The Pharma Innovation Journal 2022; 11(12): 5364-5367

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(12): 5364-5367

www.thepharmajournal.com
Received: 09-10-2022
Accepted: 12-11-2022

Shubha Singh
Department of Livestock Products Technology, College of Veterinary Sciences and Animal Husbandry, LUVAS, Hisar, Haryana, India

Meena Goswami
Department of Livestock Products Technology, College of Veterinary Sciences and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India

Vikas Pathak
Department of Livestock Products Technology, College of Veterinary Sciences and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India

Vivekanand
Veterinary Officer, Animal Welfare and Training-Brooke, Uttar Pradesh, India

Corresponding Author:
Shubha Singh
Department of Livestock Products Technology, College of Veterinary Sciences and Animal Husbandry, LUVAS, Hisar, Haryana, India

Study of storage stability of hurdle technology based shelf stable chicken pickle at ambient temperature

Shubha Singh, Meena Goswami, Vikas Pathak and Vivekanand

Abstract
The present study was carried out to evaluate the effect of acidulants and humectants on storage stability of shelf stable chicken pickle. Chicken pickle was prepared as per method, prescribed by Das *et al*. (2013) with slight modifications. An attempt was made to improve the shelf life of steam cooked chicken pickle by incorporating acidulant i.e. lactic acid at 1% level and humectants i.e. glycerol at 3% level (GY), honey at 6% level (HY) and sorbitol at 6% level (SB) separately. It can be concluded that the more stable chicken pickle could be prepared by incorporating 1% lactic acid and 6% honey.

Keywords: Chicken pickle, acidulant, humectant, shelf stable, microbial count

1. Introduction
Chicken meat pickle is a shelf stable intermediate moisture type product. It is value added convenient product containing various ingredients like meat, spices, condiments, oil, vinegar and other food additives. Cooking also improves palatability of meat pickle and causes destruction of microorganisms present in meat. In meat pickle spices are added primarily to enhance the flavour however some spices are known to exert antibacterial and antioxidant effects. Chicken meat pickle has appreciable sensory quality with lower microbial, yeast and mould counts. However quality characteristics of chicken pickle are also dependent on characteristics of meat, formulation and processing technique as well as time/temperature evolution during cooking. Any ingredient or technique leading to higher moisture content or high pH may spoil the pickle with flourish growth of micro-organisms and lipid oxidation. Such adverse changes can be prevented in terms of microbial stability and safety of traditional and novel foods like chicken pickle with combination of several preservation factors known as hurdles. Therefore, present study was planned to evaluate the effects of acidulant and humectants on shelf life of chicken pickle.

2. Material and Method
The experiments were conducted in the Department of Livestock Products Technology, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura. Live spent poultry birds were procured from Department of Poultry Science, DUVASU, Mathura. These birds were taken, given rest for 1-2 hours and then slaughtered at Meat Processing Laboratory at Department of Livestock Products Technology, College of Veterinary Sciences and Animal Husbandry, DUVASU, Mathura following the standard procedure (Halal method). The lean carcass was eviscerated and dressed carcass was kept for conditioning in a refrigerator at 4±1 °C for 4–6 hours and then frozen at -18 °C till further processing. All other ingredients like salt, mustard oil, vinegar, spices of Agmark grade and condiments etc required for product preparation were procured from local market of Mathura. All the chemicals used in the study were procured from Hi Media Laboratories (P) Ltd, Mumbai, India. Thermo rigid air tight PET containers were sourced from local market for packaging and were pre-sterilized by exposing to U.V. light for 30 minutes before use.

2.1 Preparation of Spice Mix
The ingredients in desired ratio were procured from local market, dried at 45±2 °C for 2 hours followed by grinding in food grinder (Inalsa Mixie) and sieving through mesh. The spice mix was stored in pre sterilized low density polyethylene bags and used as per requirement. The composition of spice mix is given in Table 1.

