Research Article

# Comparative Efficacy of Natural Antioxidants on Physico-Chemical and Microbial Stability of Cooked Pork Patties during Refrigerated (4±1°C) Storage

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## Abstract

The comparative antioxidant and antimicrobial efficacy of grape seed (*Vitis vinifera*) extract (GSE) at 0.1 % and green tea *(Camellia sinensis)* extract at 0.1 % with synthetic antioxidant ie) butylated hydroxy anisole (BHA) at 0.01 % was studied in cooked pork patties under aerobic packaging conditions during refrigerated storage (4±1°C). The pork patties treated with GSE had significantly ($P < 0.05$) lower thiobarbituric acid reactive substance (TBARS) values and free fatty acids (FFA) content compared to control (C) and other samples in aerobic packaged conditions. A significant ($P<0.05$) lower microbial counts were noted in the pork patties added with natural antioxidants. The GSE treated pork patties rated significantly ($P < 0.05$) superior scores of color, flavor, tenderness, juiciness and overall palatability than control, BHA and GTE treated samples. Based on the results obtained, it can be concluded that GSE has excellent antioxidant and antimicrobial properties compared to control, BHA and GTE treated pork patties during refrigerated storage under aerobic conditions.

**Keywords:** Grape seed extract, green tea extract, storage stability, quality characteristics, pork Patties

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## Introduction

Cooked meat is more susceptible than raw meat during storage due to the heating process resulting in acceleration of oxidative reactions with the lipids in meat [1]. Many mechanisms and ways are applying to control lipid oxidation of cooked meats during storage. Pork is higher degree of unsaturation of muscle lipids compared to other species meat and more susceptible to lipid oxidation. Addition of antioxidants during processing, cooking or packaging is used to inhibit/delay oxidation in processed cooked meat products. Various synthetic antioxidants like butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), propyl gallate (PG), tertiary butyl hydroquinone (TBHQ), tri hydroxy butyro phenone (THBP), nor dihydro guaiaretic acid (NDGA) and ethoxyquin have extensively utilized to delay lipid oxidation in meat products [2]. However, use of these types of synthetic antioxidants is controlled due to their carcinogenic potential. Natural antioxidants extracted from various parts of plants such as rosemary, green tea, grape seeds, grape peel, citrus peel, sesame seeds, olives and avacodo are using alternate to synthetic antioxidants because of their equivalent or greater effect of antioxidant activity.

Green tea leaf extracts are becoming increasingly important as a functional food in the diet because of their high polyphenols contents. Its polyphenols contents can increase up to 36% (dry basis) due to climate, season or variety [3]. The antioxidative property of green tea extract is due to the presence of catechins, apicatechins, epicatechin gallate, epigallocatechin, and epigallocatechin gallate [4]. Grape seed extracts (GSE) are industrial derivatives from whole grape seeds that have a great concentration of vitamin E, flavonoids and linoleic acid. The aim of the present study was to assess the comparative efficacy of synthetic (BHA) and natural antioxidants ie) green tea extract and grape seed extracts on oxidative, microbial and sensory quality of cooked pork patties during refrigerated storage (4±1°C).

## Experimental

### *Pork patties development*

Fresh lean meat (ham and loin) and other non-meat ingredients procured from local market of Tirupati and analytical chemical and food grade additives were procured from standard companies. The natural antioxidants grape seed extract and greed tea extract obtained from Lactonova Pvt. Ltd, Hyderabad, India. The fresh lean meat cut into small chunks and minced in a meat mincer (Sirman, TC 12 E, Italy) through 4 mm plate. The emulsion was prepared by chopping the minced meat along with other non-meat ingredients in a bowl chopper (Scharfen, Model No: TC 11, Germany). The emulsion was prepared by using minced pork is mixed with salt @ 1.5 %, STPP @ 0.4 %, sodium nitrate @ 150 ppm, sodium ascorbate @ 500 ppm, sugar @ 1 % and ice flakes @ 8 % and chopped for one min followed by addition of oil @ 6 % and again chopped for one min and add corn flour @ 3 %, spice mix @ 1.6, condiment mix @ 3 % (onion and garlic: 3:1) and chopped for 3 min. The temperature of the emulsion was maintained between 18 to 20ºC. The emulsion was separated into four parts and first part was control (C) (without addition of any antioxidant) and second part was added with synthetic antioxidant i.e.) 0.01% butylated hydroxy anisole (BHA) and third part was added with 0.1 % green tea extract (GTE) and fourth part was added with 0.1% grape seed extract (GSE). In each part, 60 g of pork emulsion was taken for preparation of each raw patty and moulded in round flat shape by using 9.0 cm diameter and 1.2 cm height of bottom glass of petridish and cooked in hot air oven till the patty core temperature reached to 75±3ºC. The control and treatments separately cooked, cooled and packaged separately in low density polyethylene bags and stored in refrigerated temperature (4±1°C). The patties were evaluated for their quality at five days interval up to 20 days.

