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Efficacy of natural preservatives on the shelf-life of raw chicken meat emulsion under refrigeration storage

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Abstract

The research aimed to evaluate the shelf-life and microbiological quality of raw chicken infused with natural preservatives, namely lemon grass and clove powder, stored at 4 °C. Four chicken meat emulsion batches were studied: Control (no preservatives), T1 (0.2% clove powder), T2 (1% lemon grass), and T3 (combination of 0.2% clove powder and 1% lemon grass). During the 9-day refrigerated storage, the presence of natural preservatives significantly improved oxidative stability and reduced microbial counts compared to the control. The combination of clove powder and lemon grass was found to be the most effective antioxidant and antimicrobial in preserving raw chicken meat emulsion.

Keywords: Chicken meat, lemon grass, clove powder, physico-chemical properties, oxidative quality, refrigerated storage

Introduction

Poultry meat is the fastest-growing segment of the world meat demand. India being the world's second-largest developing country, is rapidly expanding its chicken sector due to its cheap production value and excellent nutritional value, as well as customer acceptability (Elshebrawy et al., 2022)^[8]. Owing to its rich nutritional properties, most of the meat and meat products are highly perishable. Microbial growth (spoilage or pathogens) and oxidative rancidity are two major concerns that supports the shortening of such products' shelf-life. These processes lead to discoloration, the development of off-flavors and odors, and alterations in organoleptic properties that make the meat unsuitable for consumption (Sivarajan *et al.*, 2017) ^[27]. The microbial safety of the meat is greatly influenced by the type of meat, as well as its processing, distribution, and storage methods. Poultry meat, can be affected by the growth of bacteria as well as yeast and mold, which leads to deterioration of the meat. (Arvanitoyannis & Stratakos, 2013) ^[2]. To overcome this, natural preservatives of plant origin can be used to preserve the food. These natural preservatives contain active components such as phenols, alcohols, aldehydes, ketones, ethers, and hydrocarbons, may improve the storage quality as well as taste and flavour. Essential oils (EOs) and diverse plant extracts serve as excellent substitutes for chemical preservatives in a range of natural antimicrobial/antioxidant ingredients. Their use in meat products addresses customer desire for minimally processed, natural products while also providing additional functional benefits to both the food and the consumers.

Clove, is the dried floral bud of *Syzygium aromaticum*, is commonly utilized to add a distinct flavor to food and has been observed to possess inhibitory properties against several foodborne pathogens. Several researchers have found that clove extract has an inhibitory effect on various types of gram-positive and gram-negative bacteria found in meat and meat product (Blaszyk & Holley, 1998)^[5]. Eugenol, active agent present in clove oil was found to be having antimicrobial properties against many food borne pathogens. (Beuchat, 2000; Cressy *et al.*, 2003)^[4, 7].

Lemon grass (*Cymbopogon citratus*) has a high concentration of citral as well as beneficial components (flavonoids and vitamin C), which is why employed in the pharmaceutical and fragrance industries. Natural flavonoids are also garnering more and more interest due to their anti-inflammatory and anti-carcinogenic properties and antioxidant properties (Marin *et al.*, 2002) ^[19]. Lemongrass flavonoids, which are naturally occurring plant components with antioxidant and antifungal activity, are of great interest. Glabrene exhibits three times greater activity than vitamin E when compared to the flavonoids present in lemongrass, namely licochacone A and licochacone B. These flavonoids demonstrate antioxidant capabilities similar to those of vitamin E.

Various scientific studies have proven that preservatives, such as clove powder in pork (Shan *et al.*, 2009) ^[25], chevon patties (Raj *et al.*, 2005) ^[23], minced chicken meat (Asha *et al.*, 2014) ^[3] and in chicken nuggets (D. Kumar & Tanwar, 2011) ^[17] and lemon grass in beef burger (Hussein *et al.*, 2015) ^[11], chicken patties (Ibrahim & Salem, 2013) ^[12], and in pork patties (Olorunsanya *et al.*, 2010) ^[21] can be used in meat systems and have been documented individually but their combined effect needs to be ascertained fully. Therefore, in this present discourse we made an effort to compare their effectiveness to determine which natural preservative is best for extending the shelf-life of the chicken meat.

