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Improving the Storability and Quality Characteristics of Cooked Chicken Patties by Using Fruit Extracts with Different Packaging Materials

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Abstract. Plant extracts are required for processing into food due to antioxidant and antimicrobial properties compared with toxic effects of synthetic antioxidants during storage. In this study, pomegranate peel extract (PPE) and grape seed extract (GSE) were incorporated in chicken patties and subjected to aerobic and vacuum packaging under storage at 4±1°C to prevent lipid oxidation and microbial spoilage of patties during storage. Samples stored and analyzed at interval of 0, 5, 10, 15 and 20 days. PPE and GSE prevented the lipid oxidation and microbial deterioration of treated samples and increased the nutritional profile of cooked chicken patties. Vacuum packaging aided in controlling moisture loss. Sensorial results showed that incorporation of PPE and GSE extracts was helpful strategy in retaining the keeping quality of patties. Fruit extracts improved the quality of patties during storage of 20 days as compared to controlled samples. Consequently, PPE and GSE can be used as a natural preservative coupled with vacuum packaging for meat-based products.

Keywords: quality, shelf life, storage, preservatives, packaging, fruits

Introduction

People now a days prefer to eat easily prepared food products because of their busy lifestyle and complex cooking processes. Moreover, they want healthy food products that are more nutritious and less time consuming. Raw meat and products made from meat both are important sources of protein, fat and vitamins. Their likeliness by consumers are based mainly on the amount of fat, which provide delicious taste, texture and a good mouth feel (Lorenzo and Franco, 2012).

Due to high fat and protein, meat-based products are more prone towards lipid oxidation as well as microbial contaminations which cause unwanted reactions that spoil color, odor, flavor and texture. Lipid peroxidation and microbial spoilage are the main causes of meat quality spoilage resulting in loss of flavor, color, textural and nutritional properties (Jayawardana *et al*., 2015; Giannenas *et al*., 2010). Among meat products, poultry meat is said to be more susceptible to lipid oxidation as compared to other red meats (Ali and Zahran, 2010). This is due to the higher number of phospholipids present in poultry meat.

Lipid or fat oxidation is generally a process during which unsaturated fatty acids of membrane phospholipids become oxidized when exposed to air. Phospholipids of membrane are more susceptible towards oxidation of lipids present in meat. This is due to the presence of increased amount of unsaturated fatty acids as compared to other lipids. This oxidation results in the formation of hydrogen peroxide (H_2O_2) and its secondary products that are ketones and short chain aldehydes. These secondary products are responsible for the deterioration

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in colour and flavour of meat-based products (Maqsood and Benjakul, 2011).

To restrict the growing conditions of unwanted micro-organisms as well as to hinder lipid oxidation process of lipids in processed meat-based products, antioxidants might be integrated into the food product during processing, apply a layer on its surface or to be applied onto the packaging substance being used (Hashemi *et al*., 2017). To prevent these deteriorations some chemical based synthetic antioxidants and antimicrobials are being utilized that restrict oxidation and microbial contamination. This includes BHT (Butylated Hydroxy toluene), BHA (Butylated Hydroxy anisole), TBHQ (Tertbutyl hydroquinone), PG (Propyl Gallate). These are effective in lipid oxidation but these synthetic preservatives are considered unsafe for health and causes toxicity in humans (Naveena *et al*., 2008).

Due to toxicological effects of synthetic antioxidants consumers do not prefer them and are moving towards natural ones. Consumers also prefer natural plant-based extract as preservatives and their consciousness regar-ding synthetic preservatives safety has led the food technologists to move towards natural sources (Hashemi *et al*., 2017; Naveena *et al*., 2008). Consumer approval and general satisfaction have been demonstrated to correlate with instrumental and trained sensory attributes associated with the flavor, tenderness and juiciness of meat (Miller *et al*., 2023).

Natural plant extracts are now a days gaining more popularity in the field of food technology being as antioxidants and antimicrobials. Hence being considered as GRAS (Generally Recognized as Safe) and must not affect negatively in the sensorial attributes like odor, colour, taste or flavour (Fernandes *et al*., 2016). They should be effective at low concentrations, show compatibility with the foods in which they are being used and should be easily useable (Horita *et al*., 2018; Vincekoviæ *et al*., 2017). Many useable compounds having antioxidant potential had been discovered in plant- based extracts, among them are the phenolic compounds that are most effective (Koubaa *et al*., 2016). The antioxidants with their highest efficiency are recognized by their free radical scavenging ability, their action as reducing agents, their chelation potential of pro-oxidant metals, and singlet oxygen quenching activity (Mohajer *et al*., 2016).

Pomegranate production in Pakistan has been growing steadily, and the country is known for its significant pomegranate cultivation. Pakistan has favorable climatic conditions for pomegranate production, particularly in the provinces of Punjab and Balochistan (Ali *et al*., 2020). Pomegranate (Punica granatum) is a major source of bioactive components that has been utilized in herbal medicine since old times. Pomegranate fruit pulp and juice has been discovered as antioxidant. Its juice has some quantity of ascorbic acid (Vitamin C), polyphenolic flavonoids and pectin (Aviram *et al*., 2000). Pomegranate peel is found to be a large source of tannins and anthocyanins (Özkal and Serpil 1994). Extract obtained from pomegranate peel has been discovered to contain high level of antioxidant activity other than the 'pulp extract' related to scavenging activity against hydroxyl radicals, reduction in lipoprotein oxidation that have low density and superoxide anion (Li *et al*., 2006; Ghasemian *et al*., 2006).

