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Influence of custard apple (*Annona squamosa*) peel extract on quality and shelf life of chicken momo

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Abstract
Chicken momos were prepared by incorporating the custard apple peel extract (CAPE) @ 1% (T_1) & 2% (T_2) in momo filling and compared with the control momos (T0) prepared without CAPE. The momo samples were stored under refrigeration (4±1 °C) and analyzed on the 0th, 5th, 10th and 15th day of storage for sensory, physicochemical and microbiological parameters. Total phenolic content and DPPH radical scavenging assay of CAPE ranged between 407.55 to 1983.92 µg GAE/mg and 62.58 to 79.96% respectively. Further, the antioxidant activity of the extract was comparable with that of butylated hydroxytoluene (60.12%). momos treated with 2% CAPE had significantly (p<0.01) lower score than the T_1 and control momos for colour and appearance, flavour, meat flavour intensity and overall acceptability during the entire period of storage. Both the treatment samples were acceptable to the panellist till the 10th day, whereas, the control was acceptable till the 5th day of storage. The TBARS, tyrosine value, total plate count and psychrophilic count of both the treatments and control samples increased significantly (p<0.01) with the advancement of the storage periods. However, CAPE significantly (p<0.01) prevented the increase in all the values of the treated samples. The present study concluded that CAPE extended the shelf life of chicken momos stored at 4±1 °C up to 10th day compared to 5 days shelf life of the control momo samples.

Keywords: chicken momo, custard apple extract, antioxidant activity, total phenolic content

Introduction
Rancidity and microbial growth are the major factors limiting shelf life of the meat and meat products; therefore, suitable technologies are required to preserve safety and quality of meat and meat products (Aymerich *et al*., 2008) [3]. Addition of preservatives during production of meat and meat products is one of the ways to provide protection against spoilage and pathogenic microorganisms (Chouliara and Kontominas, 2006) [6]. The preservatives include antioxidant and antibacterial compounds of synthetic and natural origin. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are examples of synthetic preservatives, whereas, natural preservatives include extracts of herbs and spices (Botsoglou *et al*., 2003) [4], vegetables, fruits etc. which exhibit antioxidant and anti-microbial properties (Matan *et al*., 2006) [13]. It has been well documented that *Annona squamosa* peel, pulp, seeds, leaves, bark and roots are good sources of natural antioxidants and antimicrobial compounds (Vanitha *et al.*, 2011, Srivastava *et al.*, 2013, Roy and Lingampeta 2014) [22, 19, 15]. However, very less literature is available on its use in preservation of meat and meat products.
Momo (type of dumpling), the famous street food throughout the world, are native of Tibet and brought to the Nepal by the traders before 1930. The product is very popular in Himalayan states of India especially Sikkim and Ladakh (Tanuja, 2016) [21]. A wheat flour dough is used to make the outer momo covering. The dough is rolled into small circular flat pieces. The filling is then enclosed in the circular dough cover either in a round pocket or in a half-moon or crescent shape. Ground/minced meat with spice, condiments and vegetables is used as filling. The momos are then cooked by steaming or pan frying after steaming. There is increasing trend of popularity of chicken momos in the Indian population.
Considering the above facts the investigation was undertaken for examining the potential of custard apple peel as a natural source of antioxidant compounds for preservation of the chicken momos at refrigerated storage (4±1 °C).

2. Materials and Methods

2.1 Preparation of custard apple peel extract (CAPE)

Fresh, healthy and ripened custard apple (*Annona squamosa*) fruits procured from local market were washed with the help of distilled water so as to remove the dirt, dust or any other foreign material on their surface. Washed fruits were manually opened to separate the peel from the pulp. Peel was washed with the help of distilled water, cut into small pieces and then dried in hot air oven at 50±1 °C till constant weight was obtained. Dried peel was powdered using heavy duty mixer grinder, sieved through sieve no. 30 to maintain particle size of 0.49 mm, packaged in LDPE pouches, labelled and stored at refrigeration (4±1 °C) till further use. The powder was used to prepare the extract-CAPE by the method suggested by Jagtap and Bapat (2012) [8]. The powder was mixed in 50 per cent aqueous ethanol solution in 1:5 ratio. The mixture was stored at room temperature (27±2 °C) for 24 hr with occasional stirring. It was then strained through four layered muslin cloth and filtered through Whatman filter paper no. 1. The filtrate was concentrated in stainless steel plates by keeping at 50 °C in hot air oven till constant weight was obtained. Dried extract was collected, labelled and stored in LDPE container at refrigeration (4±1 °C) till further use.

