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ANALYZING PRESERVATIVE EFFECT OF POMEGRANATE *(PUNICAGRANATUM L.)* PERICARP EXTRACT ON RESTRUCTURED CHICKEN BLOCKS

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ABSTRACT

Recent developments in the food industry have led to the massive marketing of reconstructed meat due to its associated benefits. Incorporating plant-based preservatives, rich in antioxidants and antimicrobial property is a safe solution to extend the shelf-life of meat and meat products. They reduce the toxic repercussions of chemical preservatives, furthermore has favorable health benefits. The present study examined the preservative effect of pericarp extract of *Punica granatum L.* on restructured chicken blocks under refrigerated storage conditions. Restructured chicken blocks were prepared by the addition of salt, sugar, phosphates and nitrate for the test samples along with 1.5% of pomegranate extract. The control samples were prepared without the natural preservative and a reference product with a chemical preservative (BHT) was also prepared. The experiment was done in triplicates and various factors such as pH, cooking yield, sensory attributes and microbiological properties (SPC) were recorded using standard procedures at frequent intervals for 20 days at 4 ± 0.5°C. The pericarp extract showed a favourable result by obstructing microbial growth as well as enhancing the shelf-life of restructured chicken blocks up to 20 days compared to the control.

Keywords: Natural preservative; Restructured chicken blocks; Pericarp extract; Refrigerated storage

Background:

The Indian poultry industry is booming with great potential for production and export due to the availability of sufficient resources in the market. Almost 97 per cent of poultry meat is utilized fresh and the remaining 3 per cent, around 33000 tons is processed [1]. Convenience or restructured meat products are preferred among consumers as they are inexpensive and ready to consume. But one of the major problems in the development of convenient meat products or

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processes is the deterioration of flavour, texture and nutritive value of poultry meat due to oxidation and microbial safety at refrigerated storage temperature [2]. The growth and metabolic activity of contaminating microbial communities lead to organoleptic defects besides causing gastrointestinal problems, consequently having an impact on the financial state of poultry meat production [3]. To overcome these drawbacks, and in line with the rising awareness by consumers of the relationship between diet and health, in the last few years, the meat industry has had to face the double challenge of offering healthier meat processed products and guaranteeing their stability.

Contrasting reformulation strategies or vegetative extenders with antioxidant and antibacterial properties are being applied to enhance the attribute of meat products and to improve their storage stability [4]. Synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) etc. usage in meat products are not encouraged as it has been observed to possess toxicity, carcinogenic effects thus have adverse effects on human health [5]. Therefore, research on safe and natural sources of antioxidants to enhance the quality of meat products is preferred and is being done extensively.

In recent years, various plant materials like fruit peel, seeds, leaves containing phenolic compounds have been demonstrated to be effective antioxidants and of therapeutic value. The effect of grape seed extract and extracts of litchi fruit in poultry meat has been reported [6]. Pomegranate fruits, juice, seeds, and peels are known to have an increased quantity of bioactive compounds, such as phenolic acids, flavonoids, and hydrolysable tannins. The peels of pomegranate fruits, major by-products produced during food processing of pomegranate are known to have high levels of numerous phytochemicals, including phenolic acids, flavonoids, tannins, proanthocyanidin compounds and many vital minerals that can prevent food deterioration[7,8]. Recently, by-products of pomegranates, especially pomegranate peel extract (PPE) has gained attention due to its scientifically confirmed therapeutic properties such as antioxidant, antimicrobial, anticancer, antiulcer, and anti-inflammatory activities[9,10,11] Diverse scientific investigations have recommended that PPE exhibits excellent antimicrobial activity against assorted foodborne pathogens and improves the postharvest storability of food products [12,13].

Although the report on the application of pomegranate by-products on various food is available, their effect on physicochemical, sensory acceptability, microbial load and storage stability of restructured chicken blocks are nil. Hence the present study was organized to inspect the antioxidant additionally the preservative effect of pericarp extract of pomegranate on the reconstructed chicken block under refrigerated storage.

Materials and methods

Preparation of pomegranate fruit pericarp extract

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Fully grown, ripe pomegranate fruits were acquired from a local market in Chennai. The smooth reddish-brown outer covers (pericarp) were removed carefully from the fruits and thoroughly washed to remove any extraneous matter and dried under shade. After drying it was ground to powder and sieved through a fine mesh.

15 grams of pomegranate pericarp powder was added in 100ml of boiled distilled water and left for 1 hour. The extract was then filtered using a clean sterile muslin cloth to obtain a water extract (1.5%) of pomegranate fruit pericarp. The functional groups present in the extract were ascertained by FTIR analysis.