Materials and Methods

Raw materials

The broiler spent hens (70-75 weeks age) were purchased from local meat retail shop in Hyderabad. The birds were hygienically slaughtered, de-boned, and packed in lowdensity polyethylene (LDPE) bags and chilled for 24 hours. Clove buds and lemon grass required for the experiment were procured from local shops. Clove buds were dried and ground using a mixer to make powder. Lemon grass was also rinsed with pure water and air-dried at 25 °C for one week. Then it was milled to fine powder. Both clove powder and lemon grass powder were stored separately in an air-tight jar for further use.

Preparation and packaging of chicken meat emulsion

Any connective tissue and fat to the meat if any present were trimmed off with a sterile knife. Meat was made into small portions and subjected to mincing by the use of meat mincer (Sirman T212E, Italy). Four batches i.e., Control (C), T_1 , T_2 , T_3 of minced chicken meat, each contain about 100 gm of meat were prepared. In addition, 0.2% Clove powder, 1% Lemon grass, and combination of 0.2% clove powder and 1% lemon grass were added to T_1 , T_2 , and T_3 batches respectively. Control group (C), was prepared without any natural preservative. All the groups were packaged in LDPE bags individually and stored under refrigeration for 9 days. The sample was drawn for analysis on each alternative day i.e., 1, 3, 5, 7, 9 days for various Physico-chemical, Microbial, and Sensory characteristics.

Physico-Chemical Properties

pH: The pH measurement of meat samples was conducted using a digital pH meter (Systronics, μ pH System, Type 361, Sr.No.7856). Initially, 10 grams of the meat sample were thoroughly homogenized with 100 ml of distilled water for 1 minute, using a homogenizer. Subsequently, the resulting suspension was analysed for pH by immersing the pH meter's probe (Troutt *et al.*, 1992) ^[32].

Extract release volume

The determination of Extract Release Volume (ERV) (Jay & Kontou, 1964) ^[13] involved homogenization of 15 g meat sample with 60 ml of a 0.05 M phosphate buffer solution at pH 5.8. The mixture was then filtered through Whatman filter paper No. 1 for 15 minutes. The volume of the resulting filtrate was measured and recorded as the extract release volume in millilitres.

Oxidative Stability Thiobarbituric acid (TBA) value

The TBA value was determined by following Witte *et al.* (1970) ^[35]. For preparation of the sample, 4 g of the sample was homogenized with 20 ml of pre-cooled 20% TCA solution using a homogenizer for 2 minutes. After a 10-minute extraction, the mixture was centrifuged at 3000 rpm for 10 minutes using a CPR-24 centrifuge (Remi Instruments, Mumbai, India). Next, 3 ml of the supernatant was mixed with an equal volume of 0.1% TBA reagent and boiled in a water bath for 30 minutes. Following cooling, absorbance was measured at 532 nm by using a UV-VIS spectrophotometer. The spectrophotometer readings were taken as TBA values and expressed as mg malonaldehyde/kg. The blank was prepared similarly, using 3 ml of 20% chilled TCA solution instead of the TCA extract.

Peroxide value

To determine the peroxide value (PV) (Koniecko, 1979) ^[16] of 5 g of CME, it was blended with 30 ml of chloroform and anhydrous sodium sulphate. The filtrate was mixed with 30 ml of glacial acetic acid, 2 ml of saturated KI solution, 100 ml of distilled water, and 2 ml of 1 percent starch solution. The resulting mixture was titrated against 0.1N sodium thiosulphate until the non-aqueous layer became colourless. PV was then calculated using the specified formula.

PV (meq/kg sample) = $[(0.1 \times ml \text{ of } 0.1N \text{ sodium thissulphate}) / \text{sample weight } (g)] \times 1000$

Microbiological Analysis

Bacterial counts were estimated by using pour plate method. Meat samples (10 g) were homogenized with 90 ml of 0.1% sterile peptone water. Serial 10-fold dilutions were prepared and plated on agar for total plate and psychrophilic counts. Incubation was done at 37 °C for 48 hours for total plate count and at 4 ± 1 °C for 14 days for psychrophilic count. Yeast and mold counts were determined by pouring 20 ml of melted Potato Dextrose agar onto plates and incubating them at 25°C for 7 days. Colony counts were converted to log10 CFU/g based on the dilution factor.

