RESEARCH ARTICLE

Effect of Vacuum-Packed Refrigeration Storage on Low-Fat Antioxidant-Rich Kadaknath Chicken Sausages

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Abstract

This study was aimed to investigate the impact of vacuum-packed refrigeration storage on quality of low-fat-antioxidant rich Kadaknath chicken sausages. The study examined the changes in physico-chemical, microbiological properties and sensory attributes. The pH, thiobarbuturic acid (TBA) and free fatty acid (FFA) values were lower as compared to control throughout the storage. A progressive and significant ($p<0.05$) increment in the pH, TBA and FFA values of control as well as treatment was observed with the advancement of storage in vacuum packaged sausage. The total plate count followed a significantly ($p<0.05$) increasing pattern from 0 to 28th day. Psychrotrophic count under vacuum packaging was not detected upto 21st day of storage either in control or in treatment and was detected on 28th day of storage. Lipolytic count was not detected on initial day in control or treatment. It was detected from 7th day to 28th day of storage both in control as well as in treatment. Yeast and mold as well as coliform were not detected during the entire period of storage. The mean scores for all the sensory attributes for both control as well as treatment sausage decreased gradually with increasing storage period. Results showed that vacuum packaging helped in preserving the antioxidant properties of the sausage, thereby extending its shelf life without compromising quality.

Key Words: Antioxidant, Kadaknath, Low fat, Sausage, Vacuum packaging.

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Introduction

Growing consumer demand for processed and finished chicken products requires the ability to develop poultry products and add new value to them (Maradini *et al,* 2017). Poultry meat like Kadaknath was used for these studies. Kadaknath meat, into processed products like sausages, offer a healthier alternative to consumers. Vacuum packaging is a widely used technique in the food industry to extend the shelf life of products, vacuum sealing sausage preserve its nutritional value and sensory characteristics for an extended period, ensuring consumers receive high-quality product. Kadaknath is the only indigenous breed of black chicken from India which originates from the Jhabua district of Madhya Pradesh. Kadaknath was granted Geographical Indication (GI) by the government as per the norms set on 30 July 2018. Kadaknath chicken contains 25-27% protein compared to other chicken breeds. Its meat has lower cholesterol (0.73-1.05%) than white chicken (Sharma *et al.,* 2022).

To change consumer attitudes towards meat consumption, adding non-meat ingredients such as animal fat substitutes, natural antioxidants and antimicrobials, preferably from plant sources is practiced to improve the nutritional quality and shelf life of meat products (Hygreeva *et al.,* 2014). Fat is responsible for quality characteristics such as juiciness, texture, meaty flavor, cooking yield and characteristic aroma. Eating foods high in fat and calories has various long-term risks of hypertension and heart disease. Fat substitute plays a role in the physical and sensory properties of conventional fat (Tay *et al.,* 2018). Sago (Metroxylonsagu) is used as a fat substitute in meat products (Nayak and Pathak, 2018). Sago provides only carbohydrate value and is low in protein, vitamins and minerals and is used as an adhesive, binder, gelling agent and emulsion stabilizing agent. Grape (*Vitis vinifera*) seed extract (GSE) retards lipid oxidation in meat during storage, most likely due to the fact that GSE is a rich source of polyphenolic compounds, especially proanthocyanidins. They suppress the levels of reactive oxygen intermediates and used as antioxidant in products (Kaur *et al*, 2015). Extending the shelf life of meat products is one of the technological requirements to fulfil the consumer's interest. The use of packaging techniques is therefore

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key to meeting these needs. The overall intent of vacuum packaging is to eliminate some or all of the oxygen that is largely responsible for chemical or microbial degradation of the product (Perdue, 2009). This study investigated the effectiveness of vacuum-sealed, low-fat, antioxidant-rich Kadaknath sausages on refrigeration storage.