Table 1: Composition of Spice Mix

Serial No.	Ingredients	Percentage (%)
1.	Coriander powder (Dhaniya)	15
2.	Cumin seed (Jeera)	15
3.	Caraway seed (Ajwain)	10
4.	Fennel seeds (Soanf)	10
5.	Black pepper (Kalimirch)	10
6.	Red chilli powder	08
7.	Dried Ginger powder (Soath)	08
8.	Cinnamon (Dalchini)	05
9.	Clove (Loang)	05
10.	Black cardamom (Badi elaichi)	05
11.	Mace (Javitri)	05
12.	Nutmeg (Jaifal)	02
13.	Green cardamom (Choti elaichi)	02
	Total	100%

2.2 Preparation of chicken pickle

The chicken pickle was prepared as per method followed by Das *et al*. (2013) [3] with slight modifications (Singh *et al*., 2019) [11]. Frozen dressed meat was thawed at refrigeration temperature overnight and thawed meat was cut into smaller chunks of 1-2 inch. All the ingredients *i.e.* common salt, mustard oil, vinegar, turmeric powder, condiments and spice mix were weighed accurately as per the formulation. Meat chunks were mixed with 1% salt and 1% turmeric powder and kept for 30 minutes for marination. The marinated chicken meat was then pre-cooked with appropriate cooking method for optimum time. Pre-cooked meat chunks were then fried at 175±5 °C in pre warmed mustard oil to get golden brown colour. Condiments were prepared and fried in separate kadahi in preheated mustard oil along with mustard seeds and then spice mix and salt were added to it. Finally precooked-fried meat chunks and vinegar were added to it and cooked for 5 minutes. Thus, prepared chicken meat pickle was cooled down to room temperature and then packed in pre sterilized air tight PET container, where remaining heated mustard oil was filled in container till it's top without leaving any air space. Chicken pickle was left for next 2 days at ambient temperature for maturing and then used for further analysis. The formulation used for preparation of chicken pickle is given in Table 2.

Table 2: Formulation used for preparation of chicken meat pickle

S.N.	Ingredients	Weight
1	Chicken meat	1000 gm
2	Mustard oil	500 gm
3	Salt	30 gm
4	Dry Spice mix	30 gm
5	Condiments	80 gm
6	Vinegar	100 ml
7	Turmeric powder	10 gm
	Total	100

2.3 Analysis of product

The developed chicken meat pickle were evaluated for their storage stability at ambient temperature (25±2 °C) according to APHA (1992) [1]. Each sample was opened in an inoculation chamber of a laminar flow (Model: RH-58-03. Science tech, India) pre-sterilized by ultra-violet (UV) radiation. 10 g of sample from this was aseptically weighed and transferred to a pre-sterilized mortar containing 90 ml of sterile 0.9% saline solution. The sample was homogenized using a sterile pestle for 2 min for uniform dispersion and to get a 10^{-1} dilution of the sample. To prepare 10^{-2} dilution, 1 ml of this diluted solution was quantitatively transferred and then mixed uniformly in a test tube containing 9 ml of sterile 0.9% saline solution. Again 1 ml of 10^{-2} dilution was added to 9 ml of 0.9% sterile saline solution and mixed to obtain 10^{-3} dilution and so on. Preparation of samples and serial dilutions were done near flame in a horizontal laminar flow apparatus observing all possible aseptic conditions. Serial dilutions were made as per the requirement. Sample were analysed for total plate count, *Coliform* count, *Salmonella* count, lipolytic count, yeast and mould count at every 6^{th} day till one month or incipient spoilage is evident.

2.4 Statistical analysis

The data generated from various trials under each experiment were pooled and analyzed by statistical method of one way-ANOVA and mean±S.E using SPSS-16.0 software package developed as per the procedure of Snedecor and Cochram (1995) [13], and means were compared by using Dunkan's multiple range test at 5% level (Dunkan, 1995) [4].

3. Result and Discussion

3.1 Total plate count

The results pertaining to total plate count (TPC) of control and treatments are presented in table 3. There was no significant difference between control and treatments on 0 day, however TPC of SB, GL and HY were significantly (p<0.05) lower than treatments from 10^{th} to 60^{th} day of storage. Lower TPC in treatments might be due to addition of acidulants and humectants in chicken pickle providing oxidative and microbiological stability to products. These findings may also be supported by results in present study, where addition of different humectants significantly (p<0.05) reduced water activity of chicken pickle. There was no significant difference among the treatments upto 30^{th} day; thereafter HY had significantly (p<0.05) lower TPC than GL and SB from 40^{th} to 60^{th} day due to osmotic effect, high sugar concentration, presence of bacteriostatic and bactericidal factors (hydrogen peroxide, antioxidants, lysozyme, polyphenols, phenolic acids, flavonoids, methylglyoxal, and bee peptides) in honey (Israilli, 2014) [6]. The mean total plate count of control as well as treatments increased significantly (p<0.05) with progression of storage, however microbial count was reported to remain satisfactory even after 60 days of storage in all products as the counts remained in the range of 2.47-3.64 log cycles. The microbiological standard suggested by USDA in cooked meat in respect to SPC is 10^5/g for acceptability of the product and log 7.0/g is considered as indicative of starting of spoilage (Panda, 1971) [9].