Preparation of restructured chicken block

Fresh broiler chicken bought from the local market was deboned, cleaned and cut into small chunks and stored in the freezer for further use. The frozen deboned meat was thawed in a refrigerator overnight. It was then minced twice using a meat mincer. After mincing, the meat samples (500g each) were mixed manually with salt, sugar and other ingredients as shown in Table 1 and tumbled in a home mixer grinder.

Table 1: Restructured chicken block formulations

Treatments	Ingredients
Control	Meat with salt and without antioxidant
Test	Meat + 2%salt + 2% sugar + 1.5% PFP extract
Reference	Meat + 2%salt + 2%sugar + 200 ppm BHT

The groundmass was loaded into stainless moulds lined with food-grade aluminium foil and placed in the refrigerator overnight for curing reaction to take place. Further cooking was carried out at 90ºC by placing the moulds in water for 45mins. This was followed by cooling under tap water and chilling overnight for setting. Later, the moulds were taken out from the refrigerator to procure a restructured chicken block. These blocks were then packed individually in low-density polyethylene (LDPE) bags and stored at 4±1ºC for 20 days. Samples were withdrawn on 1, 6, 12 17 and 20th days of storage to assess the physicochemical and microbiological properties.

Analysis of restructured chicken block

pH analysis- The pH was determined by blending 10g of sample with 50ml distilled water for a minute in a homogenizer and estimated as per Trout [14] using a digital pH meter. The pH was calibrated with known buffers of pH**7** and **4** before every use.

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Cooking yield

The cooking yield of the restructured chicken blocks was determined by recording the weight per meat block prior to and post-cooking for each of the products[15]. Determining the yield percentage in advance will lead to greater efficiencies and a more productive operation. The yield was calculated and expressed in percentage as the weight of cooked meat block/ weight of raw meat block x 100.

Sensory Evaluation

The sensory qualities of cooked chicken blocks were evaluated by the meat descriptive analysis method. The sensory quality of samples was evaluated using an 8-point descriptive scale [16] where 8 denoted extremely desirable and 1 represents extremely poor. A sensory panel of five judges evaluated the product for different properties: appearance, flavor, juiciness, tenderness and overall acceptability.

Microbiological quality

The microbiological parameters viz, standard plate count and yeast and mould counts were determined for the restructured chicken block following the standard procedures recommended by APHA [17]. Samples after serial dilution were plated onto sterile nutrient agar (NA incubated at 37ºC for 24 hours) and Sabouraud's Dextrose agar (SDA incubated at room temperature for 2-3days) to determine bacterial and mould count respectively. The average number of colonies was multiplied with the dilution factor to obtain the total count as a colony-forming unit (CFU) per g of the sample. This count was then converted to the total plate count of log cfu/g of the sample.

Statistical analysis

Each of the analytical methods (such as pH and SPC) were done in triplicates and each parameter was analyzed in duplicate. One-way ANOVA was conducted using for the calculation of different mean values and statistical significance was identified at the 95 % confidence level ($p<0.05$).

Results and discussion:

FTIR analysis

The presence of phenolic content in pomegranate pericarp powder was determined using the FTIR analysis. Fourier transform infrared spectroscopy is a sensitive technique particularly for identifying organic chemicals. It relies on the fact that the molecules absorb light in the infrared region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency range is measured as wave numbers typically over the range 4000-600 $cm^{-1.}$

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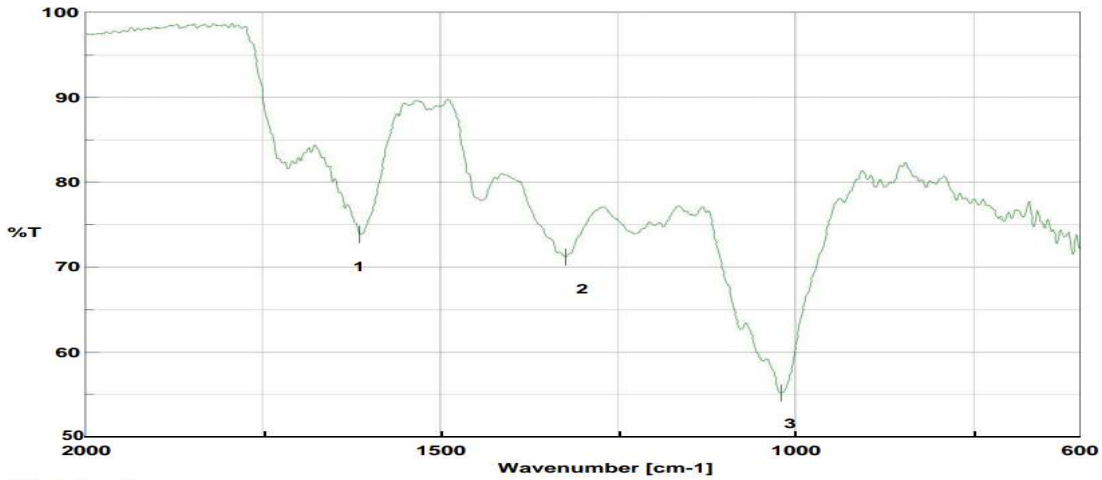
The phenolic content of the pomegranate fruit pericarp extract was determined using Fourier transform infrared spectroscopy and was found to be in the second most range next to aromatic compounds as shown in Fig.1

