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Effect of ginger extract and papain powder incorporation on physico-chemical and microbial quality of buffalo male calf meat rolls during refrigeration (4±1 °C) storage studies

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Abstract
The study was conducted with an objective to evaluate the effect of Ginger Extract and Papain Powder on shelf life of buffalo male calf meat rolls at refrigeration (4±1 °C) storage. The meat rolls were prepared with incorporation of 5% Ginger Extract (GE1) and 0.25% Papain Powder (PP1) and Combination of Ginger extract and Papin Powder in ration of 1:3 and compared with control (C) meat rolls. The products were assessed for their physico-chemical, sensory and microbiological quality at 0, 4, 8, 12, and 16 days. However, the sensory scores and moisture content decreased and microbial load, TBA value and free fatty acids (FFA) increased as the days of storage increased for control as well as treated products. The rate of decline in sensory score and moisture content and rate of increase in microbial load, TBA vale and FFA during storage were higher in control samples as compared to treated meat rolls. It was concluded that Ginger Extract (5%) and Papain Powder (0.25%) incorporation also showed their antioxidant and antimicrobial effects, by keeping the treated products organoleptically acceptable and microbiologically safe up to16 days of refrigeration storage.

Keywords: ginger extract, papain powder, buffalo calf, rolls, shelf-life

Introduction
While food has long been used to improve health, our knowledge of health is now being used to improve foods. The understanding of relationship between nutrition and health has resulted in the development of concept of functional foods. The processing of meat leads to generation of many functional compounds beneficial to human health but also pose a health hazard due to high amount to added salt and fat that somehow is proved to be a predisposing factor for cardiovascular diseases, diabetes mellitus and cancer (Cross *et al*., 2010) [3].
Ginger extract by regulating blood pressure, improving circulation of the blood, lowering cholesterol and making blood less sticky may be able to help lower the risk of heart disease. Its ingredients are active against bacteria and also help to treat fungal and viral infections while antioxidant effects are becoming of interest.
Papain is more effective when injected into the product due to its poor ability to penetrate surfaces (Brooks, 2007). However, another study by Maiti *et al.* (2008) showed that papain infusion with forking technology was more effective for tenderizing hen meat cuts than injection. While Grover *et al.* (2005) [5] concluded that sodium tri polyphosphate has a synergistic effect on papain in increasing the tenderness of chicken gizzard.
Hence, this study was conducted to evaluate the effect of Ginger extract and Papain powder on sensory and sensory and microbial qualities of buffalo meat rolls during refrigeration storage.

Materials
Buffalo male calf
Healthy male buffalo calves of 10-12 months age reared under similar feed and management conditions were obtained from the Livestock farm of the College of Veterinary Sciences, LUVAS, Hisar.

Papain powder and Ginger extract
Readily available papain enzyme powder procured directly from standard reputed firm (SRL,

Bombay) was used throughout the study. For ginger extract, local variety of fresh ginger procured from Hisar market was washed, peeled and sliced. This fresh ginger was then homogenized with distilled water, mixed and filtered through muslin cloth.

Condiments mix and Salt, additives and water

Fresh onion and garlic were procured from the local market of Hisar. They were separately peeled and a fine paste was prepared in domestic grinder. The condiment mix was prepared by mixing onion and garlic paste, respectively in 2:1 ratio and packed in LDPE bags and stored at -18±1 °C till further use.

Table salt (Tata Chemicals Ltd., Mumbai), binder (semolina) and ground nut oil (Ginni brand) were procured from the local market. Trisodium polyphosphate (Hi-Media Laboratories Pvt., Ltd., Mumbai) and sodium nitrite (SD Chemicals) and chilled water were used.

Spice mix

The fresh spice ingredients were procured from local market of Hisar. After cleaning, the spices were oven dried at 45±2ºC for 2 h. These ingredients were then ground in domestic grinder and sieved through fine mesh. The fine powders of spice ingredients so obtained were mixed in standardized proportion (Table 4) to prepare the spice mixture and were stored in a moisture proof polyethylene (PE) bags till further use.

Table 1: Composition of spice mix

Ingredients	Per cent (w/w)
Aniseed (Soanf)	10.40
Black pepper (Kalimirch)	07.40
Capsicum (Mirch powder)	10.40
Caraway seeds (Ajwain)	10.40
Cardamom dry (Badi Elaichi)	05.15
Cardamom dry (Chhoti Elaichi)	02.00
Cinnamon (Dalchini)	05.15
Cloves (Laung)	05.00
Coriander (Dhania)	18.60
Cumin seeds (Zeera)	15.50
Dry ginger powder (Soanth)	06.00
Mace (Javitri)	02.00
Nutmeg (Jaifal)	02.00
Total	100

Methods

Processing of meat

The calves were slaughtered and dressed as per the standard procedure in the slaughter house of the LPT department, COVS, LUVAS. Carcasses were washed thoroughly and deboned manually after trimming of fat and connective tissue. Deboned meat was frozen for 24 hours and then minced in an electrical mincer and used for preparation of meat rolls.

