Microbiological stability of aerobically packaged quail meat (bone-in) pickle at room temperature (30 ± 2^0C)

H.O. Singh, H.N. Singh, V.P. Singh* and A. Kumar

U.P. Pt. Deen Dayal Upadhyay Veterinary University and Go Anusandhan Sansthan, Mathura, U.P. India 281001
Corresponding author: vetvpsingh@rediffmail.com Ph. +91-9412190865

Journal of Livestock Science (ISSN online 2277-6214) 6:16-22
Received on 16/02/2015; Accepted on 27/03/2015

Abstract

In the present study suitability of different packaging materials for microbiological quality of Japanese quail *(Coturnix coturnix)* meat (bone in) pickle at room temperature for three months was assessed. Pickle was prepared using common products recipes contains common salt, sodium pyro-phosphate, spice ingredients etc. Pressure cooking with water was applied for 20 minutes (400 ml water/ kg of meat). After that cookout was separated and acidification was done with suitable juice @ 2.5% (w/v) of cookout (amla *Emblica officinalis* juice, lemon *Citrus limonium* juice and mango *Mangifera indica* juice but no acid is added in control). The cooked meat was fried in deep fat till brown colour develop (4.5% w/v of total meat) and excess oil was removed. Simultaneously, condiments and spice ingredients were mixed and fried in oil (7.5% w/v of total cooked meat) in same way as the cooking of meat in oil. Acidified cooked out was added and material was heated to constant stirring till boiling. After Addition of fried meat pieces, material was boiled for 2-3 minutes. After cooling packaging was done and prepared quail meat pickle was stored at room temperature for storage study. All products were stable for 90 days at room temperature (30 ± 2^0C) under aerobically packaged containers. Microbiological parameters like Total plate count (TPC), Halophilic count (HC), Enterobacteriacae count (EC), Yeast and mould counts (YMC) were very well under the prescribed limit for meat products. Products were also assessed on TBA and pH basis and found good in condition. Amla juice was found best for quail meat pickle followed by lemon juice, mango juice.

Keywords: Japanese quail; *Coturnix coturnix;* pickle; *Emblica officinalis*; *Citrus limonium*; *Mangifera indica*.

Introduction

In the diversified Indian tradition and culture, many meat products are emerging to fulfill the human needs besides rapid industrialization, rising income, changing food habits, more women now entering the work force, exposure of the population to various mass media particularly television, with preference for processed products, proliferation of fast food outlets. Refrigeration and freezing facilities are not available in most of the rural areas of the country, which gives an opportunity to develop shelf stable food at room temperature. Pickling is one of the methods commonly practiced for preserving fruits and vegetables for human consumption and could be a suitable method for preservation of avian products like egg and meat. Besides its preservative effect, pickling is also considered to be a means of imparting desirable characteristics such as flavour and taste to the food. Pickles are the appetizers and are referred as ready to eat food products. It can be prepared by the use of edible organic acids mainly vinegar and edible oils, table salt, sugar, spices and condiments. Meat pickles have the potential of becoming a ready-to-eat, highly acceptable convenient meat product of indigenous origin. It needs appropriate technology for preparation, packing and preservation in commercial production. Make over pickling methods can be easily practiced under rural conditions with minimum infrastructure and technological facilities, which may help in developing the cottage industries and generate self employment.

Pickle is a food which is relished most by the Indians. By exploiting the likings of people, a good quality pickle can be developed from spent quails which can be carried everywhere with their tiffin and can be kept for longer duration of time at room temperature without utilizing refrigeration and freezing facilities (Gadekar *et al.* 2010). By this way we can better utilize the spent quails in the form of palatable pickle. The stability and suitability of pickle as a food is prime concern for human health. In the light of these observations the present study was designed to assess suitability of different packaging materials for microbiological quality of quail meat (bone in) pickle at room temperature for three months.

Materials and Methods

Source of materials

Spent quails were procured from Central Avian Research Institute (CARI), Izatnagar. Slaughtered, dressed and cut into bone-in pieces of about 3-4 cubic cm in the processing unit of Department of Poultry Science, Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura. The food grade oil and ingredients for spices and condiments were procured from local market of Mathura, dried in hot air oven and ground in grinder. Common salt and sodium pyro-phosphate of food grade were also obtained from local market of Mathura. The packaging materials for storage were purchased from local market Mathura. The media used for microbiological study i.e. nutrient agar, violet red bile agar, potato dextrose agar (make for all: HiMedia lab. Pvt. Ltd., Mumbai) and sea water agar was prepared as per the ICMSF formula.

