g, 28.0g and 32.0g of cure-mix which corresponds to concentration of 4.0%, 6.0%, 8.0%, 9.8%, 10.0%, 10.2%, 12%, 14%,and 16.0g of salt (by weight) in meat respectively. Thereafter, each cure-slice of meat was hanged on a hook in a well ventilated room and contained in a netting to protect them from insect. The meat slice was allowed to dry for 12hours after which it was picked into polyvinyl bags and heat scaled ready for analysis.

Determination of moisture content: The moisture content was determined by the gravimetric method of James, 1995 as reported by Okoronkwo et al., (2005)[a].

Determination Of The Hydrogen Ion Concentration (pH)

The pH was measured directly by Genway pH meter model 3310. As reported by Okoronkwo *et al.,* (2013)[b]

Fig 1: Production of dried cured meat



2.3 Microbial Analysis

Preparation Of Media: All the media were prepared according to the manufacturer's instruction.

2.4 Determination Of Microbial Load

The method of the International Commission on Microbiological Specifications for Foods (ICMSF, 1982) as reported by Dosunmu and Bassey, (2003) was adopted. One millilitre of the test sample was diluted on 9ml of sterile distilled water and the diluents mixed and shaked vigorously by shaking 1ml of the resultant mixture, these were aseptically transferred to 9ml of sterile water in a test tube. It was done in an aseptic condition and serially until the sixth dilution was attained. One tenth milliliter (0.1ml) of the 4^{th} and 6^{th} dilution was inoculated with a sterile potato dextrose agar (PDA and nutrient agar (NA) plates respectively in a spread plate technique as reported by Varnam and Sutherland, (1994).

A flamed glass stick shape rod was used to spread the inoculums on the surface of the agar in the plate. The arrangement was done in triplicate for each of the sample. The PDA plates were inoculated at room temperature for 2 to 5days whereas the nutrients agar culture plates were inoculated at 37^0c for 24hours in the incubator. All the plates were observed daily on a Gallenkamp electronic colony counter. The counts from the triplicate plate was obtained and multiplied with the dilution time to obtain the microbial load as the viable microbial colonies per unit weight and expressed in colony forming unit (CFU) per gram of the sample. In all cases, the microbial counts were taken from the plates supporting not less than 300 colonies as reported by Chesbrough, (2005).

2.5 Statistical Analyses

The data generated was analyzed using the analysis of variance (ANOVA) as described by Ihekoronye and Ngoddy (1985). Least significant difference (LSD) test was used to determine if there was a significant difference between means. Significance was accepted at $p<0.05$ degree of freedom.

III. Results And Discussion

2.0 Table 1 Microorganism Identified In The Test Sample Of Cured Meat

Characteristic	Meat	Samples % conc. Of salt (NaCl)									
Morphology	2	4	6	8	9.8	10	10.2	12	14	16	Probable bacterial isolate
Round shape with reddish pigment.	-	-	-	-	-	-	-	+	+	+	-ve halobacter
Ellipsoidal, pink, plump rod with polar flagella	+	+	-	-	-	-	-		-	-	-ve acetobacter
Straight or curved. Spiral purple rod with single flagellum	+	+	-	-	-	-	-	-	-	-	+ve Vibro spp.
Spherical cells in cubical packets	+	+	+	-	-	-	-	-	-	-	-ve Micrococci spp.
Straight inter linking purple chains	-	-	+	+	+	-	-	-	-	-	+ve Staphylococci spp.
Curved irregular pink rods	+	-	-	-	-	-	-	-	-	-	+ve Bacilli spp.
Swollen white ovoid cells	-	+	+	+	-	-	-	-	-	-	+ve Clostridium spp.
Light purple chain varying from slender to short	-	-	-	+	+	+	+	-	-	-	+ve Lactobacillus specie

Key:
+ = positive
− → negative
+ve → gram positive
− ve → gram negative
spp → species

The microbial analysis as shown in table 1 revealed the probable microorganisms that dominated the examined cured meat sample having different salt concentrations.

These probabilities were due to the exhibited morphological characteristic which was distinct among varieties of microorganisms, as well as the observation and inference got from the gram stain test. From the above result, 2% and 4% concentration of salt housed pathogenic and toxic microbes which were also predominant. At this concentration, psychotrophic and pseudomonas cannot be dominated entirely as reported by Murano, (2003). This means that at 2% and 4% salt concentration, meat samples can still undergo bacterial spoilage which can be characterized by surface slimness. The causative organisms can always be isolated and identified from this sliming portion as described by Orjimelukwe et al., 2005. At 6%, 8%, 10% salt concentration, gram positive *lactobacillus*, gram positive *Clostridium* and gram positive *Staphylococcus* were identified as the percentage concentration increases, the population of microorganism on the meat shifted from primarily gram negative bacterial such as *Pseudomonas* to mostly gram positive bacteria. The microbes in these salt concentrations were observed as *Lactobacillus*, *Micrococcus*, *Staphylococci* and *Clostridium*. These organisms can cause a decrease on the pH of the product during growth, which is inhibitory to food born microorganisms. The moisture content of the sample significantly decreased at $P<0.05$ significant. The reduction of the moisture content might be due to the increase in salt concentration which is in tandem with the work of Orjimelukwe et al., (2005) who inferred that sodium chloride (NaCl) removes water from a food product by osmosis. When salt content of food increases, its water decreases thereby plasmolizing the cell walls of pathogenic microbes. Therefore sodium chloride (NaCl) is a water binding agents as described by Murano, (2003).