Sensory Evaluation

Experienced panelists, well-versed in the AMSA guidelines, used a five-point scale (AMSA, 2015) to evaluate the sensory attributes of meat samples. The scale ranged from 5 (indicating "Very desirable") to 1 ("Very undesirable") for attributes like color and odor. Evaluations were conducted in individual booths under controlled conditions of light, temperature, and humidity. Prior to assessment, the samples were placed in labelled petri dishes at room temperature.

Statistical analysis

The data obtained from multiple experiments, each repeated three times, was compiled and analyzed using a SPSS version 16.0 for Windows (SPSS Inc., Chicago, USA). Statistical analysis was conducted to assess significant differences (p<0.05) among groups, storage periods, and their interactions for various quality characteristics. A two-way analysis of variance (Snedecor & Cochran, 1994) ^[28] was conducted,

followed by Duncan's multiple range test (Steel, 8 C.E.) to compare means and identify variations between the groups, storage periods, and their interactions in different experiments.

Results and Discussion

Physico-chemical characteristics

The pH values of both the treatment and control groups showed a significant increase (p<0.05) as the storage days progressed, as shown in Table-1. Starting from day 5, there was a noticeable and significant difference between the control group samples and the other treatment groups. Throughout the storage period, T2 and T3 consistently maintained lower pH values compared to both the control and T1 meat samples. These findings align with a study by (D. Kumar & Tanwar 2011)^[17], where they observed a similar pH increase in chicken nuggets that contained clove powder. Similar trends were also noted in other research involving chicken meat products and chevon meat during refrigerated storage, as reported by (Hussein *et al.* 2015; Verma & Sahoo 2000) ^[11, 34], respectively. The rise in pH during the refrigerated storage period could be attributed to the accumulation of metabolites generated by bacterial activity (McDowell *et al.* 1986) ^[20].

ERV of control and treatment samples showed significantly (p < 0.05) reduction along the storage period (Table-1). Control group samples showed significant difference with treatment groups from day 3 of refrigeration storage. T3 showed significantly (p < 0.05) higher ERV value by the end of storage period compared to other treatment and control group samples. The decrease in ERV values irrespective of group is might be due to increased bacterial activity and changes in protein structure during refrigerated storage. This decrease in ERV is in correlation with total plate count of present study. The outcomes aligned with the research conducted by (R. R. Kumar & Sharma, 2004)^[18], which also noted a noteworthy reduction in the ERV of chicken meat patties throughout their refrigerated storage period. Similar findings were noticed in (Singh et al., 2014; Verma & Sahoo, 2000) [26, 34] during 9 days refrigeration storage.

Table 1: Impact of clove and lemon grass on the physico-chemical quality of chicken meat emulsion stored under refrigeration

Treatments	Storage days						
	1	3	5	7	9		
Ph							
С	5.60±0.11 ^{aA}	5.67±0.15 ^{aA}	5.78±0.12 ^{bB}	5.89±0.09 ^{cB}	5.95±0.10 ^{dC}		
T1	5.56±0.09 ^{aA}	5.62±0.12 ^{bA}	5.67±0.08 ^{bA}	5.72±0.11 ^{cA}	5.85±0.03 ^{dB}		
T2	5.52±0.13 ^{aA}	5.57±0.08 ^{aA}	5.64±0.18 ^{bA}	5.70±0.19cA	5.83±0.14 ^{dB}		
T3	5.54±0.09 ^{aA}	5.59±0.05 ^{aA}	5.62±0.12 ^{bA}	5.68±0.15 ^{cA}	5.75±0.04 ^{dA}		
Extract Release Volume (ml)							
С	24.14±1.65 ^{eA}	20.22±1.17 ^{dC}	19.33±1.84 ^{cB}	17.65±1.09 ^{bC}	15.47±1.80 ^{aC}		
T1	23.17±1.79eA	22.43±1.09 ^{dA}	21.83±1.54 ^{cA}	20.33±1.06 ^{bA}	17.50±1.54 ^{aB}		
T2	24.54±1.32eA	21.40±1.75 ^{dB}	20.67±1.65 ^{cB}	19.23±1.41 ^{bB}	18.10±1.34 ^{aB}		
T3	24.43±1.54 ^{eA}	22.17±0.45 ^{dA}	21.43±1.09cA	20.21±1.12 ^{bA}	19.98±1.54 ^{aA}		

Control (C) - without any natural preservatives; T1- 0.2% clove treated samples; T2- 1% lemon grass treated samples; T3- combined (0.2% clove + 1% lemon grass).