Recipe and formulations

Spent quail meat (bone-in) pickle was prepared by the standardized formulations based on recipe formulated after several trials. The formulations for preparation of pickle recipes using amla juice (*Emblica officinalis*), lemon juice (*Citrus limonium*), mango (*Mangifera indica*) juice and control are presented in Table-1. To make dry spice mixture, 4.0% cumin- *Cuminum cyminum*, also known as zeera, 4% coriander- *Coriandrum sativum* also known dhania, 3.0% black pepper-*Piper nigrum* also known as *kali mirch*, 2.5% red chilli-*Capsicum annum, also known as Lal mirch*, 5.0% kashmiri chilli- *Capsicum frutescens*, 2.0% turmeric powder-*Curcuma longa, also known as haldi*, 1.5% anise- *Pimpinella anisum*, also called aniseed, 1.0% clove-*Syzygium aromaticum, also known as laung*, 1.0% cinnamon *Cinnamomum verum* also known as dalchini, 1.0 cardamom-*Elettaria cardamomum* also known as choti elaichi were utilized while for green curry stuff, Onion (*Allium cepa*): Ginger (*Zingiber officinale*): garlic (*Allium sativum*) were used in a ratio of 1:1. For preparation of dry spice mix, all ingredients used in preparation were first heated in hot air oven then grinded in mixture for powder preparation. For green curry stuff preparation all three ingredients were peeled and then crust in the grinder to convert it in paste form. The minimum quantity of oil required for pickling was standardized on the bases of oil absorption by cooked meat during deep fat frying and subsequent frying of green curry stuff (condiments) and dry spice mixture during pickle preparation. Preliminary standardization showed that cooked meat had an oil intake of 4.5% during frying of meat pieces whereas,

condiments and dry spice mixture frying (DSM) required about 7.5% oil, thus making a total requirement to be about 12%. The standardized bone-in quail meat pressure cooking 1.2 kg per cm^2 pressure at 120 ^{0}C time was standardized to 20 minutes. This combination was found to be optimum after sensory evaluation.

Table1. Standardized recipes of quail meat (bone-in) pickle

S. N.	Items	Quantity of ingredients			
		C	T1	T2	T3
1	Spent quail meat with bones (g)	1000	1000	1000	1000
2	Acids (amla juice, mango juice, lemon juice (ml)	0	10	10	1 0
3	Common salt (g)	40	40	40	40
4	Condiments (g)	200	200	200	200
5	Spice mix (g)	40	40	40	40
6	Phosphate (g)	3	3	3	3

C= Control; T1= amla juice; T2= lemon juice; T3= mango juice

Methodology

Spent quail meat with bones was cut into small pieces (3-4 cubic cm). They were then cured with common salt and sodium pyro-phosphate. Pressure cooking with water was applied for 20 minutes (400 ml water/ kg of meat). After that cookout was separated and acidification was done with suitable juice @ 2.5% (w/v) of cookout (amla juice, lemon juice and mango juice but no acid is added in control). The cooked meat was fried in deep fat till brown colour develop (4.5% w/v of total meat) and excess oil was removed. Simultaneously, condiments and spice ingredients were mixed and fried in oil (7.5% w/v of total cooked meat) in same way as the cooking of meat in oil. Acidified cooked out was added and material was heated to constant stirring till boiling. After Addition of fried meat pieces, material was boiled for 2-3 minutes. After cooling packaging was done and prepared quail meat pickle was stored at room temperature for storage study.

Analytical Techniques

The physico-chemical characteristics like pH were determined using the method of Strange *et al.* (1977). Thiobarbituric acid value was estimated as per procedure given by Tarladgis, *et al.* (1960). Total plate count (TPC), Enterobacteriaceae count (EC), Halophilic count, Yeast and Mould count (YMC) in different pickles were determined by following the methods as described by ICMSF (1978). Data were analysed statistically following the procedure of Snedecor and Cochran (1994).

Results and Discussion

The mean values of total plate count (TPC), Halophilic count (HC), Enterobacteriacae count (EC), Yeast and Mould counts (YMC) per g of quail meat pickle are shown in table 2 whereas vales for pH and TBA is depicted in fig 1 and 2 respectively.