### *Analytical Parameters*

*Physico-chemical characteristics*

The pH of pork patties were determined by homogenizing 10 g of sample with 50 ml distilled water with the help of tissue homogenizer (Daihan Scientifics, WiseMix, HG-15D, Korea) for 1 min. The pH was recorded using digital pH meter (Thermo Orion, Model 420A+, USA). The TBARS value was determined adopting the procedure of Witte [5]. Free fatty acids per cent was determined according to method described by Koniecko [6]. The quantity of potassium hydroxide consumed and expressed as percentage of oleic acid. Free fatty acids (% oleic acid): 0.1 X ml of 0.1 N alcoholic KOH X 0.282/ weight of sample (g) X 100.

*Microbiological profiles*

All the microbiological parameters of pork patties were determined as per the methods described by APHA [7].

*Sensory evaluation*

The pork patties were warmed and served to trained panelists and evaluated for sensory characteristics like appearance, flavour, juiciness, tenderness and overall acceptability using a 8-point hedonic scale (where, 8=extremely desirable, 1= extremely undesirable) as described by Keeton [8].

*Statistical analysis*

The experiments were repeated three times and the data was analyzed using General Linear Model procedure of statistical package for social sciences (SPSS) $15^{th}$ version.

## Results and Discussions

### *Physico-chemical characteristics*

Addition of natural antioxidants i.e., GTE and GSE did not significantly ($P>0.05$) influence the overall mean pH values of pork patties during refrigerated storage period (**Figure 1**). Bozkurt [9] also noted similar findings in dry fermented sausages and confirmed that addition of green tea extract and BHT were not significantly affected ($P > 0.05$) pH during refrigerated storage. The refrigerated storage had significantly ($P<0.05$) influenced the pH values of pork patties during storage. As the storage period progressed, There was a significant ($P<0.05$) increase in pH values between storage days in control and antioxidants added pork patties. This increase in pH during storage could be due to protein denturation and liberation of protein metabolites, mainly amines due to bacterial activity. These results are corroborated with Lara [10] in cooked pork patties.