Preparation of papain powder extract

Papain, which is available in the purified form as a powder was used throughout the study.

Different concentrations of papain (0.25%, 0.50% and 0.75% W/W) were used for marination of buffalo male calf meat chunks. Required concentration of papain was dissolved in distilled water and used 15%W/V to facilitate uniform mixing and distribution of enzymes. Thus it comprises of following four treatments:

1. 0% control : only 15ml distilled water.
2. 0.25% (W/W) papain : 0.25 g papain + 15 ml distilled water.
3. 0.50% (W/W) papain : 0.50 g papain + 15 ml distilled water.
4. 0.75% (W/W) papain : 0.75 g papain + 15 ml distilled water.

Preparation of Ginger extract (GE)

As the ginger rhizome is easily available in the market, it is used in the fresh form for further studies. 100 g fresh ginger was homogenized with 100 ml chilled distilled water in a mixer and filtered through muslin cloth. Crude extract was used at different concentrations. 3cm×3cm × 3cm^3 uniform sized buffalo calf meat chunks were randomly divided into 4 groups and they were marinated with different concentrations (0, 5, 7, 9% W/V) of GE.

Required concentration of G.E. was diluted in distilled water and used @ 15% W/V of meat chunks. Thus it comprises four different treatments:

1. 0% control : only 15 ml distilled water.
2. 5% (W/V) G.E : 5 ml extract + 10 ml distilled water.
3. 7% (W/V) G.E : 7 ml extract + 8 ml distilled water.
4. 9% (W/V) G.E : 9 ml extract + 6 ml distilled water.

Preparation of combination of Papain powder (PP) and Ginger extract (GE)

Required concentration of combination of selected papain 0.25% (W/W) and ginger extract 5% (W/V) was made 100ml for each and than mixed in different ratios. Thus it comprises five different treatments:

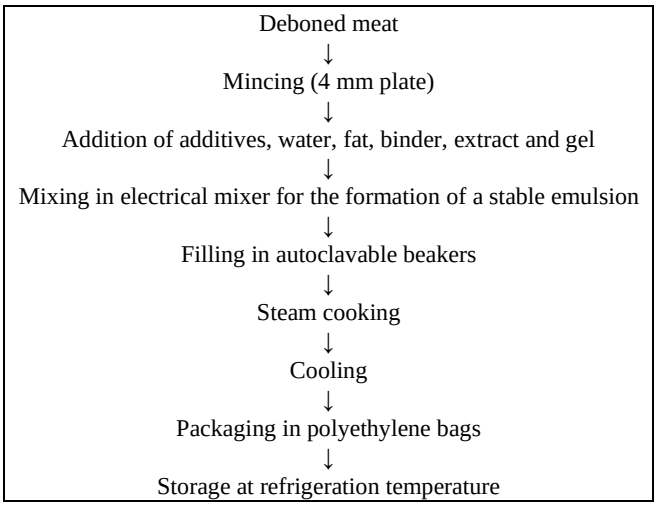
1. Papain and Ginger in the ratio of (100:0): 100 ml PP1 extract + 0 ml GE1.
2. Papain and Ginger in the ratio of (75:25): 75 ml PP1 extract + 25 ml GE1.
3. Papain and Ginger in the ratio of (50:50): 50 ml PP1 extract + 50 ml GE1.
4. Papain and Ginger in the ratio of (25:75): 25 ml PP1 extract + 75 ml GE1.
5. Papain and Ginger in the ratio of (0:100): 0 ml PP1 extract + 100 ml GE1.

After thorough mixing by hand, the chunks treated with papain, ginger and combination were placed in polythene bags and kept at (4±2 °C) for 12, 24 and 36 h. After marination at different time interval the chunks were washed, drained and were cooked in oven to an internal temperature of 75±1 °C monitored using probe thermometer. Samples were evaluated for cooking yield, pH, moisture, shear force and sensory attributes.

Preparation of buffalo meat rolls

For preparation of control meat rolls, 100 g of minced meat obtained from marinated chunks was mixed with sodium chloride (2.5 g), sodium tripolyphosphate (0.5 g), sodium nitrite (150 ppm), spice mix (2 g), condiments paste (3g), chilled water (12 g) and groundnut oil (5 g). Treatments consisted of addition of minced meat obtained from marinated meat chunks with papain powder extract at three different levels viz. 0.25%, 0.50% and 0.75%, besides other additives which were used in control in similar concentrations. Ginger extract was also added at three different levels viz. 5%, 7% and 9%, the other additives were same as the control meat rolls except for chilled water which was added 7 ml, 5 ml and 3 ml in respective levels of ginger extract. Similar scenario was followed for combination treatment. After mixing of additives and extract minced meat obtained from marinated chunks was thoroughly mixed in an electric mixer for 4-5 minutes to prepare emulsion.