Table 3: Total Plate Count (log_{10}cfu/g) (Mean±SE) of hurdle technology based shelf stable chicken meat pickle during storage at ambient temperature

Treatment	Storage Period (Days)							
	0 Day	10 Day	20 Day	30 Day	40 Day	50 Day	60 Day	Treatment Mean
S2	1.08^{e}±0.02	1.74^{dA}±0.07	2.14^{cdA}±0.20	2.68^{bcA}±0.10	3.17^{bA}±0.19	2.96^{abA}±0.05	3.64^{aA}±0.16	2.49±0.13
GL1	0.73^{f}±0.23	1.14^{efB}±0.05	1.51^{deB}±0.08	1.90^{cdB}±0.08	2.24^{bcB}±0.02	2.39^{bB}±0.08	3.03^{aB}±0.13	1.87±0.12
HY2	0.70^{de}±0.22	1.14^{cdB}±0.03	1.39^{cdB}±0.08	1.72^{bcB}±0.17	2.08^{bC}±0.29	2.14^{abC}±0.07	2.47^{aC}±0.05	1.78±0.12
SB2	0.72^{d}±0.23	1.17^{cdB}±0.04	1.47^{cB}±0.07	1.83^{bcB}±0.06	2.22^{bB}±0.31	2.45^{abB}±0.06	3.10^{aB}±0.09	1.85±0.12
Storage Mean	0.81±0.09	1.30±0.05	1.63±0.08	2.03±0.09	2.47±0.13	2.57±0.05	3.18±0.07	

Overall means bearing different superscripts in a row (a, b, c, d.....) differ significantly (p<0.05)
Overall means bearing different superscripts in a column (A, B, C, D....) differ significantly (p<0.05)

3.2 Yeast and mould count

The results pertaining to yeast and mould of control and treatments are presented in table 4. There was no yeast and mould growth in control and treatments on 0 day. The growth was detected on 10th day onwards in all products, where no significant difference was observed between control and treatments upto 20th day. Soldatou *et al.* (2009) [14] also reported negligible yeast and mould count on 0 day of storage of chicken meat snacks. With further progression of storage, S2 had significantly (p<0.05) higher growth than treatments from 30th to 60th day. Lower yeast and mould count in treatments proved the antifungal activity of humectants viz. sorbitol, glycerol and honey. There was no significant different among the treatments throughout the storage period. Rout *et al.* (2015) [10] also observed no significance difference in yeast and mould count of chicken pickle incorporated with different organic acids during storage for 90 days. The mean yeast and mould count of control as well as treatments increased significantly (p<0.05) with progression of storage. The results are also in agreement with Shukla and Srivastava (1999) [12] who reported significant (p<0.05) increase in of yeast and mould count during storage of acidic meat products due to low moisture, high acidity, salt contents. The microbiological quality of chicken pickle remained satisfactory for entire period of storage as the counts remained in the range of 3 log cycles which is similar to the observations of Pal and Agnihotri (1994) [8].

Table 4: Yeast and mould count (log_{10}cfu/g) (Mean±SE) of hurdle technology based shelf stable chicken meat pickle during storage at ambient temperature

Treatment	Storage Period (Days)							
	0 Day	10 Day	20 Day	30 Day	40 Day	50 Day	60 Day	Treatment Mean
S2	ND	0.59^{e}±0.26	1.37^{de}±0.06	1.94^{cdA}±0.29	2.27^{bcA}±0.23	2.83^{abA}±0.21	3.47^{aA}±0.06	1.78±0.18
GL1	ND	0.37^{d}±0.23	0.97^{cd}±0.19	1.39^{cB}±0.13	1.61^{bcB}±0.11	2.12^{abB}±0.15	2.65^{aB}±0.16	1.30±0.14
HY2	ND	0.34^{d}±0.21	0.76^{cd}±0.24	1.29^{bcB}±0.07	1.61^{bB}±0.16	2.01^{abB}±0.18	2.40^{aB}±0.18	1.20±0.14
SB2	ND	0.37^{d}±0.23	0.94^{cd}±0.18	1.39^{cB}±0.14	1.56^{bcB}±0.05	2.05^{abB}±0.11	2.53^{aB}±0.16	1.26±0.13
Storage Mean	ND	0.40±0.11	1.01±0.09	1.50±0.09	1.76±0.09	2.25±0.10	2.76±0.11	