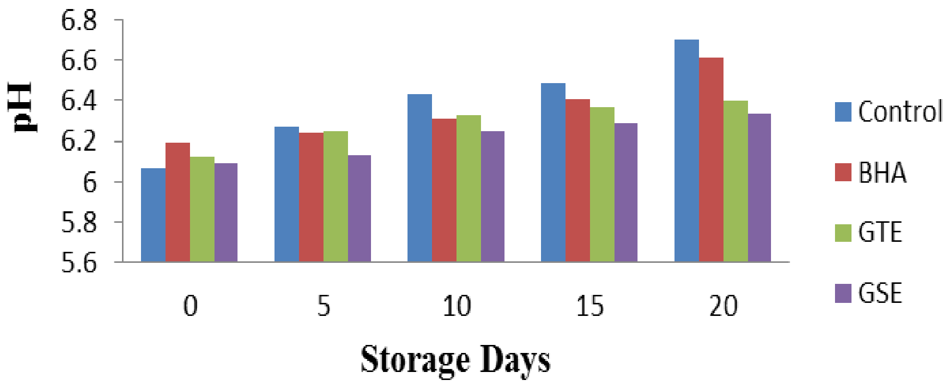


**Figure 1** Influnce of natural antioxidants and storage period on pH of aerobic packaged pork patties

Addition of natural antioxidants and refrigerated storage significantly (P<0.05) affected the 2-TBARS values of cooked pork patties during storage (**Figure 2**). Patties with GSE recorded significantly (P<0.05) lower 2-TBARS values compared with control, BHA and GTE added samples. Compare to synthetic antioxidant added pork patties natural antioxidants added pork patties had lower lipid oxidation throughout storage period. Green tea extract also showed reduced lipid oxidation compared to control and BHA added patties. This might be due to anti-oxidative property of GTE. Various active components like catechins, apicatechins, epicatechin gallate, epigallocatechin, and epigallocatechin gallate are abundantly available in GTE which might be responsible for antioxidant activity of green tea extracts [4]. Compare to GTE the pork patties added with GSE had lower lipid oxidation. This might be due to the potential antioxidative property of GSE than GTE. The antioxidative property of GSE is mainly due to phenolic compounds, especially polyphenols, such as proanthocyanidins. These procyanidins are dimers, trimers and oligomers of the monomeric flavan-3-ols (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-O-gallate [11]. These findings are in congruent with Bhaskar Reddy [2] in restructured mutton slices. The refrigerated storage period significantly (P<0.05) increased the 2-TBARS values. This could be due to the fact that, as the storage period is progressing, the intensity of lipid oxidation enhanced and production of more secondary products of lipid oxidation formed from the decomposition of oxidized lipid molecules which yields more TBARS values [12].

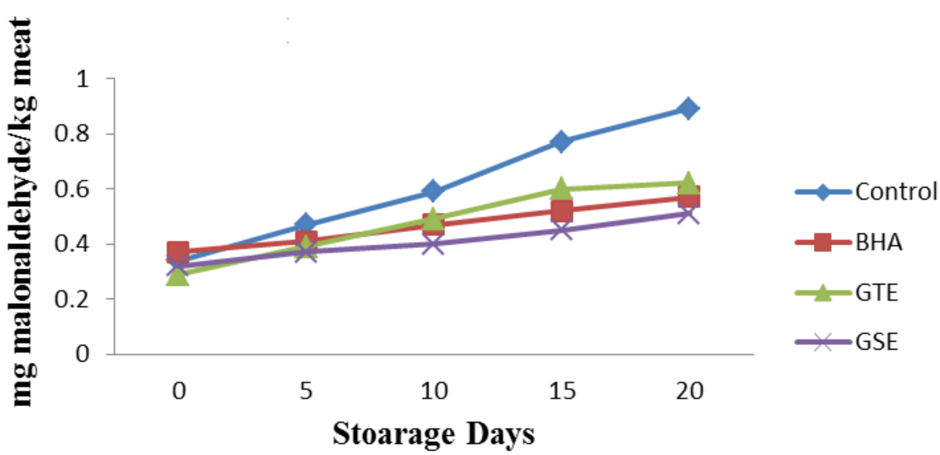


**Figure 2** Influnce of natural antioxidants and storage period on 2-TBARS value of aerobic packaged pork patties

Both natural antioxidants and refrigerated temperature had significantly (P<0.05) influenced the FFA per cent values during storage of pork patties (**Figure 3**). GSE recorded significantly (P<0.05) lower FFA per cent than control, BHA and GTE. This might be due to antimicrobial activity of GSE which causes reduction in microbial growth and subsequent microbial lipolytic activity and generation of free fatty acids. These results are in congruent with Sahoo and Anjeneyulu [13] in buffalo meat nuggets. Storage period also significantly (P<0.05) affected the FFA formation and with the progress of refrigerated storage, FFA level significantly increased. The increased FFA levels during storage might be due to microbial lipolytic activities.