The main grape growing regions in Pakistan include Punjab, Sindh, Khyber Pakhtunkhwa and Balochistan. Punjab, particularly the districts of Sargodha and Multan, is known for its grape orchards. Sindh's districts of Hyderabad, Tando Allahyar and Mirpurkhas are also significant grape-growing areas (Shah *et al*., 2022). Grape (Vitis vinifera) seeds can be separated during grapefruit juice extraction and during wine production. These seeds can be dried, purified and extracted. This seed extract obtained from grapes has many phenolic compounds (Lau and King, 2003). Moreover, it is considered as major source of catechins which is a free radical scavenging compound and strong antioxidant (Liang *et al*., 2004). Grape seed extract has an effective antimicrobial activity against many microbes. High number of flavonoids, phenolic acids and stilbenes in grape plant stems are accountable for the antimicrobial ability (Anastasiadi *et al*., 2009; Vitseva *et al*., 2005).

Vacuum packaging is mostly being used for products like fresh raw meat, cured meat and processed meat products. Vacuum packs consist of pouches or vacuum skin packets, in which a low gas porous film is closely smeared on the product. Preservation can be developed by developing an anaerobic environment inside the package (Gill and Gill, 2005). Respiration created just after its packing will use the remaining O_2 left after packing, it converts it into CO_2, whose concentration is increased with in the package (Carpenter *et al*., 2007). The objective of this research was to evaluate the potential 'antioxidant' and 'antimicrobial' effects of extracts obtained from pomegranate peel and grape seed separately and their combined effect in cooked chicken patties both in aerobic and vacuum-packed environment under refrigerated storage temperature of 4 ± 1 °C.

Materials and Methods

Procurement of materials. Fresh boneless chicken meat was purchased from a local poultry processing plant in Multan. Three healthy birds were selected, slaughtered and de-feathered. Excessive fat was removed from the meat. The chest part was separated from leg portion and then deboned to obtain boneless chicken meat. About 2 Kg of boneless meat was procured and transferred to refrigeration temperature of about 4±1 °C till. Fresh pomegranates were obtained from local fruit market of Multan. About 3 Kg of fresh, healthy clean pomegranates were purchased.

Grape extract was purchased from supplier (Herba diet) that was distributed by Aruke health care rohtak haryana, India. It was obtained in a 100 g packet which contained 95% of GSE powder. Vacuum machine and vacuum packaging material (Always fresh seal vac) were purchased online from a local supplier in Pakistan. The package contained a sealing machine with six reusable bags. The vacuum machine removes air from the bags to keep food fresh for a long time.

Pomegranate peel extraction. Extract of pomegranate peel was obtained by using method described by Naveena *et al*. (2008). Healthy and mature pomegranate fruits were selected, washed and cut into two halves to remove its seeds. After removal of its seeds, its peel was cleaned with knife to remove any seed residues. The peel was cut into smaller pieces and placed under in drying. The partially dried pieces were placed in hot air oven (Memmert UN30-230V Universal Oven, Germany) at 60 for 24 h. to ensure its proper drying. The dried pieces were ground in a grinder (Moulinex-MC300161, France), to obtain its powder. This powder was stored in polythene bags till its sieving. For its sieving a sieve with a mesh size of 63 microns with the diameter of 200mm was used. It was subjected to Electromagnetic sieve shaker (Filtravibracion C/Bronce, Pol. Ind. Les Guixeres Barcelona) for 15 min. The powder was collected in a polythene bag till extraction. For extraction about 20g of dried powder was mixed with 500 mL of boiled distilled waterand mixed on magnetic stirrer (Velpscientifica *Via* Stazione, 16, 20865 usmate velate (MB) Italy) at 350 rpm. After complete mixing of powder with water it was left for 20 min at room temperature and filtered (Whatman Paper No. 42). Extract was collected in a volumetric flask, poured in a sampling bottle and stored at 4±1 °C.

Patties preparation. Fresh boneless chicken meat was washed and minced in a 4mm plate meat mincer (Anex Ag 3044 deluxe chopper). A pre-mix was made in which 85% of minced meat was taken and the following ingredients were added (2% salt, 0.4% red chili powder, 0.4%, black pepper and 10% corn flour). All material was mixed and divided into 50 g fractions each. Seven different chicken patty treatments were made by using PPE and GSE along with packing materials. (i) Control, no extract; (ii) PPE+AP, treated with pomegranate peel extract and packed in aerobic packaging material; (iii) PPE+VP, treated with pomegranate peel extract and packed in aerobic packaging material; (iv) GSE+AP, treated with grape seed extract and packed in aerobic packaging material; (v) GSE+VP, treated with grape seed extract and packed in vacuum packaging material; (vi) PPE+GSE+AP, treated with pomegranate peel extract, grape seed extract and packed in aerobic packaging material; (vii) PPE+GSE+VP, treated with pomegranate peel extract, grape seed extract and packed in vacuum packaging material. After adding above mentioned extracts, patties were cooked in a microwave oven (Dawlance cooking series) set for grilling for 10 min. After proper cooking, patties were kept for cooling at room temperature before packing.

After cooling, patties were packed in its respective packaging material. Aerobic packaging was done in polythene zip bags, while aerobic packaging was done in a nylon packaging bags by using a vacuum sealing machine. After packing the samples were stored at 4±1 °C. All the analyses of controlled samples were performed at Day 0.

pH and water activity determination. For pH determination, 1g of each sample was weighed and mixed in 100 mL distilled water. After proper mixing it was subjected to pH meter (MILWAUKEE pH and temperature laboratory bench meter-Mi150, Ontario, Canada) by placing its electrodes in the solution and kept for 5 min until its reading become stable. Water Activity was determined by Humi-meter (Schaller GmbH, Austria) by filling the sample in its glass container and kept for about 8-10 min until reading becomes stable (Sohaib *et al*., 2017).

Moisture determination. Moisture was determined using moisture analyzer (ADAM PMB-53, hollow road, Oxford USA) For this, about 5 g of sample was weighted and crushed in smaller pieces using a spatula. This crushed sample was placed in the tray of moisture analyzer and set for moisture analysis with the temperature set at 130 °C.