Figure 1and Table 2: FTIR Spectrumof Pomegranate pericarp extract



[Comments]
Sample name PROMOGRANATE POWDER
Comment B NO M-8531 TNTH
User MOP
Division REF NO 1 27/2/19
Company TNTH

[Measurement Information]
Model Name FT/IR-4600typeA
Serial Number D103461786

Accessory ATR-PRO-ONE
Accessory S/N B111061809
Incident angle 45 deg

Measurement Date 27/02/2019 14:02

Light Source Standard
Detector TGS
Accumulation 32
Resolution 4 cm-1
Zero Filling On
Apodization Cosine
Gain Auto (32)
Aperture Auto (7.1 mm)
Scanning Speed Auto (2 mm/sec)
Filter Auto (30000 Hz)

Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity
1	1613.16	73.8208	2	1323.89	71.1607
3	1021.12	55.1523			

FREQUENCY RANGE	ABSORPTION	APPEARANCE	GROUP	COMPOUND
2000-1650 cm^{-1}	2000-1650	Weak	C-H bending	Aromatic compound
1390-1310 cm^{-1}	1390-1310	Medium	O-H bending	Phenol compound
1400-1000 cm^{-1}	1400-1000	Medium	C-N stretching	Amine

Thus, the phenol content was found to be present at an intensity of 71.1607at the second position of absorbance range 1323.89as shown in Table.2. It was found to be of medium-range and aromatic compounds were also present which prevents the auto-oxidation and lipid oxidation of meat products. In 2018 also experimented and reported the presence of high levels

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of phenolic compounds in the peel portion of the pomegranate fruit[18].

3.2 pH and cooking yield

The pH of the chicken blocks was checked frequently during the storage period. All the three i.e, control, reference and test product were checked for any difference in pH using the digital pH meter (Table.3).

Table 3: Effect of Pomegranate pericarp extract and BHT on pH and cooking yield of restructured chicken blocks

MEASUREMENT	CONTROL	REFERENCE(BHT 200PPM)	TEST (PFP 1.5%)
pH	6.68 ± 0.01	6.61 ± 0.04	7.03 ± 0.08
Cooking yield (%)	90.0 ± 5.2	95.7 ± 0.67	96.7 ± 0.27

There was not much significant difference in pH and cooking yield (p-value < 0.05) among the groups. pH was found to be near neutral and the addition of PFP extract or BHT did not alter the pH during the storage period.[6] reported that sheep meat nuggets prepared with 1% LFP extract had a comparably low pH value than those of control and BHT nuggets but nuggets with 100ppm BHT had lower pH compared to others.

The cooking yield of the restructured chicken blocks recorded in this study ranged from 90% to 97% and no significant changes were observed among the three products. The addition of PFP extract also did not have any drastic influence. Similar to the present study, [19] reported that the addition of curry leaf powder and extract of drumstick leaves in raw goat meat and cooked buffalo meat respectively did not show any significant differences in cooking yield compared with the control product. In one study reported a decrease in cooking yield at 40% level by incorporation of gizzard as well as fat [20]. In another study reported a cooking yield of 95.5 to 97.8%. [21].

3.3 Sensory analysis

The sensory attributes of cooked restructured chicken blocks concerning appearance, flavour, juiciness, tenderness and overall acceptability are presented in Table 5 and there exists significant(p-values<0.05) among the groups. The use of BHT (200 ppm) and PFP (1.5%) extract influenced the sensory attributes when compared to control.

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Table 5: Sensory attributes of cooked restructured chicken blocks treated crude extract of Pomegranate pericarp extract and BHT

SENSORY ATTRIBUTES	CONTROL	REFERENCE (BHT 200PPM)	TEST (PFP 1.5%)
Appearance	6.25 ± 0.21	7.12 ± 0.41	7.38 ± 0.27
Flavor	6.12 ± 0.41	7.12 ± 0.41	7.38 ± 0.27
Juiciness	6.12 ± 0.41	7.12 ± 0.41	7.38 ± 0.27
Tenderness	6.12 ± 0.41	7.12 ± 0.41	7.50 ± 0.28
Overall acceptability	6.12 ± 0.41	7.12 ± 0.41	7.50 ± 0.28

Number of observations (n)=8

General appearance and flavour scores of test and reference were not affected significantly even up to 20 days of storage under refrigeration and the scores remained above 7 indicating that all the products are well accepted. In [22] have stated that raw ground pork meat containing mint and curry leaf extracts exhibited better colour stability when compared to those treated with sodium nitrite. These scores were found to resemble the appearance scores of similar products when tested [23, 24].