Emulsion was stuffed in autoclavable beakers manually and steam cooked in a closed container for 40 minutes. After cooking, rolls were taken out and cooled to room temperature, packaged in polythene bags and stored at refrigerated temperature for further study.



Flow diagram for preparation of buffalo male calf meat rolls

Initial treatments were as follows

1. Control – Meat rolls without any treatment at 12 h, 24 h and 36 h of marination.
2. PP – Meat rolls treated with 0.25%, 0.50% and 0.75% papain powder extract
3. GE – Meat rolls incorporated with 5%, 7%, and 9% ginger extract
4. PG – Meat rolls incorporated with combination o papain and ginger in different percentage.

One treatment each from papain powder, ginger extract and combination was selected on the basis of sensory evaluation for 24 h of marination.

Final treatments were as follows

1. Control - Meat rolls without any treatment for 24 h of marination.
2. PP1 - Meat rolls with 0.25% papain powder
3. GE1 -Meat rolls with 5% ginger extract.
4. PG4 – Meat rolls with 0.25% papain powder and 5% ginger extract in ratio of 1:3.

Both control and treated rolls were subjected to physicochemical, sensory and proximate evaluation.

Studied Parameters

TBA value (Witte *et al*., 1970) [11] and free fatty acids (Koniecko, 1979) were estimated to evaluate the physico-chemical properties. Microbiological (Total plate counts, Psychrotrophic counts and Yeast and mould counts) quality parameters of the treated and control products were analyzed at 0, 4, 8, 12, and 16th day of storage following the methods as described by APHA (1984) [2].

The experiment was repeated thrice in duplicate and the results were analyzed using completely randomized design as per Snedecor and Cochran (1994) [10].

The addition of papain powder, ginger extract and their combination caused a decrease in the thiobarbituric acid content of the developed products. The pH values for all the treatments were similar and there was no significant variation. The shear press value was lower for papain powder and higher for the ginger extract and combination treated product as compared to the control samples.

There was an increase in TBA values and Free Fatty Acid content in meat rolls with advancement in storage interval (Table 2). The products containing 0.25% papain powder, 5% ginger extract and combination (PP 0.25% + GE 5% in 1:3) had the lower TBA and FFA content than control products. This was due to antioxidant property of Papain powder and Ginger extract.

Table 2

Treatment	Day 0	Day 4	Day 8	Day 12	Day 16
pH value of male buffalo calf meat rolls					
Control	5.94^{aA}±0.02	5.91^{aA}±0.02	6.11^{bA}±0.01	6.35^{bA}±0.03	6.55^{bA}±0.01
PP1	5.96^{aA}±0.01	5.92^{aA}±0.02	6.00^{bA}±0.01	6.22^{bA}±0.02	6.42^{cA}±0.03
GE1	6.02^{bB}±0.02	5.97^{aA}±0.01	6.04^{bA}±0.02	6.25^{bA}±0.01	6.45^{cA}±0.02
PG4	6.04^{bB}±0.03	5.93^{aA}±0.01	6.02^{bA}±0.01	6.24^{bA}±0.03	6.44^{cA}±0.02
TBA value (mg malonaldehyde/kg) of male buffalo calf meat rolls					
Control	0.56^{aB}±0.04	0.62^{bB}±0.02	0.76^{cB}±0.04	0.92^{dB}±0.03	1.15^{eB}±0.06
PP1	0.47^{aA}±0.01	0.52^{bA}±0.03	0.60^{cA}±0.02	0.67^{dA}±0.04	0.85^{eA}±0.03
GE1	0.49^{aA}±0.04	0.56^{bA}±0.02	0.64^{cA}±0.04	0.71^{dA}±0.03	0.89^{eA}±0.01
PG4	0.48^{aA}±0.03	0.56^{bA}±0.02	0.62^{cA}±0.04	0.70^{dA}±0.01	0.88^{eA}±0.04
Free Fatty Acid (% oleic acid) of male buffalo calf meat rolls					
Control	0.141^{aC}±0.01	0.202^{bB}±0.02	0.261^{cB}±0.01	0.280^{cB}±0.01	0.391^{dB}±0.03
PP1	0.350^{aD}±0.01	0.421^{bD}±0.03	0.530^{cD}±0.02	0.643^{dD}±0.01	0.791^{eD}±0.04
GE1	0.071^{aA}±0.02	0.112^{bA}±0.03	0.171^{cA}±0.01	0.201^{dA}±0.02	0.282^{eA}±0.01
PG4	0.091^{aB}±0.01	0.262^{bC}±0.06	0.393^{cC}±0.002	0.513^{dC}±0.03	0.623^{eC}±0.01

There was no significant differences in Total Plate Count, Psychrotrophic Count and Yeast and Mould Counts (log cfu/g) between control and treated rolls during 0-8 days of storage then the counts increased significantly as the storage period increased in control as well as treated products (Table 3). The increase was seen to be more for control samples than the treated products. Control meat rolls were visibly spoiled after 16 days of storage at refrigerated temperature (4±1 °C) but both the developed products were within the safety limits microbiologically up to 16th day of storage.