Mean values bearing different superscripts are significantly varied (p < 0.05).

Oxidative quality

Thiobarbituric acid (TBA) values of control and treatment samples were significantly (p < 0.05) increased as the storage period progressed (Table-2). The TBA values were influenced by the addition of lemon grass and clove powder during the storage period. The chicken samples treated with both lemon grass and clove powder (T3) showed significantly lower TBA values than other treatment and control group samples. Lemon grass powder contains bioactive compounds such as flavonoids and citral, while clove powder contains phenols and eugenol. These compounds likely inhibited chain reactions during lipid oxidation in chicken meat samples when stored. Hussein et al., 2015; Ibrahim & Salem, 2013)^{[11,} ^{12]} reported that addition of lemon grass significantly reduced the lipid oxidation compared to untreated samples. Similarly (Singh et al., 2014; Tareq et al., 2018; Vasavada et al., 2006) ^[26, 31] reported delayed lipid oxidation in chicken meat samples incorporated with clove powder during refrigeration storage. (Kandeepan & Biswas, 2007)^[14] concluded that the

TBA value increased significantly during the storage period. Likewise, (Sudheer et al., 2011) [30] observed a rise in TBA values for refrigerated storage of restructured chicken meat products. Peroxide value of T3 showed significantly (p < 0.05) lower values compared to other treatment and control samples along the refrigeration storage (Table-2). Control samples showed high peroxide value throughout the storage period compared to treatment groups. The lowest peroxide values recorded by T3 among all treatments and indicating that most preferable for human consumption. In a study conducted by Al-Kutby in 2012 ^[1], it was found that the use of alcoholic extracts of cinnamon, clove, and sumac had a positive impact on the microbial safety and shelf-life of high-fat meat products (doner kebab). These extracts effectively slowed down the process of lipid oxidation, contributing to the improved quality and preservation of the cooked meat. Combined use of lemon grass and clove powder significantly influenced the peroxide values of T3 chicken samples during storage period.

Treatments	Refrigerated storage days						
	1	3	5	7	9		
TBA values (mg malonaldehyde/kg)							
С	0.34±0.016 ^{cA}	0.43±0.019cA	0.59±0.012 ^{bA}	0.67±0.018 ^{bA}	0.92±0.017 ^{Da}		
T1	0.30±0.015 ^{cA}	0.36±0.020 ^{bB}	0.56±0.015 ^{bB}	0.62±0.017 ^{bA}	0.75±0.018 ^{cB}		
T2	0.21±0.013 ^{bB}	0.31±0.018 ^{bC}	0.48±0.018 ^{aC}	0.54±0.015 ^{aB}	0.69±0.014 ^{bB}		
T3	0.17±0.018 ^{aC}	0.28±0.014 ^{bD}	0.43±0.013 ^{cD}	0.48±0.012 ^{dC}	0.56±0.018 ^{eC}		
Peroxide (mg/kg)							
С	1.22±0.01 ^{aA}	1.30±0.04 ^{bA}	1.45±0.11 ^{cA}	1.59±0.09 ^{dA}	1.73±0.04 ^{eA}		
T1	1.19±0.04 ^{aA}	1.25±0.09 ^{bA}	1.34±0.14 ^{cB}	1.42±0.12 ^{dB}	1.56±0.07 ^{eB}		
T2	1.17±0.07 ^{aA}	1.29±0.12 ^{bA}	1.32±0.15 ^{cB}	1.40 ± 0.11^{dB}	1.45±0.05 ^{eC}		
T3	0.89±0.11 ^{aB}	0.95 ± 0.05^{bB}	0.98±0.09 ^{cC}	1.14±0.09 ^{dC}	1.20±0.12 ^{eD}		

Table 2: Impact of clove and lemon grass on oxidative stability of chicken meat emulsion under refrigeration storage.

Control (C)- without any natural preservatives; T1- 0.2% clove treated samples; T2- 1% lemon grass treated samples; T3- combined (0.2% clove + 1% lemon grass).

Mean values bearing different superscripts are significantly varied (p<0.05).