2.2 Preparation of chicken momo

Momos were prepared according to the method of Tanuja *et al.* (2016) [21] with slight modification. Thawed chicken meat and other ingredients viz. green onion, green coriander, ginger and garlic were chopped separately with a hand vegetable chopper and mixed as per the formulation to prepare the filling material of the momos. Control momos were prepared without the addition of CAPE whereas; treatment momos were prepared by addition of 1% (T_1) and 2% (T_2) CAPE in filling material. The dough was prepared by kneading refined and whole wheat flour with refined vegetable oil, salt and water. 5 g dough was manually rolled in a flat round shape, filled with 15 g filling material and edges were closed manually. The momos were shaped manually at ambient temperature and steam cooked for 30 min. After cooling at ambient temperature, the momo samples of different treatments were packaged separately in LDPE pouches of 55µ thickness, labelled and stored under refrigeration (4±1 °C).

Table 1: Formulation of chicken momo

Formulation for filling (%)		Formulation for dough (%)	
Minced chicken meat	89.00	Refined wheat flour	54.00
Soya sauce	00.80	Whole wheat flour	43.00
Green onion	03.00	Salt	01.20
Green coriander	03.50	Refined vegetable oil	01.80
Ginger	00.60	Water	QS
Garlic	01.20		
Chili powder	01.20		
Salt	00.70		

2.3 Analysis of samples

Total phenolic content and DPPH radical scavenging assay of CAPE were estimated as per the method of Singleton & Rossi (1965) [17] and Harbarne (1973) [7] respectively. The pH, TBARS value and tyrosine value of momo samples were estimated as per the method of AOAC (2012) [1], Witte *et al.* (1970) [24] and Strange *et al.* (1977) [20] respectively. Total plate count (TPC) and psychrophilic count (PSC) were determined as per the standard procedures of APHA (1984) [2]. The sensory evaluation of chicken momos was conducted by semi-trained sensory panellists for various sensory attributes *viz.*, colour & appearance, flavour, juiciness, meat flavour intensity and overall acceptability by using 8 point hedonic scale (Keeton *et al.*, 1983) [11]. The data generated was analysed by analysis of variance following the procedure described by Snedecor and Cochran (1989) [18].

3. Results and Discussion

3.1 Total phenolic content of CAPE

Total phenolic content of CAPE increased proportionately with increasing concentration of the extract.

Table 2: Total phenolic content of CAPE

CAPE (mg/ml)	2	4	6	8	10
TPC (µgGAE/mg)	407.55 ±0.48	787.27 ±0.86	1197.33±0.56	1579.35±0.58	1983.92±0.36

The results were in agreement with Kadam *et al.*, (2018) [10] who reported that 50% ethanolic extract of custard apple peel contained 209.52 µg GAE/mg total phenolics.

3.2 Antioxidant activity of CAPE

CAPE possessed antioxidant activity comparable with butylated hydroxytoluene (BHT) which is a commercial antioxidant being widely used in meat industry all over the world.

Table 3: Antioxidant activity of CAPE (DPPH Radical Scavenging Assay)

CAPE (mg/ml)	10	12	14	16	18	20	BHT 100 ppm
DPPH Radical scavenging assay (% RSA)	62.58 ±0.53	65.57 ±0.35	69.08 ±0.51	73.48 ±0.34	76.88 ±0.39	79.96 ±0.66	60.12 ±0.69

The antioxidant activity increased proportionately with increase in concentration of the extract. The results were in agreement with Seema *et al.*, (2008) [16] who reported that antioxidant activity of methanol extract of custard apple peel by DPPH method was 90.6%.

3.3 Sensory analysis of chicken momos

The scores of three different parameters *viz.* colour and appearance, flavour and meat flavour intensity of the treatment samples decreased with increase in concentration of CAPE in the samples. The scores of these three parameters of T_2 samples were significantly ($p<0.01$) lower than the T_1 and control samples irrespective of storage periods. This may be attributed to disliking of colour and flavour of the extract imparted to the treated samples. Further, the colour and appearance, flavour and meat flavour intensity scores of both the treatments and the control samples were significantly ($p<0.01$) and progressively decreased with increase in the storage periods. This could be attributed to fading of colour of the samples and production of off odours due to increase in bacterial load of the samples with storage periods (Khare *et al.*, 2016) [12], however the flavour of both the treatments was within the acceptable limit till 10th day, whereas, that of the control was acceptable till 5th day. Maintenance of flavour of the samples by CAPE within the acceptable limit for longer period as compared to the control could be attributed to the antioxidant and antibacterial activity of CAPE demonstrated during the experiment. Similar findings have been

documented by Badee *et al.* (2013) [5] for chicken drumstick treated with marjoram essential oil and Tanuja *et al.* (2016) [21] for chicken momos incorporated with corn starch.

The effect of CAPE on juiceness of samples was non-significant on zero day but it was significant ($p<0.01$) with the advancement of storage period. The juiciness scores of all the samples gradually and significantly ($p<0.01$) decreased with the advancement of storage periods. Similar results have been recorded by Yadav *et al.* (2020) [25] for chicken nuggets.