Fat, protein and ash determination. Fat was determined using Soxhlet extractor unit (Behr labor technik™ behrotest™ R 104 S), protein by Kjeldahl apparatus (Velp Scientifica Via Stazione, 16, 20865 Usmate velate (MB) Italy) and ash using muffle furnace (Vetrotecnica, Uruguay) following the official standard method (AOAC, 2016).

Texture profile analysis Hardness. Texture analysis in terms of hardness was performed by using TA-XT plus Texture Analyzer (Kassama *et al*., 2003).

TBARS determination. TBARS values of chicken patties were determined by extraction method as suggested by Sohaib *et al*. (2017).

Microbial analysis. Analysis for total plate count, total coliform, *E. Coli*, *S. Aureus*, salmonella and yeast/ mold were determined by dissolving agar broth in 105 mL of water. Then it was placed in water bath (Memmert, Germany) for 20 min at temperature of 95 °C for its proper dissolving. After removing from water bath agar bottle was shaken properly and placed in autoclave (Hirayama, hiclave HVE50, Japan). In an autoclave heating, leading to sterilization at 121 °C and then exhausting was performed for cooling as described by Gadekar *et al*. (2009) and Alabdulkarim *et al*. (2012).

Results and Discussion

pH and moisture. pH values of controlled samples showed a significant decrease between samples as shown Table 1. This decrease in pH values could be due to growth of lactic acid bacteria present in them (Sylvestre *et al*., 2001). For all other samples a gradual increase in values of pH was noted as storage time increased. This increment in pH values during refrigerated storage might be due to breaking of protein molecules and release of protein metabolites chiefly amines during storing of patties (Vaithiyanathan *et al*., 2011; Sylvestre *et al*., 2001) stated about the treatment of pomegranate fruit juice on meat samples. The phenolics present in pomegranate fruit juice increased the pH of meat during its storage period. In addition to this, the storage at refrigeration temperature also caused many enzymatic and chemical reactions. In contrast to this, Sarýçoban and Yilmaz (2014) found decrease in pH values in chicken patties due to addition of thyme/cumin essential oils in one treatment and addition of BHA and BHT in other treatments. In another study, the addition of moringa leaf powder resulted in decrease in pH in cooked chicken patties. Related to these results, decrease in pH value of moringa leaf used in herbal sausages has recently been stated (Jayawardana *et al*., 2015).

Patties with aerobic packaging were found to have more moisture loss as compared to vacuum packaging Table 2. Treatments T_1 and T_2 showed significant decrease in moisture from day 0 to day 20 of storage. Samples treated PPE showed more difference as compared to GSE treated samples This could be due to increased amount of total soluble solids in GSE treated patties as compared to PPE treated patties (Elhadi *et al*., 2017). Treatments T_5 and T_6 showed a significant decrease in moisture from day 0 to day 20. T_6 showed less decrease in moisture as compared to T_5. This could be due to more moisture permeability in aerobic packaging material as compared to vacuum packaging material. The reason could be the difference in type of packaging material. Aerobic packaging material was a poor barrier of moisture and oxygen while vacuum

Table 1. Effect on pH values of PPE and GSE treated cooked chicken patties during storage

Treatment	0 day	5 day	10 day	15 day	20 day
Control (T_0)	$6.61±0.8_b$	$6.56±0.5_{ab}$	$6.57±0.7_c$	$6.6±0.08_b$	$6.56±0.08_c$
PPE+AP (T_1)	$6.73±0.1a_b$	$6.78±0.4_{ab}$	$6.84±0.3_{bc}$	$6.96±0.37_b$	$6.84±0.35_b$
PPE+VP (T T_2)	$6.91±0.2_a$	$6.76±0.2_{ab}$	$6.91±0.2_b$	$6.75±0.2_b$	$6.99±0.21_b$
GSE+AP (T_3)	$6.56±0.4_b$	$6.58±0.3_a$	$6.62±0.4_{bc}$	$6.77±0.29_b$	$6.63±0.15_c$
GSE+VP (T_4)	$6.59±0.2_b$	$6.51±0.7_b$	$6.59±0.9_c$	$6.65±0.1_b$	$6.64±0.09_c$
PPE+GSE+A P (T_5)	$6.62±0.7_b$	$6.8±0.2_a$	$6.75±0.9_{bc}$	$6.7±0.12_b$	$6.63±0.09_c$
PPE+GSE+V P (T_6)	$6.63±0.9_b$	$6.57±0.8_a$	$6.81±0.5_{bc}$	$6.67±0.6_b$	$6.67±0.05_c$

Means ± standard deviation, PPE = pomegranate peel extract; GSE = grape seed extract; AP = aerobic packing; VP = vacuum packaging.

Table 2. Effect on moisture percentage of PPE and GSE treated cooked chicken patties during storage

Treatment	0 day	5 day	10 day	15 day	20 day
Control (T_0)	56.61±0.75$_{e}$	55.47±0.39$_{c}$	54.07±0.61$_{e}$	54.02±1.22$_{c}$	52.94±2.2$_{b}$
PPE+AP (T_1)	62.71±1.5$_{a}$	61.71±1.7$_{a}$	60.62±0.72$_{a}$	59.17±1.5$_{a}$	56.75±2.5$_{a}$
PPE+VP (T_2)	61.23±1.2$_{a}$	60.39±1.9$_{a}$	59.17±0.4$_{b}$	58.97±0.4$_{a}$	56.47±3.1$_{a}$
GSE+AP (T_3)	58.35±0.8$_{d}$	58.18±0.4$_{b}$	57.79±0.43$_{d}$	57.52±0.7$_{b}$	56.48±0.3$_{a}$
GSE+VP (T_4)	59.09±0.7$_{c}$	58.62±0.43$_{b}$	58.5±0.7$_{c}$	58.44±0.2$_{a}$	57.74±0.7$_{a}$
PPE+GSE+AP (T_5)	60.3±0.3b$_{c}$	58.98±0.7$_{b}$	59.32±0.3$_{b}$	58.69±0.9$_{a}$	57.9±0.71$_{a}$
PPE+GSE+VP (T_6)	58.57±1.3$_{d}$	57.99±1.8$_{b}$	58.41±0.4$_{c}$	58.45±0.7$_{a}$	57.82±0.5$_{a}$