Microbiological profile

As the storage period increased, the total plate count of chicken meat in both control and treatment groups increased. Notably, among the treatment groups, the samples from T3 exhibited significantly lower total plate counts (p<0.05). This reduction could be attributed to the combined effects of lemon grass and clove powder, which contain active components like phenolics and eugenol known for their antimicrobial properties. These findings are consistent with prior studies; for instance, Georgantelis *et al.* (2007) ^[10] observed lower microbial counts in pork treated with chitosan and rosemary, suggesting a possible synergy. Similarly, Reddy *et al.* (2023) ^[24] reported that the addition of lemon grass essential oil significantly reduced total plate counts in refrigerated ground pork meat. Furthermore, a significant difference in coliform count during refrigeration storage was observed among the

various treatment groups and the control. At the end of the storage period, T3 exhibited significantly lower counts compared to other treatment and control groups. This aligns with (Fyfe, 1998), demonstrating the antimicrobial properties of clove and other natural preservatives in broth models. Regarding yeast and mold count, the presence of lemon grass and clove powder had a significant impact (p < 0.05) on chicken samples during refrigeration storage. Both T2 and T3 showed similar yeast and mold counts over the storage period, with slightly lower values noted in T3. This reduction could be attributed to the antimicrobial activity of neral, geranial, and geraniol present in lemon grass. These results are consistent with the findings of Tareq et al. (2018)^[31] and Zaki et al. (2018) [36], who observed lower yeast and mold counts in meat samples treated with clove and lemon grass, respectively.

Table 3: Impact of clove and lemon grass on the microbial quality of chicken meat emulsion under refrigeration storage

Treatments	Storage days						
	0	3	6	9	12		
	Total Plate Count (log ₁₀ cfu/g)						
С	4.52±0.14 ^{eA}	4.74±0.05 ^{dA}	4.97±0.09 ^{cA}	5.12±0.27 ^{bA}	5.33±0.17 ^{aA}		
T1	4.43±0.36 ^{eB}	4.78±0.26 ^{dA}	4.95±0.41 ^{cA}	5.15±0.11 ^{bA}	5.25±0.17 ^{aA}		
T2	4.21±0.07 ^{dC}	4.39±0.33 ^{cB}	4.62±0.11 ^{bB}	4.69±0.25 ^{bB}	4.87±0.19 ^{aB}		
T3	3.98±0.34 ^{dD}	4.25±0.12 ^{cC}	4.48 ± 0.09^{bC}	4.57±0.18 ^{bC}	4.76±0.37 ^{aC}		
Total Coliform Count (log10 cfu/g)							
С	2.30±0.05 ^{aA}	2.45±0.11 ^{bA}	2.65±0.13 ^{cA}	2.84±0.12 ^{dA}	2.95±0.08eA		
T1	1.87 ± 0.09^{aB}	2.15±0.15 ^{bB}	2.44±0.09 ^{cB}	2.59±0.13 ^{dB}	2.73±0.11 ^{eB}		
T2	1.72±0.31 ^{aB}	2.10±0.10 ^{bB}	2.38±0.07°C	2.54 ± 0.09^{dB}	2.68±0.05 ^{eC}		
T3	1.54 ± 0.44^{aC}	1.87±0.08 ^{bC}	2.24±0.09 ^{cD}	2.38±0.07 ^{dC}	2.51±0.08 ^{eD}		
Yeast and Mold count (log10 cfu/g)							
С	1.60±0.03 ^{eB}	1.75±0.02 ^{dA}	1.91±0.07 ^{cA}	2.29±0.04 ^{bA}	2.62±0.09 ^{aA}		
T1	1.62±0.04 ^{eA}	1.70±0.03 ^{dA}	1.92±0.07 ^{cA}	2.41±0.06 ^{bA}	2.50±0.05 ^{aB}		
T2	1.53±0.05 ^{dBC}	1.59±0.03 ^{dB}	1.71±0.08 ^{cB}	1.98±0.03 ^{bB}	2.25 ± 0.02^{Cc}		
T3	1.48±0.11 ^{dC}	1.53±0.04 ^{dB}	1.67±0.10 ^{cB}	1.81 ± 0.06^{bC}	2.15±0.09 ^{aC}		

Control (C)- without any natural preservatives; T1- 0.2% clove treated samples; T2- 1% lemon grass treated samples; T3- combined (0.2% clove + 1% lemon grass).