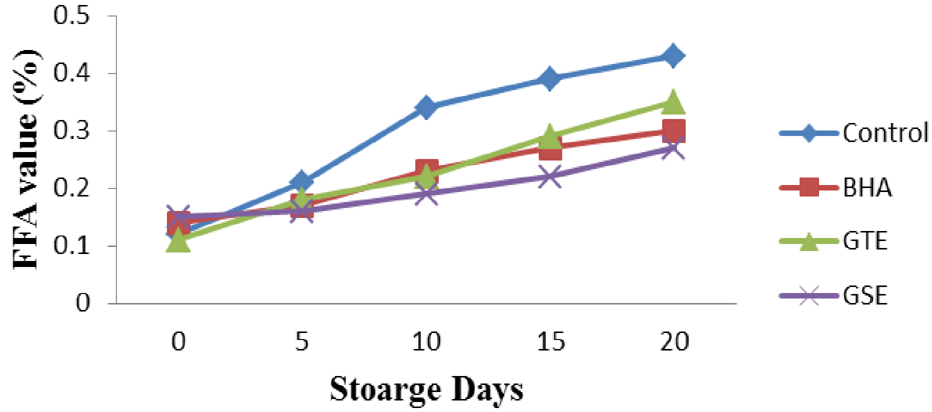


**Figure 3** Influnce of natural antioxidants and storage period on Per cent FFA value of aerobic packaged pork patties

***Microbiological profiles***

Addition of natural antioxidants and refrigerated storage significantly (P<0.05) influenced the microbial profiles *viz.*, total plate count, total psychrophilic count and yeast and mould counts of aerobic packaged pork patties during refrigerated storage (**Table 1**). Among all treatments, pork patties added with GSE recorded significantly lower total plate counts than control and other treatments. The pork patties added with GTE also showed lower total plate counts than control and BHA added samples. Grape polyphenols have a certain antibacterial activity in vitro, and the partial hydrophobic nature of the phenolic compounds of GSE is responsible for the antimicrobial activity of GSE [14]. Irrespective of treatments, refrigerated storage period significantly (P<0.05) influenced the total plate counts of aerobic packaged pork patties. As the storage period increased, total plate counts significantly (P<0.05) increased from 2.27 ($0^{th}$ day) to 4.65 log10 cfu/g after 20 days of refrigerated storage in control samples. This might be due to conducive water activity, change in pH and packaging conditions.

**Table 1** Mean ± S.E values of microbial counts of aerobic packaged pork patties affected by natural antioxidants during refrigerated storage (4±1°C)*

| Pork patty samples | Storage Period (days) | | | | |
|---|---|---|---|---|---|
| | **0** | **5** | **10** | **15** | **20** |
| **Total plate count ($log_{10}$ cfu/g)** | | | | | |
| Control | $2.27±0.17^{eA}$ | $3.48±0.14^{dA}$ | $3.68±0.11^{cA}$ | $3.89±0.12^{bA}$ | $4.65±0.22^{aA}$ |
| BHA | $2.24±0.12^{eA}$ | $3.35±0.09^{dB}$ | $3.57±0.26^{cB}$ | $3.72±0.25^{bB}$ | $4.58±0.13^{aB}$ |
| GTE | $2.28±0.06^{eA}$ | $3.12±0.11^{dC}$ | $3.20±0.15^{cC}$ | $3.31±0.08^{bC}$ | $3.79±0.16^{aC}$ |
| GSE | $2.25±0.08^{eA}$ | $2.94±0.17^{dD}$ | $3.07±0.09^{cD}$ | $3.11±0.23^{bD}$ | $3.59±0.08^{aD}$ |
| **Total psychrophilic count ($log_{10}$ cfu/g)** | | | | | |
| Control | ND | $2.64±0.1^{d}$ | $3.12±0.15^{cB}$ | $3.44±0.11^{bB}$ | $3.89±0.10^{aA}$ |
| BHA | ND | ND | $3.27±0.10^{cA}$ | $3.52±0.22^{bA}$ | $3.76±0.13^{aB}$ |
| GTE | ND | ND | $2.26±0.21^{cD}$ | $2.75±0.15^{bC}$ | $3.12±0.17^{aC}$ |
| GSE | ND | ND | $2.42±0.28^{cC}$ | $2.68±0.23^{bD}$ | $2.94±0.09^{aD}$ |
| **Yeast and mould count ($log_{10}$ cfu/g)** | | | | | |
| Control | $1.71±0.29^{eA}$ | $2.18±0.44^{dA}$ | $2.61±0.18^{cB}$ | $3.02±0.33^{bA}$ | $3.47±0.61^{aA}$ |
| BHA | $1.78±0.07^{dA}$ | $2.21±0.42^{cA}$ | $2.72±0.08^{bA}$ | $2.76±0.12^{bB}$ | $3.42±0.42^{aA}$ |
| GTE | $1.69±0.33^{dA}$ | $2.01±0.19^{cB}$ | $2.15±0.27^{bD}$ | $2.52±0.08^{aD}$ | $2.80±0.20^{aC}$ |
| GSE | $1.79±0.25^{eA}$ | $2.13±0.16^{dA}$ | $2.27±0.22^{cC}$ | $2.67±0.14^{bC}$ | $2.92±0.15^{aB}$ |