Means ± standard deviation, PPE = pomegranate peel extract; GSE = grape seed extract; AP = aerobic packing; VP = vacuum packaging.

packaging material was good barrier of moisture and oxygen (Gill and Gill, 2005). As storage time increased more moisture was lost from patties due to which a significant decrease in moisture values was recorded. According to another observation, addition of dried plum ingredients in raw and pre-cooked patties caused an increase in moisture percentage as compared to controlled samples (Nunez de Gonzalez *et al*., 2008). Similarly, a non-significant alteration in moisture of fresh sausages was recorded when treated with extract of microencapsulated jabuticaba (Baldin *et al*., 2016).

Fat, protein and ash. Storage days did not have any significant effect on percentages of protein, fat and ash. According to results, for treatment T_0 a significant reduction in protein values was recorded from day 0 to day 5, then a significant increase at day 10 and a non-significant decrease at day 15 was recorded in Table 3. Moreover, a significant reduction in values was recorded on day 20. For treatment T_1, a non-significant difference is observed from day 0 to day 15 and an observed significant decrease at day 20. For T_2, a non-significant reduction in values was recorded from day 0 to day 5 then a significant increase at day 10 was recorded which led to non-significant increase up to day 20. The increased amount of protein in samples that were treated with pomegranate peel extract could be due to some amount of protein present in pomegranate peel (Pande and Akoh, 2016). Addition of *Aloe Vera* gel, ginger paste and tomato paste significantly increased the amount of protein percentage in the last two months of frozen storage and showed insignificant increase in first 15 days (Reddy *et al*., 2013).

During storage of samples, controlled samples showed a significant increase from day 0 to day 5, a significant decrease from day 5 to day 15 and a significant increase at day 20 as shown in Table 4. For treatment T_1, a significant reduction in values was recorded from day 0 to day 20. With the increase in storage days, a significant decrease in values was recorded in treatments T_2 and T_3 from day 0 to day 20. A similar trend was recorded for treatments T_4, T_5 and T_6, with the increase in storage period a significant decrease in fat % values was recorded from day 0 to day 20. Controlled samples had the least fat percentage as compared to the other two treatments. PPE contained more fat than controlled samples because pomegranate peel contains some amount of fat in it.

Table 3. Effect on protein of PPE and GSE treated cooked chicken patties during storage

Treatment	0 Day	5 Day	10 Day	15 Day	20 Day
Control (T_0)	23.49±1.1$_{d}$	22.65±0.4$_{c}$	23.62±2.34$_{b}$	23.45±1.34$_{b}$	23.16±1.3$_{cd}$
PPE+AP (T_1)	34.7±3.15$_{a}$	36.35±2.4$_{a}$	34.03±2.4$_{a}$	34.79±3.1$_{a}$	34.32±2.9$_{b}$
PPE+VP (T_2)	34.02±0.7$_{ab}$	33.46±2.2$_{ab}$	35.99±1.5$_{a}$	33.21±2.8$_{a}$	35.34±2.3$_{a}$
GSE+AP (T_3)	27.27±0.8c	25.05±2.5$_{c}$	25.56±2.7$_{b}$	24.32±1.8$_{b}$	26.4±1.6c
GSE+VP (T_4)	25.49±0.6$_{cd}$	25.9±2.8c	26.48±1.12$_{b}$	23.84±2.8$_{b}$	25.48±0.9$_{c}$
PPE+GSE+AP (T_5)	32.68±1.9a$_{b}$	31.69±1.7$_{b}$	32.51±2.4$_{a}$	31.75±2.3$_{a}$	31.39±2.9$_{b}$
PPE+GSE+V P (T_6)	31.07±1.9$_{b}$	30.65±2.4$_{b}$	32.78±2.1$_{a}$	31.6±1.9$_{a}$	32.94±1.7$_{b}$

Means ± standard deviation, PPE = pomegranate peel extract; GSE = grape seed extract; AP = aerobic packing; VP = vacuum packaging.

Fat percentages in patties treated with both PPE and GSE were in between the ranges of PPE and GSE treated patties.

For ash percentages, at day 0, a significant increase in ash percentages was recorded from T_0 to T_1 and a non-significant increment in values was recorded from T_1 to T_2 in Table 5. The increase in PPE treated samples as compared to controlled samples could be due to presence of some amount of ash content present in pomegranate peel as pomegranate peel contains about 3.30% of ash content in it (Pande and Akoh, 2016). Furthermore, a significant decrease was recorded from T_2 to T_3 and T_4. This decrease in ash% could be due to less amount of ash content present in grape seeds as compared to pomegranate peel *i.e*., 2.05-2.08%. Similar results were found by Elhadi *et al*. (2017), according to which increase in ash% was recorded due to incorporation of moringa leaf powder in chicken patties.