The non significant effect of treatment on TPC was observed on day 0, significant ($P<0.05$) effect on day 60 and highly significant ($P<0.01$) effect on day 30 and 90. Overall non significant effect of packaging material on TPC and in two way interaction between container and treatment was observed. Increasing trend in microbial number was found with increasing storage period from 0 to 90 days and was reported as log 4.47 to 4.89 in product C, 3.42 to 3.86 in T1, 6.81 to3.75 in T2 and 3.62 to 3.82 per g in product T3. However, total plate count of the quail meat pickle was not affected by the types of rigid jars. These reporting's were in agreement with the finding of Singh and Panda (1984); Chatterjee *et al.* (1969) and Shukla (1997). These authors have observed the total aerobic mesophilic count to the tune of log 4.0 per gram in traditionally pickled quail and spent hen poultry pickle respectively after 120 / 90 days of storage at room temperature.

The results for the effect of packaging materials and storage period on log halophilic counts per gm of quail meat pickle stored at room temperature have been shown in table 2. The log halophilic counts per g was highly significantly($P<0.01$) affected by treatment during whole storage period and significantly ($P<0.05$) affected container on day 0 and 30^{th} whereas, non significantly affected on day 60^{th} and 90^{th}. In this study no significant

effect in two way interaction between container x treatment was observed. In general a numerical increase in the log halophilic count of the quail meat pickle with increasing storage interval was observed. Shukla (1997) and Pal and Bacchil (1990) also reported the increase in halophilic counts of pork and spent hen pickle with the increasing storage period up to 150 days and 90 days respectively. Halophilic bacteria require certain minimal concentration of

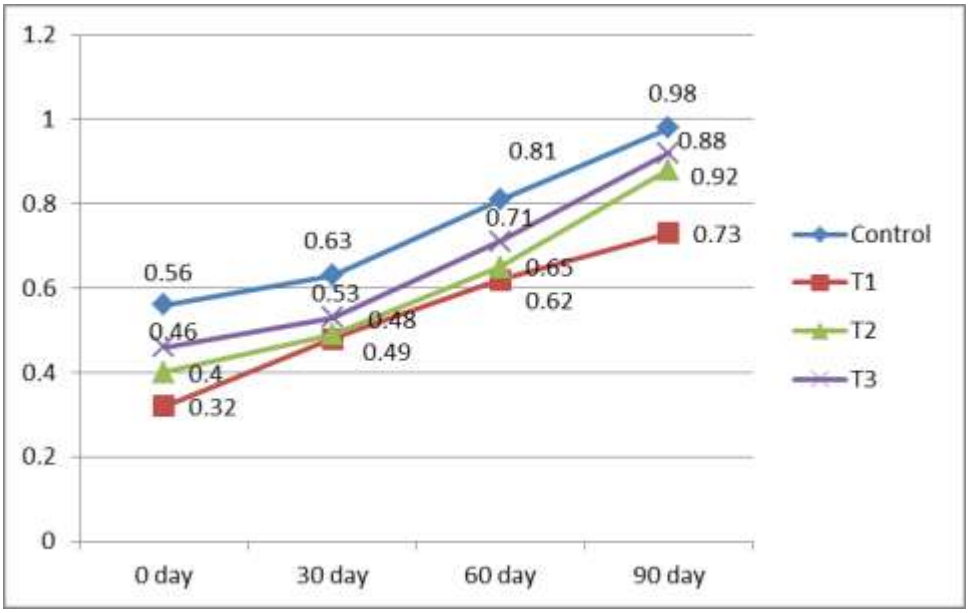

Figure1 . TBA values (mg malonaldehyde per g) in quail meat pickles under storage