Materials and Methods

Source of Raw Material

Kadaknath chicken of 5-6 months of age was procured from the Department of Poultry Science, NDVSU, Mhow (India). The chicken was slaughtered using a scientific procedure and meat packed in low-density polyethylene (LDPE) bags was brought to the lab. The meat was deboned. The samples were frozen at -18°C until further usage and stored for conditioning in a refrigerator at 4±1°C for 6-8 h. After being partially thawed for 15 h at 4±1°C, the samples were used. Ingredients like spice mix, sago flour, GSE used in the study were procured from standard firm and local market as per availability and requirement of the study. All the chemicals and microbiological media used in the study were of analytical grade and procured from Hi Media laboratories (P) Ltd, Mumbai. Low density polyethylene (LDPE) bags of 250 gauge thickness were sourced from local market for packaging and were pre-sterilized by exposing to U.V. light for 30 min before use.

Preparation of Kadaknath Chicken Sausage

Formulation is shown in Table 1. Kadaknath Chicken meat was partially thawed overnight, cubed and twice minced in an Electronic mincer. A bowl chopper was used to prepare the meat emulsion (Seydelmann K20, Ras, Germany). Salt and sodium tripolyphosphate were added to a pre-weighed quantity of minced chicken meat and the mixture was chopped for 2 to 3 min. The addition of ice flakes was followed by 2 min of additional chopping. Refined vegetable oil was slowly incorporated while chopping till it was completely dispersed in the batter. Condiments paste, dry spices mix, sugar and corn flour, sago flour, GSE were added. Until the required consistency of the emulsion and uniform distribution of all the ingredients were achieved, chopping was maintained. Weighed quantity of emulsion was stuffed in natural casing using sausage filler. Then sausages were cooked in hot air oven at 90°C for 30 min. Two types of products were prepared, *viz.,* Kadaknath chicken sausage without sago flour and GSE (Control-C), and Kadaknath chicken sausage with 4% sago flour and 0.25% GSE (Treatment-T).

The product was vacuum packaged separately in LDPE films & stored under refrigeration. The samples were evaluated for changes in physicochemical, microbiological properties and sensory attributes from 0 day and then at 7 days interval of vacuum packaged till spoilage for following parameters: pH, thiobarbuturic acid value, free fatty acid value, microbiological quality (Total plate count, Psychrotropic count, Lipolytic count, Coliform count along with Yeast and Molds count) and sensory evaluation.

Table 1: Formulation for control and treatment (low-fat antioxidant-rich) Kadaknath chicken sausage

Ingredients	Control	Treatment
Meat (%)	71.0	71.0
Corn flour (%)	3.0	3.0
Vegetable oil (%)	8.0	4.0
Condiments (%)	3.0	3.0
Ice-flakes (%)	10.0	9.50
Spices (%)	1.6	1.6
Salt (%)	2.0	2.0
Sugar (%)	1.0	1.0
STPP (%)	0.4	0.4
Sago flour (%)	0.0	4.0
GSE (%)	0.0	0.25
Total	100	100

Analytical Procedure

Physico-Chemical Parameters: The pH of the samples was determined using a digital pH meter (WTW, Germany, model pH 330i). The thiobarbuturic acid (TBA) value was estimated as per procedure given by Tarladgis *et al.* (1960). Free fatty acid (FFA) value was determined by modified AOCS method (Koniecko, 1979).

Microbiological Analysis: The microbiological properties under vacuum packaging were evaluated with the Total Plate count, Psychrotrophic count, Lipolytic count, Coliform count, Yeast & mold as per the procedure described in APHA (1992) using Nutrient agar with some modifications.

Sensory Evaluation: The sensory quality of samples was evaluated by using 8 point descriptive scale (Keeton, 1983), where 8 denoted extremely desirable and 1 denoted extremely poor.

The data obtained in the study on various parameters were statistically analyzed on 'SPSS 16.0' software package as per standard methods of Snedecor and Cochran (1994).