Water activity. A significant reduction in values of water activity was recorded from day 0 to day 20 in all treatments Table 6. This decrease in water activity could be due to decrease. Moisture content with the increase

Table 4. Effect on fat percentage of PPE and GSE treated cooked chicken during storage

Treatment	0 day	5 day	10 day	15 day	20 day
Control (T_0)	$14.53\pm0.6_{c}$	$14.55\pm0.4_{b}$	$14.48\pm0.2_{b}$	$14.39\pm0.3_{b}$	$14.51\pm0.9_{cd}$
PPE+AP (T_1)	$15.03\pm0.1_{bc}$	$15.03\pm0.19_{b}$	$15.07\pm0.17_{b}$	$14.95\pm0.2_{b}$	$14.99\pm0.7_{c}$
PPE+VP (T_2)	$16.07\pm0.1_{ab}$	$15.02\pm0.1_{b}$	$14.99\pm0.12_{b}$	$14.87\pm0.88_{b}$	$15.15\pm0.8_{c}$
~~GSE+AP (T_3)~~	~~$17.06\pm0.4_{a}$~~	~~$17.61\pm0.6_{a}$~~	~~$17.02\pm1.04_{a}$~~	~~$16.85\pm1.1_{a}$~~	~~$16.92\pm1.4_{b}$~~
GSE+VP (T_4)	$17.81\pm0.5_{a}$	$16.29\pm1.2_{b}$	$15.22\pm1.01_{b}$	$14.52\pm0.67_{b}$	$13.72\pm0.5_{e}$
PPE+GSE+A P (T_5)	$14.55\pm0.4_{d}$	$13.18\pm0.2_{c}$	$13.59\pm1.14_{d}$	$13.18\pm1.16_{c}$	$14.07\pm0.6_{cd}$
PPE+GSE+V P (T_6)	$31.07\pm1.9_{b}$	$30.65\pm2.4_{b}$	$32.78\pm2.1_{a}$	$31.6\pm1.9_{a}$	$32.94\pm1.7_{b}$

Means ± standard deviation; PPE = pomegranate peel extract; GSE = grape seed extract; AP = aerobic packaging, VP = vacuum packaging.

Table 5. Effect on ash percentage of PPE and GSE treated cooked chicken patties during

Treatment	0 day	5 day	10 day	15 day	20 day
Control (T_0)	$1.46\pm0.9_{b}$	$1.34\pm0.1_{cd}$	$1.6\pm0.2_{bc}$	$1.49\pm0.1_{c}$	$1.48\pm0.5_{c}$
PPE+AP (T_1)	$1.86\pm0.1_{a}$	$1.81\pm0.3_{b}$	$1.79\pm0.2_{b}$	$2.12\pm0.6_{b}$	$1.86\pm0.1_{b}$
PPE+VP (T_2)	$1.88\pm0.6_{a}$	$1.36\pm0.8_{cd}$	$1.34\pm0.1_{d}$	1.32±0.8de	$1.38\pm0.7_{c}$
GSE+AP (T_3)	$1.46\pm0.5_{b}$	1.53±0.9c	$1.49\pm0.6_{acd}$	$1.46\pm0.7_{c}$	$1.49\pm0.5_{c}$
GSE+VP (T_4)	$1.37\pm0.4_{bc}$	$1.47\pm0.6_{cd}$	$1.52\pm0.7_{acd}$	$1.49\pm0.6_{c}$	$1.47\pm0.5_{c}$
PPE+GSE+AP (T_5)	$1.25\pm0.9_{c}$	$1.24\pm0.6_{d}$	$1.31\pm0.3_{d}$	$1.26\pm0.7_{e}$	$1.25\pm0.6_{c}$
PPE+GSE+V P (T_6)	$1.46\pm0.9_{b}$	$1.34\pm0.1_{acd}$	$1.6\pm0.2b_{c}$	$1.49\pm0.1_{c}$	$1.48\pm0.5_{c}$

Means ± standard deviation; PPE = pomegranate peel extract; GSE = grape seed extract; AP = aerobic packaging, VP = vacuum packaging.

Table 6. Effect on water activity of PPE and GSE treated cooked chicken patties during storage

Treatment	0 day	5 day	10 day	15 day	20 day
Control (T_0)	$0.92\pm0.1_{b}$	$0.82\pm0.1_{d}$	$0.78\pm0.1_{e}$	0.74±0.1e	$0.74\pm0.3_{b}$
PPE+AP (T_1)	$0.93\pm0.3_{a}$	$0.83\pm0.2_{d}$	$0.81\pm0.2_{cd}$	$0.77\pm0.3_{bc}$	$0.75\pm0.1_{bc}$
PPE+VP (T_2)	$0.93\pm0.1_{a}$	$0.89\pm0.3_{b}$	$0.85\pm0.2_{b}$	$0.79\pm0.1_{b}$	$0.71\pm0.2_{d}$
GSE+AP (T_3)	$0.91\pm0.4_{c}$	$0.88\pm0.1_{c}$	$0.83\pm0.1_{bc}$	$0.78\pm0.1_{bc}$	$0.75\pm0.1_{b}$
GSE+VP (T_4)	$0.91\pm0.1_{c}$	$0.89\pm0.7_{b}$	$0.85\pm0.3_{b}$	$0.78\pm0.01_{bc}$	$0.75\pm0.4_{bc}$
PPE+GSE+A P (T_5)	$0.92\pm0.5_{b}$	$0.89\pm0.1_{b}$	$0.84\pm0.2_{b}$	$0.76\pm0.02_{cd}$	$0.74\pm0.6_{bc}$
PPE+GSE+V P (T_5)	$0.92\pm0.1_{b}$	$0.82\pm0.1_{d}$	0.79±0.1de	$0.76\pm0.01_{de}$	$0.72\pm0.2_{cd}$

Means ± standard deviation; PPE = pomegranate peel extract; GSE = grape seed extract; AP = aerobic packaging, VP = vacuum packaging.

in storage period. The reduction of free water availability by removing of water or addition of solutes or hydrocolloids aid in lowering of water activity in a product (Nunez de Gonzalez *et al*., 2008). As more amount of moisture was lost from samples that were aerobically packed so more reduction in water activity was recorded. Whereas vacuum packed samples had less moisture loss due to which less increase in water activity was recorded. This could be due to the increased amount of total soluble solids in GSE treated samples (Elhadi *et al*., 2017; Nunez de Gonzalez *et al*., 2008).