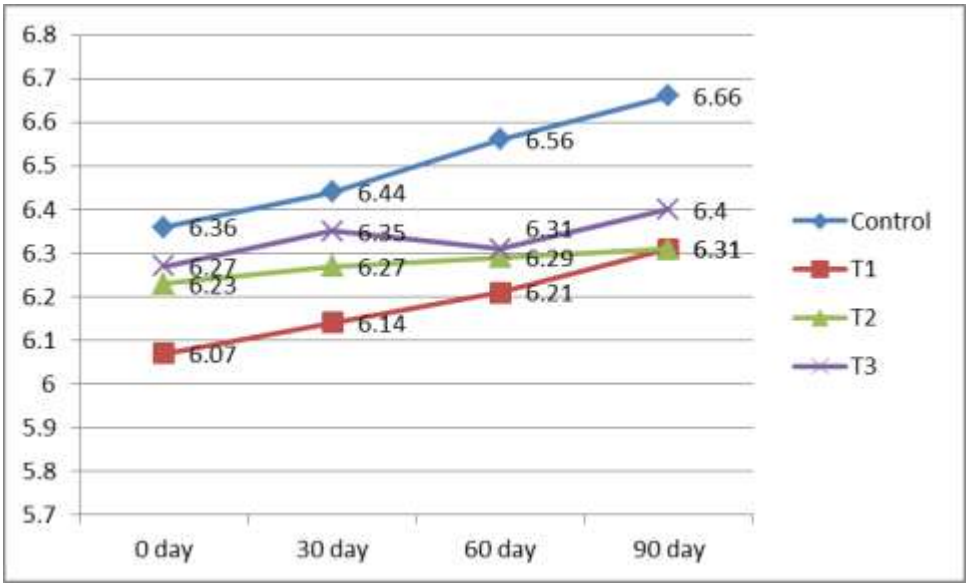

Figure 2. pH values in quail meat pickles under storage

dissolved sodium chloride for growth. Salt concentration for their growth varies from 2 to 30 % depending upon, the microbial species (Frazier, 1978). In the present study the standardized pickle contained 4 % salt.

The results obtained from statistical analysis of the data for log enterobacteriacae counts per gm of quail meat pickle as affected by storage periods and packaging materials has been presented in table 2. In this study non significant effect of containers and two way interaction between container and treatment was observed during whole storage period whereas, treatment affect was highly significant ($P<0.01$) on day 30 and 90 but non-significant in the fresh product.

In YMC study treatment, package materials, and two way interactions between container and treatment all showed non-significant effect on storage period from 0 to 90 except significant effect of treatment on 90^{th} day. In the whole storage period increased YMC count was observed and among the package PET was found better than glass.

Kumar (1987) reported yeast and mould count in the range of 6.53 to 9.56 x 10^3 /g in pork pickles after 120 days of storage at ambient temperature. Shukla (1997) also observed yeast and mould count in the range of log 3.01 ±0.43/g to 3.80±0.03/g in poultry pickle after 90 days of storage at room temperature. Thus the values of yeast and mould counts affected by storage interval of quail meat pickle reported were well in tune with the early reports of Kumar (1987) and Shukla (1997).

In the present study, over all yeast and mould count also increased significantly irrespective of all the factors taken in to account. Multiplication of yeast and mould in these pickle samples may be due to their ability to grow in acidic environment. They can also grow over a wide range of salt concentrations and moisture contents of food materials. Frazier (1978) and Fischer *et al.* (1985) had similar opinion regarding the growth of yeast and mould in pickled foods.

In the present investigation, amla juice, lemon and mango juice were used for preparing quail meat pickle. Among these amla juice was found more effective than others. Amla juice had exerted the same inhibitory effect to spoilage microbes in pickle as shown by acetic acid in the report of Anand and Johar (1958). Frazier (1978) reported that inhibitory action of organic acids is due to direct pH reduction of substrate, depression of internal cellular pH or disruption of substrate transport by alteration of cell membrane permeability. In addition to inhibitory substrate transport, organic acids may inhibit NADH oxidation, thus eliminating supplies of reducing agents to electron transport system. The inhibitory effects of these juices may also be in the line of these modes of actions.

It is also equally important to point-out that higher levels of green curry stuff used in standardized product may also help in reducing the multiplication rate of microbes in pickle stored at room temperature. Beuchat and Golden (1989) reported that most of food borne pathogenic bacteria / yeast examined were sensitive to extracts of garlic and onion. The antimycotic effect of garlic and onion is also well documented Tynecka and Gos (1973). The antimycotic effect of garlic and onion is also well documented by Moore and Atkins (1977); Ekanola *et al.* (2014).

On estimation of pH of all four combinations of quail meat pickle it was evident that control had the pH in between 6.36±0.08 to 6.66±0.07, Product T1 from 6.07±0.07 to 6.31±0.04, T2 from 6.23±0.03 to 6.31±0.05, T3 from 6.27±0.06 to 6.40±0.04. The effect of ambla and lemon was significantly ($P<0.05$) different from the control however, little variation is noticed control and mango juice combinations product. The values for TBA were in the range of 6.36±0.08 to 6.66±0.07, 6.07±0.07 to 6.31±0.04, 6.23±0.03 to 6.31±0.05 and 6.27±0.06 to 6.40±0.04 in product control, T1, T2 and T3 respectively. Chowdhury *et al.* (2002) observed that TBA content increased during refrigerated storage in 10% hydrated TSP extended patties. The increase in pH with the advancement of storage time was evidenced. Nayak and Tanwar (2004) also reported similar results on pH in tofu extended chicken patties.