Mean values bearing different superscripts are significantly varied (p < 0.05).

Sensory characteristics

The color and odour of control and treatment samples were significantly (p<0.05) decreased during the storage period (Table-4). The addition of lemon grass and clove powder significantly affected the color and odour scores of treatment group compared to control samples as the storage period advanced. The T3 samples with both 1% lemon grass and 0.2% clove powder maintained desirable color than other treatment groups. The treated samples showed improved color scores. This might be due to the action of antioxidants which prevents the oxidation of oxymyoglobin. Similar results were

reported by (Tareq *et al.*, 2018) ^[31] in which clove powder treated chicken samples showed better color scores during refrigerated storage. Similarly (Kandeepan & Biswas, 2007; Kilinc, 2009) ^[14] reported decrease in appearance and colour scores in chicken samples. The odour scores were in congruence with lipid oxidation indicators (TBA, PV) in raw chicken meat emulsion. No off-odour was initially detected on day 0 in any of the treatments. The T3 samples showed significantly better odour scores compared to other groups. Similarly (Zaki *et al.*, 2018) ^[36] noticed better color and aroma scores in lemon grass treated camel burgers.

Table 4: Impact of clove and lemon grass on colour and odour scores of chicken meat emulsion under refrigeration storage.

Treatments	Storage days					
	1	3	5	7	9	
Colour scores (5-point scale)						
С	4.75±0.11 ^{aBC}	4.00±0.13 ^{bA}	3.50±0.09cA	2.75±0.15 ^{dA}	2.15±0.17 ^{eA}	
T1	4.91±0.12 ^{aB}	4.65±0.11 ^{bC}	4.25±0.15 ^{cC}	3.92±0.16 ^{dC}	3.65±0.09 ^{eB}	
T2	4.85 ± 0.09^{aC}	4.57 ± 0.09^{bB}	4.17±0.05 ^{cB}	3.75±0.14 ^{dB}	3.44±0.11 ^{dD}	
T3	4.82±0.15 ^{aC}	4.60±0.17 ^{bB}	4.15±0.09 ^{cB}	3.84±0.13 ^{dD}	3.57±0.14 ^{dC}	
Odour scores (5-point score)						
С	4.83±0.12 ^{aA}	4.25±0.09 ^{bC}	3.79±0.17 ^{cB}	2.50 ± 0.09^{dD}	1.54±0.11 ^{eD}	
T1	4.85 ± 0.05^{aA}	4.45±0.11 ^{bB}	4.18±0.06 ^{cC}	3.52±0.05 ^{dB}	2.24±0.08 ^{eC}	
T2	4.79 ± 0.08^{aB}	4.51±0.22 ^{aB}	3.98±0.06 ^{bB}	3.32 ± 0.09^{cC}	2.59±0.09 ^{dAB}	
T3	4.80±0.05 ^{aB}	4.62±0.05 ^{aA}	4.29±0.14 ^{bA}	3.75±0.08 ^{cA}	2.94 ± 0.06^{dA}	

Control (C)- without any natural preservatives; T1- 0.2% clove treated samples; T2- 1% lemon grass treated samples; T3- combined (0.2% clove + 1% lemon grass).

Mean values bearing different superscripts are significantly varied (p < 0.05).

Conclusion

The current study's findings suggest that natural preservatives like clove powder and lemongrass have the potential to serve as superior alternatives to artificial preservatives. These natural options possess both antioxidant and antimicrobial properties, which can effectively extend the shelf life of meat and meat products when stored under refrigeration. Interestingly, when combined in a ratio of 0.2% clove powder and 1% lemongrass, these effects seem to be even more pronounced. This combination demonstrates enhanced synergistic effects across various aspects, including the meat's physico-chemical characteristics, oxidative stability, and microbiological factors throughout the refrigeration storage period. As a result, the meat industry can consider adopting the blend of 0.2% clove powder and 1% lemongrass as an effective strategy. This combination proves valuable in enhancing microbial safety, prolonging the freshness of raw chicken meat emulsion, and retarding lipid oxidation. Importantly, this improvement in preservation does not come at the cost of compromising sensory attributes.

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