Acceptability score of T_2 samples was significantly ($p<0.01$) lower than that of T_1 and control samples on 0th, 10th and 15th day of storage. The CAPE had significant effect on overall acceptability of the samples irrespective of storage periods. Both the treatment samples were acceptable to the panellist till the 10th day whereas control was acceptable till 5th day of storage. Gradual and significant ($p<0.01$) decrease in the acceptability scores was observed with advancement of the storage periods irrespective of the treatments. This could be correlated with the decreased scores of juiciness and flavour of samples observed during the experiment. The results are in agreement with those recorded by Pawade *et al.* (2018) [14] for refrigerated chicken momos.

Table 4: Effect of CAPE on sensory attributes of chicken momos stored at refrigeration (4±1 °C)

Treatments	Day 0	Day 5	Day 10	Day 15	F value
Colour & Appearance					
T_1	7.82±0.02Bd	7.52±13Cc	7.05±0.05Cb	5.15±0.07Ba	248.662**
T_2	5.87±0.12Ad	5.40±0.04Ac	4.00±0.04Ab	2.14±0.04Aa	601.862**
T_0	7.84±0.32Bd	6.86±0.08Bc	5.27±0.11Bb	2.15±0.04Aa	1226.363**
F Value	256.051**	153.678**	437.483**	1144.593**	
Flavour					
T_1	7.81±0.04Bd	7.00±0.67Bc	6.55±0.05Cb	4.11±0.16Ca	293.437**
T_2	5.93±0.12Ac	5.77±0.06Ac	5.15±0.10Bb	2.09±0.06Ba	421.983**
T_0	7.84±0.04Bd	5.86±0.72Ac	3.21±0.05Ab	1.44±0.07Aa	2318.395**
F Value	221.456**	106.796**	804.641**	165.728**	
Juiciness					
T_1	7.01±0.06Ad	6.60±0.04Bc	6.14±0.08Cb	3.04±0.05Ba	910.016**
T_2	7.03±0.02Ad	5.92±0.08Ac	5.08±0.10Bb	1.19±0.03Aa	1496.431**
T_0	7.04±0.03Ad	5.89±0.06Ac	3.04±0.04Ab	1.18±0.03Aa	3906.588**
F Value	0.279 NS	43.907**	413.129**	723.831**	
Meat flavour intensity					
T_1	7.90±0.02Bd	7.54±0.10Bc	7.03±0.96Cb	2.97±0.44Ba	954.070**
T_2	7.13±0.21Ad	5.97±0.06Ac	5.15±0.06Bb	1.08±0.03Aa	519.492**
T_0	7.93±0.02Bd	7.58±0.07Bc	3.02±0.91Ab	1.13±0.03Aa	3180.936**
F Value	13.610**	134.437**	572.927**	925.400**	
Overall Acceptability					
T_1	7.91±0.01Bd	7.54±0.03Bc	7.00±0.07Cb	4.11±0.11Ca	678.597**
T_2	6.26±0.08Ad	6.02±0.07Ac	5.06±0.07Bb	2.10±0.04Ba	881.145**
T_0	7.92±0.01Bd	6.06±0.06Ac	3.06±0.06Ab	1.08±0.03Aa	4236.374**
F value	452.334**	251.355**	848.433**	501.787**	

Means ± (S.E.) bearing different superscripts (between column small letters and between rows capital letters) differ significantly.*Significant value ($p<0.05$); **Highly significant value ($p<0.01$); NS Non-significant. n=6, T_1:1% CAPE; T_2: 2% CAPE; T_0: control.

3.4 Physico-chemical analysis of chicken momos

Control samples recorded the highest pH followed by T_1 and T_2 during entire storage period. The CAPE significantly ($p<0.01$) reduced pH of the treated samples which could be attributed to lower pH of CAPE. Highly significant ($p<0.01$) difference was recorded between the pH of both the treatments and control samples during entire storage period indicating significant change in pH of all the samples with advancement of storage periods. Similar results have been recorded by Yadav *et al.* (2020) [25] for chicken nuggets.

TBARS and tyrosine value of both the treatments was significantly ($p<0.01$) lower than that of the control samples during entire storage period. TBARS value of T_2 samples was significantly ($p<0.01$) lower than that of T_1 and control samples during 10th and 15th day of storage. The CAPE significantly ($p<0.01$) prevented increase in the TBARS and tyrosine value of the treated samples which could be attributed to the antioxidant activity of CAPE demonstrated during the experiment. There was significant ($p<0.01$) increase in TBARS and tyrosine value of the samples during the storage periods irrespective of treatments. Increase in TBARS value could be attributed to increase in the lipid oxidation (Kadam *et al.*, 2018) [10], whereas, increase in tyrosine value could be attributed to the intrinsic changes (autolysis) in momo samples and action of increased bacterial load of the samples with advancement of the storage period leading to proteolysis (Strange *et al.*, 1977) [20]. Similar results have been documented by Vanramhlimpuii (2015) [23] for chicken momos and Kanle (2017) [9] for chicken nuggets.