Hardness. According to observation, a significant increment in values of hardness was recorded in all the treatments in Table 7. Samples that were packed in aerobic packaging showed greater increase in hardness as compared to vacuum packaging. This could be due to more permeability of air and moisture in aerobic packaging as compared to vacuum packaging. This allowed less amount of water lost through vacuum packaging material (Gill and Gill, 2005). In aerobic packaging an excess amount of water was lost due to which hardness of samples increases. In contrast to this, vacuum packaging played its role in retention of moisture as well as hardness of the samples. According to results, the addition of PPE and GSE did not affect hardness of samples. Although controlled samples were harder than treated samples. Similarly, addition of PPE in chicken nuggets did not affect textural properties but vacuum packaging played its role it is retaining moisture and softness as compared to aerobic packaging (Sarýçoban *et al*., 2014). As reported the hardness was increased with the passage of storage time. Moreover, loss of moisture in meat and meat products would increase its textural parameters (Pande and Akoh, 2016).

TBARS determination. When compared, values of TBARS were significantly higher in controlled samples, followed by PPE and GSE treated samples packed aerobically and lowest in PPE and GSE treated samples vacuum packed as shown in Table 8. There was a significant lowering in TBARS values by addition of PPE and GSE and further decrease in TBARS values by use of vacuum packaging (Pande and Akoh, 2016). The least TBRAS value was of T_6 which contains both GSE and PPE and was vacuum packed (Reddy *et al*.,

Table 7. Effect on hardness of PPE and GSE treated cooked chicken patties during storage

Treatment	0 day	5 day	10 day	15 day	20 day
Control (T_0)	21.04±0.6$_{a}$	23.41±0.4$_{ab}$	24.02±0.78$_{a}$	26.49±0.91$_{a}$	27.64±0.46$_{b}$
PPE+AP (T_1)	20.03±0.8$_{b}$	20.69±0.4$_{c}$	21.06±0.78$_{c}$	25.17±0.8$_{b}$	25.89±0.2$_{c}$
PPE+VP (T_2)	19.92±0.7$_{b}$	21.16±0.9$_{c}$	22.9±0.26b	23.96±0.17$_{c}$	24.55±0.47$_{e}$
GSE+AP (T_3)	21.73±0.2$_{a}$	23.23±0.2$_{a}$	24.13±0.76$_{a}$	26.25±0.85$_{a}$	27.87±0.27$_{b}$
GSE+VP (T_4)	21.65±0.4$_{a}$	22.9±0.2$_{b}$	24.18±0.7$_{a}$	25.07±1.7$_{b}$	26.78±1.2$_{b}$
PPE+GSE+A P (T_5)	21.42±0.6$_{a}$	23.23±0.2$_{b}$	24.01±0.7$_{a}$	26.79±0.4$_{a}$	29.13±0.8$_{a}$
PPE+GSE+V P (T_6)	21.67±0.6$_{a}$	23.78±0.3$_{a}$	23.9±0.2$_{ab}$	24.11±0.5$_{c}$	24.84±0.2$_{d}$

Means ± standard deviation; PPE = pomegranate peel extract; GSE = grape seed extract; AP = aerobic packaging, VP = vacuum packaging

Table 8. Effect on TBRAS values of PPE and GSE treated cooked chicken patties during storage

Treatment	0 Day	5 Day	10 Day	15 Day	20 Day
Control (T_0)	0.34±0.1$_{ab}$	0.41±0.3$_{b}$	0.46±0.1$_{bc}$	0.56±0.1$_{b}$	0.52±0.3$_{b}$
PPE+AP (T_1)	0.33±0.2$_{a\text{-}c}$	0.39±0.2$_{b}$	0.45±0.1$_{b}$	0.56±0.2$_{b}$	0.51±0.03$_{b}$
PPE+VP (T_2)	0.31±0.2$_{bc}$	0.32±0.3$_{c}$	0.39±0.2$_{de}$	0.44±0.02$_{cd}$	0.48±0.3$_{b}$
GSE+AP (T_3)	0.35±0.1$_{a}$	0.39±0.3$_{b}$	0.48±0.5$_{b}$	0.55±0.2$_{b}$	0.51±0.4$_{b}$
GSE+VP (T_4)	0.32±0.3$_{ac}$	0.33±0.1$_{c}$	0.41±0.4$_{ce}$	0.48±0.03$_{c}$	0.41±0.03$_{c}$
PPE+GSE+A P (T_5)	0.3±0.03$_{c}$	0.31±0.1$_{c}$	0.48±0.3$_{b}$	0.41±0.3$_{de}$	0.35±0.3$_{d}$
PPE+GSE+V P (T_6)	0.25±0.2$_{d}$	0.31±0.2$_{c}$	0.36±0.3$_{e}$	0.38±0.4$_{e}$	0.31±0.3$_{d}$

Means ± standard deviation; PPE = pomegranate peel extract; GSE = grape seed extract; AP = aerobic packaging, VP = vacuum packaging.

2013). This was due to combined antioxidant effect of PPE and GSE. Similar to these results, addition of pomegranate peel extract in chicken and goat meat showed its antioxidant behavior in treated samples (Baldin *et al*., 2016). According to these findings, pomegranate peel having strong antioxidant effects is due to high amount of total phenolics present in it. Results regarding vacuum packaging are in accordance with the findings of vacuum packaged meat-based items (Carpenter *et al*., 2007).

Microbiological analysis. A significant decrease in counts of microbes was observed with the use of PPE and GSE along with vacuum packaging. A significant increase in TPC and total coliform counts was recorded from day 0 to day 20 in all the controlled and treated samples. At day 0, few of the colonies were observed on petri plates mostly on controlled samples shown in Fig. 1A and Fig. 1B, some of them were present in GSE and PPE treated samples in aerobic packaging and least were found in samples containing PPE and GSE extracts. Antioxidants added in mutton slices and packed under vacuum environment lowered the total plate counts as compared to controlled samples and BHA preserved samples. It could be due to antimicrobial activity of proanthocyandins, and polyphenolic s present in grape seed extract (Erkmen and Bozoglu, 2016). Moreover, a linear increa se in total plate counts of hurdle treated chevonkeema when vacuum packaged was recorded keeping at room temperature (Devatkal *et al*., 2014).