Mean values within row and column bearing different superscripts are differ significantly (P<0.05). * n=8
BHA-butylated hydroxyl anisole, GTE-green tea extract, GSE-grape seed extract

Similar results were also noted by and Bhaskar Reddy [2] in restructured mutton slices during refrigerated storage. The above pattern also observed in the total psychrophilic counts of pork patties during storage. During storage, overall reduction of total psychrophilic counts in GSE is mainly due to antibacterial activity of grape polyphenols. Both natural antioxidant and storage period significantly (P<0.05) influenced the yeast and mould count of pork patties. Patties added with GTE had significantly (P<0.05) lower yeast and mould count which might be due to antifungal activity of green tea extracts polyphenols. In addition to GTE, grape seed extract also showed the antifungal activity for delay of yeast and mould growth in pork patties during refrigerated storage. The broad spectrum of antifungal activity of green tea extract is found to be due to the presence of polyphenolic compounds (catechins) which act on the cell, leading to leakage of cell material [15]. Significant (P<0.05) increase in yeast and mould counts of aerobic packaged pork patties were noted with the increased storage period. Similar results were also observed by Kumudavally [15] in fresh mutton.

### *Sensory attributes*

Addition of natural and synthetic antioxidants and storage period significantly affected the sensory scores viz colour, flavour, juiciness, tenderness and overall palatability of pork patties during refrigerated storage (**Table 2**). In the present study, both natural antioxidants i.e., GTE and GSE significantly (P<0.05) improved the colour scores than control and BHA during refrigerated storage of aerobic packaged pork patties. The overall mean flavour scores of aerobic packaged pork patties during refrigerated storage were significantly (P<0.05) affected by both antioxidants and storage period. Addition of GSE recorded significantly (P<0.05) higher flavour scores than control BHA and GTE whereas, GTE and BHA was better flavour scores than control. This might be due to antioxidative effect of GSE which reduce the lipid oxidation and also off- flavour development during refrigerated storage [16]. The addition of GSE showed higher tenderness and juiciness scores than control, BHA and GTE added pork patties during refrigerated storage. The influence of antioxidants and storage days on overall acceptability scores of pork patties during refrigerated storage revealed a significant (P<0.05) difference between treatments. Among all treatments, GSE rated superior overall acceptability scores which might be due to favourable colour, flavour and juiciness scores compared to control, BHA and GTE. As the progressing of storage period, all sensory scores were gradually reduced but all scores are within the acceptable limits during entire storage.

**Table 2** Mean ± S.E values of sensory scores of aerobic packaged pork patties affected by natural antioxidants during refrigerated storage (4±1°C)*