Thus it can be concluded that Papain powder (0.25%), Ginger extract (5%) and Combination (PP 0.25% + GE 5% in 1:3) can be incorporated for development of designer male buffalo calf meat rolls without compromising the sensory quality. The sensory scores and microbial load were decreased as the storage period increased in all the samples irrespective of

treatments and the control meat rolls were visibly spoiled after 16th day of storage, but all the treated products were organoleptically acceptable and microbiologically safe till end of the storage period (16 days) at refrigeration (4±1 °C) temperature.

It is concluded that GE treated meat chunks are suitable for development of meat rolls and is comparable with PP and Combination treated with regard to product characteristics and acceptability. Shelf stability of treated rolls at refrigerated temperature is 16 days. The study revealed that GE1 treated meat rolls can be healthy alternative than control and other treated meat rolls.

Table 3

Treatment	Day 0	Day 4	Day 8	Day 12	Day 16
Total plate counts (log cfu/g) of male buffalo calf meat rolls					
Control	2.45^{aA}±0.16	3.05^{bB}±0.14	3.98^{bcC}±0.10	4.45^{cD}±0.13	5.98^{dE}±0.12
PP1	2.41^{aA}±0.08	3.01^{bB}±0.12	3.95^{bcC}±0.16	4.34^{cD}±0.11	5.91^{dE}±0.13
GE1	2.31^{aA}±0.10	2.95^{bB}±0.11	3.25^{cC}±0.06	4.10^{dD}±0.08	5.72^{eE}±0.10
PG4	2.40^{aA}±0.12	3.00^{bB}±0.13	3.32^{bcC}±0.09	4.23^{cD}±0.10	5.85^{dE}±0.12
Psychrotrophic count (log cfu/g)of male buffalo calf meat rolls					
Control	1.39^{aA}±0.33	1.64^{bB}±0.14	1.85^{bC}±0.08	1.98^{bcD}±0.12	2.45^{cE}±0.10
PP1	1.35^{aA}±0.24	1.47^{aB}±0.11	1.69^{bC}±0.09	1.86^{bD}±0.22	2.29^{cE}±0.
GE1	1.28^{aA}±0.27	1.32^{aB}±0.06	1.51^{bC}±0.07	1.72^{bD}±0.18	2.32^{cE}±0.
PG4	1.32^{aA}±0.24	1.35^{aB}±0.11	1.58^{bC}±0.09	1.85^{bD}±0.27	2.42^{cE}±0.14
Coliform counts (log cfu/g) of male buffalo calf meat rolls					
Control	ND	ND	ND	ND	ND
PP1	ND	ND	ND	ND	ND
GE1	ND	ND	ND	ND	ND
PG4	ND	ND	ND	ND	ND
Yeast and mould counts (log cfu/g) of male buffalo calf meat rolls					
Control	ND	ND	0.86^{aA}±0.44	1.22^{bB}±0.16	1.58^{cC}±0.18
PP1	ND	ND	0.82^{aA}±0.68	1.28^{bB}±0.24	1.55^{cC}±0.29
GE1	ND	ND	0.80^{aA}±0.72	1.20^{bB}±0.19	1.45^{cC}±0.18
PG4	ND	ND	0.81^{aA}±0.49	1.26^{bB}±0.19	1.48^{cC}±0.21

Conclusion

There was an increase in TBA values and Free Fatty Acid content in meat rolls with advancement in storage interval. The products containing 0.25% papain powder, 5% ginger extract and combination (PP 0.25% + GE 5% in 1:3) had the lower TBA and FFA content than control products. This was due to antioxidant property of Papain powder and Ginger extract. There was no significant differences in Total Plate Count, Psychrotrophic Count and Yeast and Mould Counts (log cfu/g) between control and treated rolls during 0-8 days of storage then the counts increased significantly as the storage period increased in control as well as treated products. The increase was seen to be more for control samples than the treated products. Control meat rolls were visibly spoiled after 16 days of storage at refrigerated temperature (4±1 °C) but both the developed products were within the safety limits microbiologically up to 16th day of storage.

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