Results and Discussion

Physico-Chemical Parameters

The pH value of C as well as T sausage increased significantly ($p<0.05$) with the advancement of storage period. However, the significant ($p<0.05$) difference in pH value between C and T on 28th day of storage was noticed. It might be due to the accumulation of metabolites of bacterial action and deamination of proteins by the growth of certain Gram-negative bacteria. Such pattern of subsequent increment in the pH value was due to the liberation of metabolites from the bacterial activities as the microbial load enhanced with the storage period (Table 2). Badole *et al.* (2019) in chicken patties noticed increasing in pH value under vacuum packaged at refrigeration temperature.

The TBA value gradually increased with advancement of storage. Significant ($p<0.05$) difference was observed from

14th day of storage. Slower increase in TBA value in treatment group might be due to vacuum packaged meat was more resistant to lipid oxidation because the availability of oxygen is more important for the lipid oxidation. Similar trends in TBA value were observed by Meena *et al* (2021) in vacuum packaged different meat products incorporated with GSE at refrigeration storage. FFA values differed significantly ($p<0.05$) between C and T throughout storage. FFA values of T were observed lower as compared to C on all days of storage (Table 2). Nayak *et al.* (2014) and Chauhan *et al.* (2018) also reported increasing FFA value with the advancement of storage period in different meat products. Devi *et al*. (2018) also reported similar pattern of increasing FFA content with the advancement of storage in vacuum packaged meat sausage.

Microbiology Analysis

The data in Table 3 show that the total plate count (TPC) followed a significant ($p<0.05$) rise from day 14th to 28th day of storage in C as well as T sausages. Significant ($p<0.05$) difference was observed between C and T throughout the storage on respective interval of storage period. It could be due to incorporation of GSE in sausages which could be attributable to phenolic acids and flavonoids present in GSE (Narkhede, 2012). The increase in total plate count in vacuum packaged chicken sausage was in accordance with Dhagare *et al.* (2022). Psychrotrophic counts were not detected upto 21 day of storage either in C or T. This could be due to sufficient heat treatment during cooking which drastically injured and killed the psychrotrophs in sausage. Psychrotrophs were detected on 28th day of storage in both C and T and showed significant ($p<0.05$) difference between C and T. This might be due to recovery of injured organism and then multiplication during subsequent period of storage. The count remained within the permissible limit of log 4.6 CFU/gm as reported by Cremer and Chipley (1977) in cooked meat products. Our findings supported the observations of Mehta *et al*. (2013) in vacuum packaged chicken patties at refrigeration temperature. Lipolytic counts were not detected on initial day of storage either in C and T, but was detected on 7th day of storage. However, count was increased significantly ($p<0.05$) from 7th day to 28th day of storage in both C and T, while significant ($p<0.05$) difference was observed between C and T on 21st and 28th day of storage. Bharti *et al.* (2011) also reported similar findings in vacuum packaged chicken tikka at refrigeration temperature. In current study, Yeast and mold as well as coliform were not detected during the entire period of storage in both C as well as T. The absence of coliform is due to their destruction during cooking above their death point of 57°C. Further hygienic practices were followed during and after preparation of product. Similar results were reported by Sudheer *et al.* (2011). Absence of Yeast and mold detection

Table 2: Effect of refrigerated storage (4±1°C) on the pH, TBA and FFA values (Mean ±SE) of vacuum packaged low-fat antioxidant-rich Kadaknath chicken sausages

Chemical values	Treatment	Storage days				
		0	7	14	21	28
pH of sausage	Control	6.10^{a}±0.05	6.20^{b}±0.13	6.24^{c}±0.16	6.29^{c}±0.08	6.43^{Ad}±0.09
	Treatment	6.05^{a}±0.08	6.17^{b}±0.07	6.26^{c}±0.11	6.28^{cd}±0.01	6.31^{Bd}±0.02
TBA (mg malonalde-hyde/kg)	Control	0.461^{a}±0.015	0.493^{a}±0.003	0.674^{Ab}±0.007	0.895^{Ac}±0.005	1.248^{Ad}±0.007
	Treatment	0.449^{a}±0.016	0.484^{a}±0.002	0.530^{Bb}±0.011	0.711^{Bc}±0.019	1.135^{Bd}±0.005
FFA (% oleic acid)	Control	0.213^{Aa}±0.001	0.257^{Aa}±0.003	0.486^{Ab}±0.002	0.674^{Ac}±0.003	0.888^{Ad}±0.002
	Treatment	0.204^{Ba}±0.001	0.229^{Ba}±0.001	0.383^{Bb}±0.003	0.645^{Bc}±0.003	0.821^{Bd}±0.002