Conclusion

The microbiological study of these products evidenced higher quality in the product treated with amla juice and these findings were very well agreed with the report of Anand and Johar (1958) as they have reported that acetic acid was inhibitor to spoilage microbes in pickle than other organic acids. There was none significant difference among the total plate counts in all pickle type. Halophilic count in pickle D was significantly different from pickle type A and B while it was none significantly different from pickle C. There were no enterobacteriacae and yeast and mould counts reported in all pickles. Products can be very well utilized up to 3 months by the room temperature storage.

Acknowledgement
Authors are thankful to the Hon'ble Vice-Chancellor of the U.P. Pt. Deen Dayal Upadhyay Veterinary University and Go Anusandhan Sansthan, Mathura, U.P. India for financial and material support to conduct the research.

References

1) Anand JC, Johar DS 1958. Action of certain organic acids on spoilage organisms in mango pickle. Journal of Scientific and Industrial Research 17: 203-205.
2) Beuchat LR, Golden DA, 1989. Antimicrobials occurring naturally in foods. Food Technology 43(1):134-142.
3) Chatterjee AK, Panda PC, Khabda VS, 1969. Poultry pickle. Food industries J. 3: 8-13**.**
4) Chowdhury J, Kumar S, Murthy TRK, 2002. Effect of texturized soy proteins on keeping quality of mutton patties. Environment and Ecology 20(4): 773-777.
5) Ekanola YA, Ogunshe AAO, OlugbodiAjibade RJ, Ojediran YO, 2014. In vitro susceptibility of clinical Candida species to some natural and synthetic antimycotic agents. International Research Journal of Biochemistry and Bioinformatics 4(1): 4-11.
6) Fischer JR, Fletcher DL, Cox NA, Baiely JS, 1985. Microbial properties of hard boiled eggs in a citric acid based preservative solution. Journal of Food Protection 48: 252-256.
7) Frazier WC, 1978. Food microbiology, 3rd Edn . Mc. Graw Hill Book company, New York.
8) Gadekar Y P, Kokane R D, Suradkar US, Thomas R, Das AK, Anjaneyulu A S R, 2010. Shelf stable meat pickles- a review. International Food Research Journal 17: 221-227.
9) ICMSF, 1978. Intern. Comm. Microbial. Specifications for food. Micro-organism in foods. 1. Their significance and methods of enumerations. 2nd edn., University of Toronto Press. Toronto.
10) Kumar V, 1987. Studies on development and keeping quality of pork pickle. M.V.Sc. thesis, submitted to the Deemed University, Indian Veterinary Research Institute, Izatnagar.
11) Moore GS, Atkins RD, 1977. The fungicidal and fungistatic effects of an aqueous garlic extract on medically important yeast-like fungi. Mycologia 69: 341-348.
12) Nayak NK, Tanwar VK, 2004. Effect of tofu addition on physicochemical and storage properties of cooked chicken meat patties. Indian Journal of Poultry Science 39(2):142-146.
13) Pal UK, Bacchil VN, 1990. Effect of chemical spray on quality and shelf life of fresh goat meat at ambient temperatures. Journal of Meat Science and Technology 1: 114-120.
14) Shukla PK, 1997. Studies on preparation and storage stability of poultry pickle. Ph.D. thesis, submitted to C.S.A. University of Agriculture and Technology, Kanpur, U.P.
15) Singh RP, Panda B, 1984. Preparation and storage stability of quail pickle. Indian Journal of Poultry Science 19: 203-206.
16) Snedecor GW, Cochran WG, 1994**.** In: Statistical Methods (9th Ed.). Iowa State University Press, Ames, Iowa.
17) Strange ED, Benedit RC, Smith JL, Swift CE, 1977. Evaluation of rapid test for monitoring alterations in meat quality during storage. Journal of Food Protection 40: 843-847.
18) Tarladgis BG, Watts BM, Yonathan M, 1960. Distillation method for the determination of malonaldehyde ın rancid foods. Journal of the American Oil Chemist's Society 37(1): 44-48.
19) Tynecka A, Gos A, 1973. The inhibitory action of garlic (Allium sativum L.) on growth and respiration of some microorganisms. Acta Microbiologica Polonica. 5B: 51-62.