| Pork patty samples | Storage Period (days) | | | | |
|---|---|---|---|---|---|
| | **0** | **5** | **10** | **15** | **20** |
| **Colour** | | | | | |
| Control | $6.82±0.25^{aB}$ | $6.48±0.13^{bC}$ | $6.30±0.41^{cA}$ | $5.82±0.46^{dA}$ | $5.45±0.30^{eB}$ |
| BHA | $6.76±0.27^{aC}$ | $6.38±0.19^{bD}$ | $5.93±0.41^{cC}$ | $5.65±0.34^{dC}$ | $5.36±0.28^{eC}$ |
| GTE | $6.85±0.41^{aB}$ | $6.57±0.23^{bB}$ | $6.12±0.30^{cB}$ | $5.75±0.21^{dB}$ | $5.55±0.19^{eA}$ |
| GSE | $6.93±0.16^{aA}$ | $6.68±0.52^{bA}$ | $6.16±0.37^{cB}$ | $5.65±0.30^{dC}$ | $5.57±0.11^{dA}$ |
| **Flavor** | | | | | |
| Control | $6.77±0.10^{aB}$ | $6.49±0.50^{bB}$ | $6.16±0.35^{cC}$ | $5.75±0.25^{dC}$ | $5.63±0.09^{dD}$ |
| BHA | $6.90±0.33^{aA}$ | $6.52±0.21^{bB}$ | $6.44±0.10^{cB}$ | $6.03±0.16^{dB}$ | $5.75±0.17^{eC}$ |
| GTE | $6.93±0.18^{aA}$ | $6.77±0.29^{bA}$ | $6.59±0.21^{cA}$ | $6.14±0.32^{dA}$ | $5.91±0.12^{eB}$ |
| GSE | $6.88±0.13^{aA}$ | $6.71±0.13^{bA}$ | $6.55±0.19^{cA}$ | $6.19±0.08^{dA}$ | $6.07±0.35^{eA}$ |
| **Juiciness** | | | | | |
| Control | $7.08±0.13^{aA}$ | $6.75±0.08^{bC}$ | $6.58±0.41^{cC}$ | $6.23±0.19^{dC}$ | $5.68±0.09^{eC}$ |
| BHA | $6.97±0.15^{aB}$ | $6.69±0.18^{bD}$ | $6.41±0.31^{cD}$ | $6.19±0.11^{dC}$ | $5.65±0.17^{eC}$ |
| GTE | $7.03±0.22^{aA}$ | $6.89±0.12^{bB}$ | $6.75±0.20^{cB}$ | $6.52±0.13^{dB}$ | $5.91±0.13^{eB}$ |
| GSE | $6.98±0.18^{aB}$ | $6.91±0.26^{bA}$ | $6.83±0.26^{cA}$ | $6.69±0.34^{dA}$ | $6.11±0.15^{eA}$ |
| **Tenderness** | | | | | |
| Control | $7.06±0.27^{aA}$ | $6.75±0.17^{bB}$ | $6.54±0.34^{cB}$ | $6.18±0.10^{dC}$ | $5.74±0.13^{eD}$ |
| BHA | $6.93±0.18^{aB}$ | $6.69±0.10^{bB}$ | $6.51±0.21^{cB}$ | $6.24±0.21^{dB}$ | $5.92±0.11^{eC}$ |
| GTE | $6.96±0.16^{aB}$ | $6.81±0.43^{bA}$ | $6.62±0.18^{cA}$ | $6.39±0.15^{dA}$ | $6.19±0.29^{dB}$ |
| GSE | $6.93±0.07^{aB}$ | $6.79±0.10^{bA}$ | $6.69±0.12^{cA}$ | $6.46±0.14^{dA}$ | $6.27±0.17^{eA}$ |
| **Overall acceptability** | | | | | |
| Control | $6.98±0.15^{aA}$ | $6.54±24^{bD}$ | $6.25±0.16^{cD}$ | $6.12±0.31^{dD}$ | $5.81±0.20^{eC}$ |
| BHA | $6.93±0.17^{aA}$ | $6.62±0.33^{bC}$ | $6.43±0.22^{cC}$ | $6.27±0.10^{dC}$ | $6.11±0.39^{eB}$ |
| GTE | $6.89±0.09^{aB}$ | $6.75±0.19^{bB}$ | $6.58±0.17^{cB}$ | $6.35±0.29^{dB}$ | $6.12±0.27^{eB}$ |
| GSE | $6.97±0.25^{aA}$ | $6.81±0.13^{bA}$ | $6.70±0.13^{cA}$ | $6.42±0.37^{dA}$ | $6.25±0.09^{eA}$ |

Mean values within row and column bearing different superscripts are differ significantly (P<0.05). * n=24
BHA-butylated hydroxyl anisole, GTE-green tea extract, GSE-grape seed extract

## Conclusions

Based on the above results it is concluded that the addition of GSE at 0.1% reduced the lipid oxidation, delays the microbial organism's multiplication and improves the sensory attributes of aerobic packaged pork patties during refrigerated storage (4±1ºC) and stable up to 20 days without any significant quality deterioration.

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