Means bearing different superscripts row wise (a, b, c, d) and column wise (A, B) within parameters differ significantly ($p<0.05$).

Table 3: Effect of refrigerated storage (4±1°C) on the microbial count (Mean ±SE) of vacuum packaged low-fat antioxidant-rich Kadaknath chicken sausages

Microbial count	Treatment	Storage (days)				
		0	7	14	21	28
Total plate count (cfu/gm)	Control	0.86^{Aa}±0.022	2.59^{Ab}±0.022	2.63^{Ab}±0.030	3.61^{Ac}±0.027	4.14^{Ad}±0.030
	Treatment	0.58^{Ba}±0.035	2.21^{Bb}±0.026	2.35^{Bb}±0.029	3.17^{Bc}±0.023	4.12^{Bd}±0.032
Psychrotrophic count (cfu/gm)	Control	ND	ND	ND	ND	0.84^{A}±0.095
	Treatment	ND	ND	ND	ND	0.62^{B}±0.26
Lipolytic count (cfu/gm)	Control	ND	0.89^{a}±0.44	1.25^{a} 0.23	1.97^{Aab}±0.18	2.98^{Ab}±0.20
	Treatment	ND	0.75^{a}±0.14	1.18^{a}±0.12	1.67^{Bab}±0.21	2.10^{Bb}±0.16

* ND= Not Detected, Means bearing different superscripts row wise (a, b, c, d) and column wise (A, B) within parameters differ significantly ($p<0.05$).

Table 4: Effect of refrigerated storage (4±1°C) on the sensory attributes (Mean ±SE) of vacuum packaged low-fat antioxidant-rich Kadaknath chicken sausages

Sensory attributes	Treatment	Storage days				
		0	7	14	21	28
General appearance	Control	7.15^{d}±0.15	7.11^{d}±0.24	6.85^{c}±0.13	6.66^{b}±0.37	6.18^{a}±0.19
	Treatment	7.20^{d}±0.02	7.15^{d}±0.02	6.90^{c}±0.02	6.71^{b}±0.04	6.22^{a}±0.03
Flavour	Control	7.16^{c}±0.21	7.04^{c}±0.16	6.85^{c}±0.36	6.37^{Ab}±0.14	5.87^{a}±0.24
	Treatment	7.18^{c}±0.22	7.07^{c}±0.18	6.80^{c}±0.33	6.32^{Bb}±0.18	5.92^{a}±0.28
Texture	Control	7.28^{c}±0.24	7.12^{bc}±0.28	6.98^{bc}±0.28	6.78^{b}±0.34	6.10^{Aa}±0.23
	Treatment	7.21^{c}±0.19	7.18^{bc}±0.17	6.95^{bc}±0.24	6.85^{b}±0.12	6.18^{Ba}±0.24
Mouth coating	Control	6.77^{c}±0.11	6.67^{bc}±0.25	6.58^{ab}±0.31	6.52^{a}±0.35	6.49^{a}±0.13
	Treatment	6.58^{b}±0.27	6.60^{b}±0.32	6.62^{b}±0.28	6.57^{b}±0.28	6.45^{a}±0.16
Juiciness	Control	7.30^{c}±0.11	7.10^{Abc}±0.14	7.00^{Abc}±0.12	6.62^{Ab}±0.16	6.18^{Aa}±0.03
	Treatment	7.36^{c}±0.18	7.28^{Bbc}±0.20	7.24^{Bbc}±0.15	6.90^{Bb}±0.17	6.48^{Ba}±0.02
Overall acceptability	Control	7.27^{c}±0.03	7.18^{c}±0.14	6.87^{b}±0.04	6.68^{Ab}±0.02	6.02^{Aa}±0.03
	Treatment	7.25^{c}±0.04	7.20^{c}±0.17	6.91^{b}±0.07	6.75^{Bb}±0.01	6.38^{Ba}±0.04