Sensory scores for juiciness and tenderness was not affected much among the treatments and after the storage period, the scores ranged from 6.2 to 7.5 indicating good juiciness of the products. In a study reported that the addition of drumstick leaf extract had improved the quality of cooked ground buffalo meat by enhancing its tenderness and juiciness [19]. Overall acceptability of the test and reference was better when compared to control. Similar studies report that the addition of pomegranate rind and seed powder does not exert any negative effect on cooked meat patties [25, 26].

3.4 Microbiological quality

Standard plate count was performed at frequent time periods during storage and they were recorded as shown in Table 6

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Table 6: Effect of PFP extract on the SPC values (log_{10} CFU/g) of restructured chicken blocks during storage

STORAGE DAYS	CONTROL		REFERENCE		PFP EXTRACT	
	NA	SDA	NA	SDA	NA	SDA
1	2.22 ± 0.06	NG	0.53 ± 0.01	NG	0.47 ± 0.01	NG
6	3.25 ± 0.05	NG	2.67 ± 0.05	NG	1.77 ± 0.05	NG
12	3.72 ± 0.05	0.53 ± 0.01	3.23 ± 0.24	TLTC	3.30 ± 0.05	TLTC
17	4.20 ± 0.01	1.00 ± 0.03	3.83 ± 0.01	TLTC	3.67 ± 0.01	TLTC
20	4.63 ± 0.08	2.00 ± 0.03	4.07 ± 0.01	0.21 ± 0.02	3.87 ± 0.01	0.90 ± 0.05

NG= No growth, TLTC-to less to count

(The bacterial colonies on nutrient agar and yeast & mould growth on Sabouraud's dextrose agar were enumerated and were tabulated accordingly by calculating Mean ± SE with log $_{10}$ CFU/g)

The product with the PFP extract showed significantly lower bacterial and mould counts compared to the control product with salt. The reference product also showed good antimicrobial activity along with the test during the 20 days' storage. The lowest SPC values were recorded till the 17th day of storage. Researcher reported a shelf life of 10 days and yeast and mould counts were not detected in all the test, reference and control products throughout the storage period in restructured chicken slices incorporated with a gizzard [21]. In recent study, reported that the total plate counts of ground buffalo meat lowered significantly by the incorporation of drumstick leaf extract at various levels. [19].

The PFP extract prevented lipid oxidation which is the main cause of microbial growth. It could be due to the higher phenolic content by inhibiting the free radical formation and through chelation of metals. A similar study with litchi fruit pericarp extract [6] has also reported retarded lipid oxidation in meat products during storage. The incorporation of plant extracts containing phenolic and flavonoid compounds also prevented oxidation in the case of curry leaves and fenugreek leaves[25]. Further, antioxidant effects of grape seed extracts, tea catechins, rosemary, sage and extract of pomegranate in poultry meat have been reported.

Among the different parts of the pomegranate fruit, the peel contained the highest content of

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hydrolysable tannins compared to the juice or seeds[27]. In one study found that meatballs incorporated with PPE exhibited greater lipid and protein stability as well as sensory scores due to high phenolic content[28]. In study 2018, used lyophilized pomegranate peel nanoparticles (LPP-NP) to extend the shelf life and enhance the safety of beef meatballs [29]. After 15 days of storage at 4 °C, the indicators of protein degradation (total volatile base nitrogen) and lipid peroxidation (peroxide value and TBARS) in the meatballs containing 1.5% LPP-NPs was lower than samples with BHT (0.01%) and control without antioxidants. This positive effect could be associated with the presence of fibre in the pomegranate peels that acts as a water-binding agent.Therefore, pomegranate fruit pericarp extract (1.5%) can be used effectively for storage of the meat products for up to 15 to 17 days under refrigerated conditions.

Conclusions

The present study indicates that the pomegranate fruit pericarp extract has a good source of phenolic compounds and has antioxidant activity. The incorporation of PFP extract did not have any adverse effect on pH, cooking yield and sensory attributes. The data on SPC scores showed that the restructured chicken slices incorporated with 1.5% PFP extract were acceptable up to 17 days and was effective in inhibiting the lipid peroxidation similar to the synthetic antioxidant BHT (200ppm). Therefore, it was concluded that being a good natural antioxidant, pomegranate fruit pericarp extract could be used effectively to improve the product quality and stability during the storage of different meat and meat products. Further research to determine the molecules responsible for the antioxidant properties in these extracts is required.

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Conflicts of Interest: The authors declare no conflict of interest.

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