Overall means bearing different superscripts in a row (a, b, c, d.....) differ significantly (p<0.05)
Overall means bearing different superscripts in a column (A, B, C, D....) differ significantly (p<0.05)

3.3 Lipolytic count

The results pertaining to lipolytic count of control and treatments are presented in table 5. Lipolytic count is the best indicator of fat oxidation and microbial spoilage of high fat foods. There was no significant difference between control and treatments upto 20th day thereafter GL, SB and HY had significantly (p<0.05) lower count than S2 from 30th to 60th day due to water binding effect of humectants and organic acid resulting into lower water activity and microbial growth in product. There was no significant difference among the treatments throughout the storage period, except on 50th and 60th day where HY had significantly (p<0.05) lower lipolytic count than GL and SB due to presence of various phyto-chemicals and phenolic compounds in honey exhibiting antimicrobial properties (Bertoncelj *et al.*, 2007) [2]. The mean lipolytic count of control as well as treatments increased significantly (p<0.05) with progression of storage. Igbinedion *et al.* (1983) [5] also observed significant increase (p<0.05) in lipolytic count of vacuum packaged fresh pork at later stage of storage under refrigeration.

Table 5: Lipolytic count (log_{10}cfu/g) (Mean±SE) of hurdle technology based shelf stable chicken meat pickle during storage at ambient temperature

Treatment	Storage Period (Days)							
	0 Day	10 Day	20 Day	30 Day	40 Day	50 Day	60 Day	Treatment Mean
S2	0.54^{f}±0.24	1.18^{ef}±0.06	1.42^{de}±0.08	1.89^{cdA}±0.18	2.32^{bcA}±0. 09	2.68^{abA}±0.19	3.21^{aA}±0.15	1.89±0.14
GL1	0.54^{d}±0.24	0.56^{d}±0.25	0.92^{cd}±0.19	1.27^{bcdB}±0.06	1.54^{bcB}±0.05	1.97^{abB}±0.22	2.49^{aB}±0.20	1.33±0.12
HY2	0.36^{e}±0.22	0.53^{de}±0.24	0.80^{cde}±0.27	1.21^{bcdB}±0.06	1.48^{bcB}±0.05	1.53^{abC}±0.20	2.18^{aC}±0.14	1.24±0.12
SB2	0.36^{e}±0.22	0.59^{de}±0.27	0.97^{cde}±0.22	1.33^{bcdB}±0.11	1.57^{bcB}±0.06	1.94^{abB}±0.05	2.48^{aB}±0.12	1.32±0.12
Storage Mean	0.45±0.11	0.71±0.11	1.03±0.10	1.43±0.07	1.73±0.07	2.03±0.10	2.59±0.10	

Overall means bearing different superscripts in a row (a, b, c, d.....) differ significantly (p<0.05)
Overall means bearing different superscripts in a column (A, B, C, D....) differ significantly (p<0.05)

3.4 Coliform count

There was no *Coliform* growth in any product throughout the storage period which due to their destruction of organisms during cooking which was much above their death point i.e.

57 °C and hygienic conditions maintained during processing and analysis of product. Kumar *et al*. (2015) [7] also reported no *Coliform* growth in chicken spread stored under refrigeration in PET jars.

3.5 Salmonella count
In present study, *Salmonella* count was not detected on any day of storage period which might be due to high processing temperature, hygienic handling and packaging of product.

4. Conclusion
The microbial count of honey, glycerol and sorbitol were significantly (p<0.05) lower than control during storage. However, all products were well accepted upto 60th day of storage. Among the treatments, HY had lower microbiological count till the end of the storage. It can be concluded that well acceptable hurdle technology-based chicken pickle may be prepared by precooking of marinated meat under steam without pressure for 15 minutes with incorporation of 1% lactic acid as acidulant and 6% honey as humectants. This product may be at least well acceptable at room temperature for 60 days on the basis of microbiological studies.

5. Acknowledgement
The authors want to show gratitude to the Department of livestock products technology, central institute for research on goat, Makhdum for providing all kind of facilities and support required to carry out the current investigation.

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