Means bearing different superscripts row wise (a, b, c, d) and column wise (A, B) within parameters differ significantly ($p<0.05$).

might be due to absence of favorable condition like humid and aerobic climate for the growth of yeast and mold during the experiment. These findings agreed with the observation recorded by Indumathi and Arun (2017) in vacuum packaged restructured chicken chunk, and also by Reddy *et al.* (2020) in vacuum packaged turkey meat sausages incorporated with carrot and radish paste at refrigeration temperature.

Sensory Evaluation

The score for general appearance did not show significant difference in between C and T sausage throughout storage period. This might be due to partly preventive nature of the myoglobin oxidation by GSE, which in turn delays the surface color deterioration in vacuum environment. However, score for general appearance remained higher in T compared to C throughout the storage. This decline in color scores encountered during storage could be due to nonenzymatic browning resulted from reaction between lipid oxidation products and amino acids (Man *et al.,* 1995). Mean flavor score between C and T sausage did not differ significantly throughout the storage period, except on 21st day. This might be due to antioxidant effect of grape seed extract, which decreases the intensity of off-flavor generation by unknown metal chelator (Brannan and Mah, 2007). No significant difference in score of texture upto 21 day of storage was recorded between C and T. However, on 28th day the difference was significant ($p<0.05$). Further, with the advancement of storage period the texture score was decreased and became significant ($p<0.05$) from 14th day of storage in both C as well as T. The score of mouth coating between C and T sausage did not differ significantly during the storage. Further, within group with the progress of the storage from 14th day, significant ($p<0.05$) difference was observed in C and T (Table 4). Nayak *et al.* (2019) reported the decreasing value of mouth coating with the advancement of storage in functional chevon patties under refrigeration.

There was no significant difference in the score of juiciness on initial day, then further significantly ($p<0.05$) higher scores for treatment from 7th day onwards were noticed as compared to C, whereas within the group the score gradually decreased and showed significant ($p<0.05$) difference with the advancement of storage. This might be due to dehydration of the product during storage period. Uikey *et al.* (2018) reported the decreasing value of juiciness with the advancement of storage in chicken meat products. The mean overall acceptability score did not differ significantly between C and T sausages upto 14th day and the value differed significantly ($p<0.05$) on days 21st and 28th of the storage. Whereas, within the group upon subsequent storage the scores gradually decreased significantly ($p<0.05$) from 14th day of storage and lowest score observed on 28th day. It might be due to synergistic effect of increasing pH and microbial load in respective treatment during the storage. The possible reason for decrease in overall acceptability during storage could be due to decline in flavor, color and juiciness as a result of protein denaturation, lipid oxidation and dehydration of the meat products. Similar observations of decreasing overall acceptability scores with increasing storage were reported by Badole *et al.* (2019) in vacuum packaged Kadaknath chicken patties and by Chauhan and Nayak (2022) in Kadaknath chicken patties under refrigeration storage.

Conclusion

The developed product was assessed for physico-chemical, microbiological and sensory attributes at regular interval (Day 0, 7, 14, 21, 28) till spoilage was evident. A progressive and significant increment in the pH, TBA and FFA values of sausage was observed with the advancement of storage. The mean score for all sensory attributes decreased gradually with increasing storage period and compare between the groups (C&T) sensory rating of T were higher than C. From the study it was concluded that microbial count as well as sensory attributes remained well

below the permissible level and product was stable up to 28 days of storage under refrigeration (4±1°C).

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