Table 2 Effects of various preservatives and packaging materials on Total Plate Count, Halophilic counts, Entrobacteriacae count and yeasts and moulds count of spent quail meat pickle at different periods of storage

Group	0 Day				30th day				60th day				90th Day			
	TPC	HC	EC	YMC	TPC	HC	EC	YMC	TPC	HC	EC	YMC	TPC	HC	EC	YMC
SQMP+ G	4.51	3.34	.00	0.00	4.81	3.38	2.01	1.77	4.80	3.48	2.11	1.93	4.91	3.67	2.18	2.04
SQMP+A+G	3.43	2.04	1.52	0.00	3.60	2.07	1.52	1.37	3.66	2.17	1.61	1.51	3.87	2.30	1.61	1.65
SQMP+M+G	3.49	2.18	0.00	0.00	3.66	2.24	1.70	1.54	3.70	2.36	1.77	1.60	3.76	2.48	1.77	1.70
SQMP+L+G	3.63	2.17	0.00	0.00	3.94	2.27	1.78	1.59	4.01	2.39	1.84	1.71	3.84	2.51	1.95	1.85
SQMP+P	4.44	3.23	0.00	0.00	4.70	3.34	1.94	1.66	3.91	3.42	2.08	1.86	4.87	3.59	2.10	1.97
SQMP+A+P	3.41	1.96	0.00	0.00	3.54	2.03	1.38	1.30	3.57	2.09	1.38	1.40	3.84	2.21	1.49	1.55
SQMP+M+P	10.13	2.09	0.00	0.00	3.61	2.16	1.59	1.43	3.86	2.29	1.68	1.52	3.73	2.42	1.73	1.62
SQMP+L+P	3.60	2.11	0.00	0.00	3.87	2.18	1.69	1.49	3.85	2.33	1.74	1.61	3.80	2.41	1.83	1.77
Container																
Glass	3.77	2.43^{a}	0.00	0.00	4.00	2.49^{a}	1.75	1.57	4.04	2.61	1.81	1.69	4.09	2.74	1.88	1.81
Plastic	5.39	2.35^{b}	0.00	0.00	3.93	2.43^{b}	1.65	1.47	3.80	2.53	1.72	1.59	4.06	2.66	1.79	1.73
Treatment																
NP	4.47	3.28^{c}	0.00	0.00	4.75^{b}	3.36^{c}	1.97^{c}	1.72	4.36^{b}	3.45^{c}	2.09^{c}	1.89	4.89^{b}	3.64^{c}	2.14^{c}	2.00^{b}
A	3.42	1.99^{a}	0.00	0.00	3.57^{a}	2.05^{a}	1.45^{a}	1.34	3.62^{a}	2.13^{a}	1.45^{a}	1.46	3.86^{a}	2.25^{a}	1.55^{a}	1.6^{a}
M	6.81	2.14^{b}	0.00	0.00	3.63^{a}	2.20^{b}	1.65^{ab}	1.48	3.78^{a}	2.33^{b}	1.73^{b}	1.56	3.75^{a}	2.45^{b}	1.75^{ab}	1.66^{a}
L	3.62	2.14^{b}	0.00	0.00	3.90^{a}	2.23^{b}	1.74^{bc}	1.54	3.93^{ab}	2.36^{b}	1.79^{b}	1.66	3.82^{a}	2.46^{b}	1.89^{b}	1.81^{ab}
SEM	.80	.11	0.00	0.00	.12	.11	.05	.06	.10	.11	.06	.06	.12	.12	.06	.05
Sig Level																
Container	NS	P<0.05	NS	NS	NS	P<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Treatment	NS	P<0.01	NS	NS	P<0.01	P<0.01	P<0.01	NS	P<0.05	P<0.01	P<0.01	NS	P<0.01	P<0.01	P<0.01	P<0.05
Container X Treatment	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

SQMP -Spent Quail Meat Pickle; G-Glass; A-Amla juice; M-Mango juice; L-Lemon juice, P- Plastic; NP- No preservative, TPC- Total Plate Count; HC- Halophilic count; EC- Entrobacteriacae count; YMC- Yeast and Mould count, NS- Non significant; Means bearing different superscripts within a column differ significantly (P<0.05)