Table 5: Effect of CAPE on Physico-chemical attributes of chicken momos stored at refrigeration (4±1 °C)

Treatments	Day 0	Day 5	Day 10	Day 15	F value
pH					
T_1	5.83±0.01Bb	5.81±0.02Ba	5.83±0.01Bb	5.96±0.01Bc	117.379**
T_2	5.77±0.01Ab	5.75±0.02Aab	5.74±0.00Aa	5.91±0.01Ac	124.026**
T_0	5.86±0.01Ca	5.84±0.01Ca	6.03±0.04Cb	6.24±0.02Cc	12.421**
F Value	42.309**	46.784**	34.136**	178.974**	
TBARS (mg Malonaldehyde/kg)					
T_1	0.14±0.00Aa	0.18±0.00Ab	0.23±0.00Bc	0.32±0.01Bd	401.731**
T_2	0.14±0.00Aa	0.17±0.00Ab	0.21±0.01Ac	0.28±0.00Ad	197.277**
T_0	0.19±0.01Ba	0.28±0.01Bb	0.35±0.00Cc	0.56±0.01Cd	627.545**
F value	55.652**	180.174**	211.269**	658.717**	
Tyrosine (mg/100 g)					
T_1	15.82±0.10Aa	17.03±0.19Bb	19.88±0.33Ac	20.92±0.20Ad	113.811**
T_2	15.60±0.19ABa	16.13±0.14Aa	18.72±0.68Ab	20.30±0.38Ac	29.730**
T_0	16.21±0.09Ba	17.70±0.18Cb	21.01±0.31Bc	23.36±0.17Bd	249.249**
F value	4.964**	20.213**	5.911**	36.816**	

Means ± (S.E.) bearing different superscripts (between column small letters and between rows capital letters) differ significantly.*Significant value (p<0.05); **Highly significant value (p<0.01); NS Non-significant. n=6, T_1:1% CAPE; T_2: 2% CAPE; T_0: control.

3.5 Microbiological analysis of chicken momos

Increase in concentration of CAPE in the samples prevented increase in TPC and PSC compared to the control samples. This could be attributed to the antibacterial activity of CAPE demonstrated during the experiment. Though there was gradual and significant (p<0.01) increase in TPC as well as PSC of all the samples with advancement of the storage period, the TPC was within the acceptable limit till 10th day and PSC till end of the storage. The results are in agreement with those reported by Kadam *et al.* (2018) [10] for the chicken breast fillets treated with CAPE.

Table 6: Effect of CAPE on microbiological attributes of chicken momos stored at refrigeration (4±1 °C)

Treatments	Day 0	Day 5	Day 10	Day 15	F value
TPC (log10cfu/g)					
T_1	1.26±0.01Aa	2.93±0.02Bb	4.15±0.01Bc	5.81±0.02Bd	17519.241**
T_2	1.25±0.01Aa	2.65±0.01Ab	3.86±0.01Ac	5.15±0.01Ad	54771.967**
T_0	1.26±0.01Aa	3.46±0.01Cb	5.74±0.01Cc	5.83±0.01Bd	71783.418**
F value	0.159NS	1283.235**	14244.628**	923.277**	
Psychrophilic count (log10cfu/g)					
T_1	00±0.00a	00±0.00Aa	0.65±0.01Bb	1.67±0.01Bc	13552.720**
T_2	00±0.00a	00±0.00Aa	0.46±0.01Ab	1.56±0.01Ac	16887.471**
T_0	00±0.00a	0.33±0.33Bb	0.94±0.01Cc	2.95±0.00Cd	22999.182**
F Value		692.779**	22.666**	10276.987**	

Means ± (S.E.) bearing different superscripts (between column small letters and between rows capital letters) differ significantly.*Significant value (p<0.05); **Highly significant value (p<0.01); NS Non-significant. n=6, T_1:1% CAPE; T_2: 2% CAPE; T_0: control.

4. Conclusion

CAPE possessed good antioxidant activity demonstrated by its total phenolic content (407.55 to 1983.92 µg GAE/mg) and DPPH scavenging activity (62.58 to 79.96%). CAPE extended the shelf life of the chicken momos stored at 4±1 °C up to10th day compared to 5 days shelf life of the control momo samples, hence, it has been concluded that 50% hydro-ethanolic extract of the custard apple peel can be used as a natural preservative for chicken momo @ 1% in momo filling.

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