Escherichia Coli was absent in all the samples. Only few colonies were found in controlled samples at day 10 in controlled samples. These colonies could be due to air contamination or contamination during handling shown in Fig. 1C. The absence of *Escherichia Coli* colonies in most of the samples could be due to use of hygienic conditions in which samples were prepared and stored. *E. coli* could be grown due to contaminated water used or due to contamination in air (Nunez de Gonzalez *et al*., 2008). A significant increase in colonies of Staphylococcus aureus was recorded for controlled samples from day 0 to day 5 and a non-significant increment up to day 20 shown in Fig. 1D. Controlled samples showed highest increase in colonies of Staphylococcus aureus as compared to other treated samples. T_1 samples treated with PPE and packed under aerobic conditions showed significant growth of colonies from day 0 to day 20 as compared to controlled samples but this growth was lesser in T_3 samples that were packed under vacuum environment. This could be due to antimicrobial effect of PPE that was incorporated in samples (Martinez *et al*., 2004). Vacuum packaging played its part in controlling microbial growth (Martinez *et al*., 2004). The GSE treated samples showed less increase in colonies of Staphylococcus aureus as compared to PPE treated and controlled samples. This showed that GSE has more antimicrobial properties than PPE as grape seeds richer quantity of flavonoids and its derivatives. Anastasiadi *et al*. (2009), also grape seeds are a good source of polymers of flavan-3-ols that are merely responsible for its antimicrobial activity.

All the samples were found to be free from the contamination of Salmonella species. No colonies were observed in any samples of all the treatments shown in

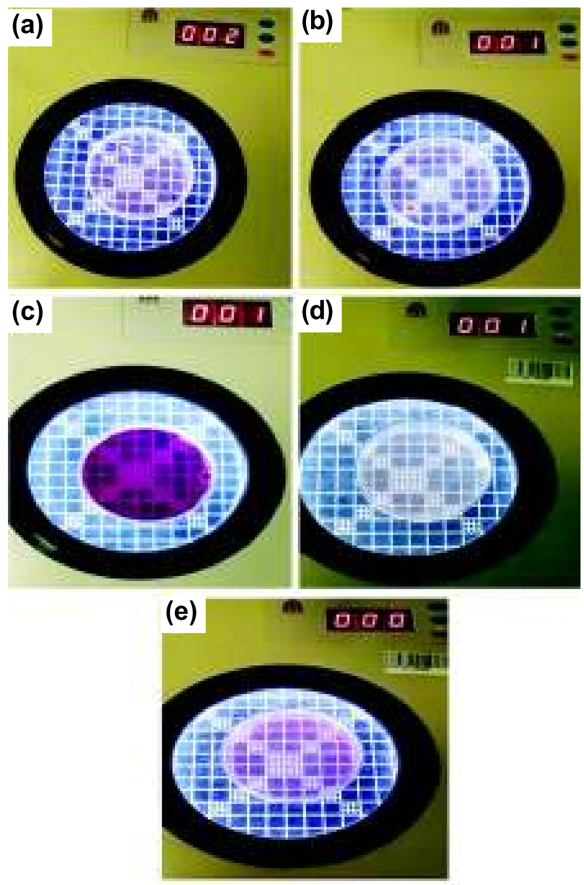

Fig. 1. Effect of PPE and GSE treated colonies of microbes during storage. **(a)** total plate count colonies at day 0 **(b)** total coliform colonies at day 0 **(c)** E.coli counts due to air contamination **(d)** S. aureus count at day 20 **(e)** No grouth of salmonella colonies.

Fig. 1E. Their absence might be due to sample preparation in hygienic conditions and use of sterilized distilled water. Antimicrobials like PPE and GSE played their part in controlling the microbial growth in samples. Moreover, packaging material like vacuum packaging prevent microbial growth by acting as a barrier against microorganism present in atmosphere.

A significant increase in yeast and mold counts was recorded during the storage. Controlled samples showed highest increase in colonies of yeast and mold as compared to treated samples as shown in Fig. 6. This might be due to absence pf any antimicrobial as well as packaging material (Reddy *et al*., 2013). The samples treated with PPE showed lesser growth of colonies as compared to controlled samples. This could be due to antimicrobial effect of PPE that was incorporated in samples. The GSE treated samples showed less increase in colonies of yeast and mold as compared to PPE treated and controlled samples. This showed that GSE has more antimicrobial properties than PPE as grape seeds have high concentrations of flavonoids and its derivatives, also grape seeds are a rich source of polymers of flavan-3-ols that are merely responsible for its antimicrobial activity.

Sensory attributes. Sensory attributes of all the samples including controlled and treated samples showed a characteristic change in its properties including colour, flavor and overall acceptability.

Colour analysis. Overall scores of colour characteristics decreased during its storage, scores of controlled samples were lower as compared to treated samples as depicted in Fig 2. PPE treated samples had highest scores as compared to PPE which showed that PPE played its role in retaining its natural colour. GSE treated sample had darkened colour due to dark grey colour of grape seed extract. GSE treated samples scores were significantly higher than controlled samples. This showed that GSE also played its role in improving its colour. GSE treatment along with vacuum packaging prevented is colour deterioration due to inhibition of myoglobin oxidation. The delay in colour spoilage due to incorporation of GSE during storage is reported (Ahn *et al*., 2004). In another research treatment of GSE in restructured mutton slices kept in both aerobic and vacuum environment. GSE played its part in preventing colour deterioration as compared to controlled and BHA treated samples. A significant increase in T_5 and T_6 color scores could be due to the combined effect of PPE and GSE in retaining its original colour. Thus, preventing its deterioration (Baldin *et al*., 2016; Devatkal *et al*., 2014).