Table 2 : Moisture Content, pH Content Of Meat At Different Salt Concentrations

	% salt concentration									
Characteristic	2	4	6	8	9.8	10	10.2	12	14	16
Moisture content	55	50	45	40	35	30	25	20	15	10
pH	7	6.5	6.3	6	5	4.8	4.7	4.6	4.5	4.0

Table 3: The Average Plate Count Of Microbial Colonies Obtained From The Cured Meat Samples After Serial Dilution.

Cure mix sample % salt concentration	Dilution 10^{-4}	CFU 10^{-5}	ML 10^{-6}
2	39	28	22
4	34	25	19
6	29	22	17
8	25	19	15
9.8	20	17	13
10	17	15	11
10.2	15	13	9
12	13	10	7
14	10	7	5
16	8	5	3

According to the findings from table 3 above, the effect of the various salt concentrations on the microbial load can be deduced. Therefore, as the concentration of salt increase the microbial load decreases significantly ($P<0.05$). Hence, for every 2% increases in the cure mix, there is roughly 12-13% decrease in the microbial load.

Sensory Evaluation of Uncured and Cookers Sample of Cured Meat

Table 4 Sensory Evaluation Of Cooked Samples Of Cured Meat

Samples	Colour	Texture	Flavour (taste and odour)
2	6.8a	8.7a	8.7a
4	6.6a	8.2a	8.5a
6	6.5a	8.0a	8.3a
8	4.8b	6.5a	6.8a
9.8	4.8b	6.1b	6.2b
10	4.7b	6.2b	4.9c
10.2	4.7b	5.9b	4.1c
12	2.1c	4.1c	2.3d
14	2.1c	4.2c	2.2d
16	1.9c	4.1c	1.1d

Samples mean with different superscript are considered significantly different at $p<0.05$ level.

Table 4 show the sensory evaluation carried out which are colour, texture and flavour (taste and odour). The results obtained revealed that at 2%, 4%, and 8%, there were a lower preference attributed to the samples in terms of colour. This might be due to the lower concentration of salt. At 10% the sample had a mean score 4.7^{b}

while at 12%, 14% and 16% the sample had high mean scores of $2.1^{c,}$ 2.1^{c}, and 1.9^{c} respectively. At these concentrations, the sample had highest preference. Potter and Hotckiss, (1996) reported that cooled cured meat (corned beef) remains red but can turn to brown when uncooked. Cooking improves the colour attributes of cured meat as reported by Rao and Machendrakar, (2003). The flavour (taste and odour) of the cured meat at 2%, 4% and 6% recorded mean scores of 8.7^{a}, 8.5^{a}, and 8.3^{a}. The 9-point hedonic scale interpreted it as having a lower preference of "moderately not improved as flavour" in terms of taste and odour between 8% and 10.2%. The preference scale increased while between 12% and 16%, the mean scores having values of 2.3^{d}, 22^{d} and 1.1^{d} respectively had the highest preference. This might be even to the increase level of salt concentration. Sofos, (2008) reported that where more effective preservation methods are available, the main purpose of curing is to produce unique flavour of meat and its product. They also opined that cooking can also improve the flovour of meat in terms of taste and odour. It was also observed that the texture of the cured meat increased in the scale of preference from the 9-point hedonic scale. The increase ranged from 8.7^{a} – 4.1^{c} as the salt concentration increase (2-16%) which agreed with the report of Murano, (2003) who said that at certain concentration and with increase in the tenderness of meat, the salt have an exact influence in tenderness by softening the connecting tissue protein (Collagen) into a more tender form.

IV. Conclusion And Recommendation

From the result obtained in this study, it can be inferred that an increase in the salt concentration has a significant ($p<0.05$) effect on the microbial quality of cured meat samples. The effect also holds in the number of microbes present in the meat samples. Thus, at higher salt concentrations like 12-16%, they were no pathogenic microorganisms present which are in tandem with the work of Soyiri, et al., (2008).

The pH and moisture were significantly different $p<0.05$ in this study, as the salt concentration increased (2%-16%), the pH became more acidic making the medium unfavourable for pathogenic organism to thrive and proliferate. This agreed with Onyeagba and Isu=, (2006). The sensory characteristics indicated that at higher salt concentrations, the organoleptic characteristics were improved, however, from the results obtained, the optimum concentration of salt can be established as better product amongst sensory appeal, shelf life stability and cost of execution was 14%. This agreed with the work of Banwatt, 1998, Orjimelukwe et al., (2005) and Bolder, (1997).

V. Recommendations

Although excess salt concentration may have undesirable effect on meat quality (Cliver, 2007), we hereby recommend that sodium chloride (NaCl) should be incorporated with other ingredients like sugar, nitrate and anti-oxidants in the meat to minimize any pathogenic or undesirable changes. It was also observed that impurities in sodium chloride (NaCl) can reduce it's effectiveness in extracting NaCl soluble protein because they interfere with the water holding capacity and the emulsifying properties of meat. We also recommend that a purified grade of NaCl should be used. Finally, if the product is not stored in a moisture-proof container, it will equilibrate with the relative humidity of the surrounding air that accounts to its sorption isotherm as described by Cliver, (2007). As a result, vacuum packaging films relatively impermeable to moisture and oxygen (high density polyethelene bag) should be used in meat packaging.

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