Flavor analysis. At day 5, scores for flavour characteristics significantly increased from T_o to T_1 and T_2 and significantly decreased at T_3 and T_4 (Fig. 3). Furthermore, a significant increase in T_5 and T_6 was recorded. At day 10, similar trend was recorded as that of day 5. At day 15, a significant increase in T_1 was recorded from T_o. Furthermore, a non-significant change in other samples was recorded. At day 20, a significant increase in scores of flavor characteristics was recorded from T_o to T_1 and T_2 which further increased significantly at T_3 and T_4. Furthermore, a significant increase in T_5 and T_6 was recorded. Increase scores for flavor in PPE treated

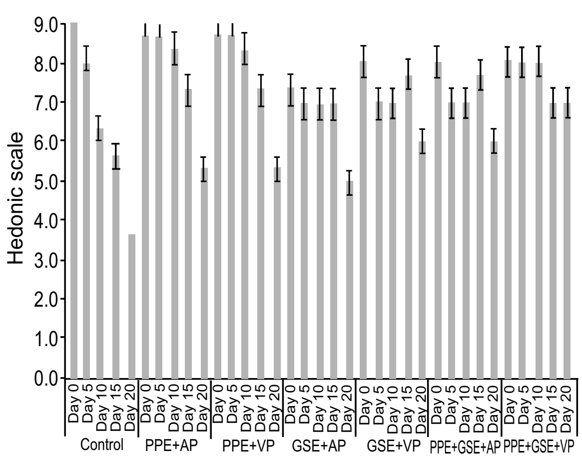

Fig. 2. Effect on color of cooked chicken patties treated with PPE and GSE during storage.

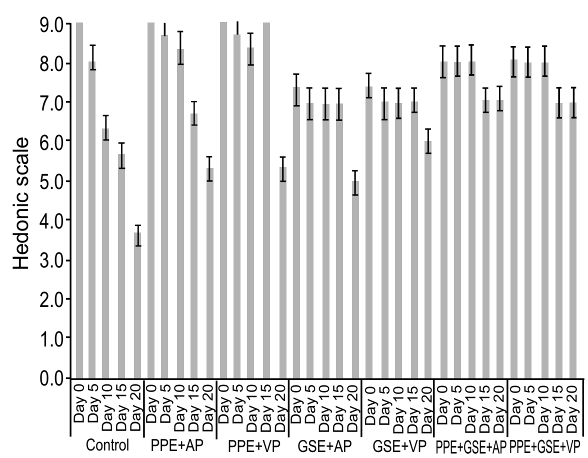

Fig. 3. Effect on flavor of cooked chicken patties treated with PPE and GSE during storage.

sample could be due to addition of pomegranate peel extract which help in retaining natural flavor of patties as compared to controlled samples. This could be due to its antioxidant effect which prevented lipid oxidation that caused off flavour. Further increase in T_3 and T_4 could be due to addition of GSE which retained more of its characteristic flavor due to its antioxidant and antimicrobial behaviour (Martinez *et al*., 2004). Increased values of T_5 and T_6 could be due to combined effect of PPE and GSE in treated samples. This might be due to retention of flavor by natural antioxidants added. Similarly, GSE was active in reducing the strength of rancid flavour of pre-cooked meat samples. According to another research, grape seed extract lowered rancid and wet cardboard off-odor scores which showed that GSE can reduce rancid flavour (Ahn *et al*., 2004).

The mean values of overall acceptability scores are presented in Fig. 4. According to which scores of overall acceptability was reduced significantly in controlled samples due to its deterioration. In PPE and GSE treated samples, acceptability was more as compared to controlled samples due to antioxidant and antimicrobial effects of PPE and GSE. In samples treated with both PPE and GSE had more overall acceptability scores as compared to other samples. In accordance with these results, Reddy *et al*. (2013) reported that restructured mutton slices treated with GSE showed better overall acceptability as compared to controlled samples. According to another research, addition of GSE in pork patties significantly improved the overall acceptability of patties (Carpenter *et al*., 2007). Contrary to above

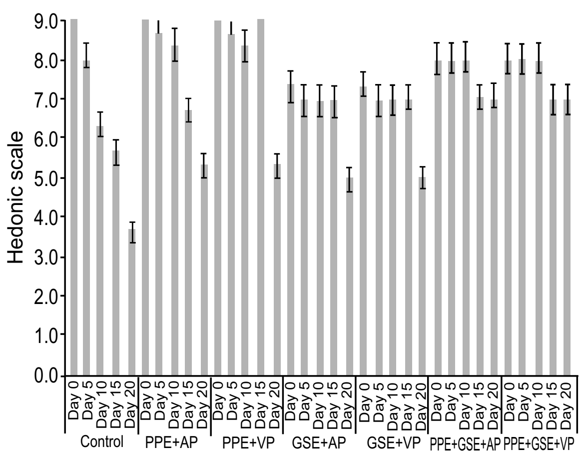

Fig. 4. Effect on overall acceptability of cooked chicken patties treated with PPE and GSE during storage.

results, decrease in overall acceptability of meat product was observed when stored under refrigerated storage (Karthikeyan *et al*., 2000).

Conclusion

Meat based products are more prone towards microbial and fungal contamination contaminations which cause unwanted reactions that spoil colour, odour, flavour, taste and rheological properties. Natural plant extracts are now a days gaining more popularity in the field of food technology being as antioxidants and antimicrobial agents. Samples incorporated with both pomegranate peel and grape seed extract followed by vacuum packaging showed least changes in color, flavour and overall acceptability compared with controlled samples.

Conflict of Interest. The authors declare that they have no conflict of interest.

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