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# Phytochemical and Antioxidant Activity of Some Agriculture Waste as A Functional Food in Meat Technology

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**ABSTRACT**

Antioxidant effect of pomegranate rind powder (PRP), orange peel powder (OPP) and tomato pomace powder (TPP) to retard lipid oxidation and protein decomposition was investigated in beef burger during frozen storage at -18±1°C for 6 months. Powders of pomegranate rind, orange peel and tomato pomace were incorporated into freshly minced beef meat at different concentration (1%, 2% and 3%) for PRP, (2%, 3% and 4%) for OPP and (2%, 4% and 5%) for TPP and compared with control sample. Chemical compositions of beef burger prepared by powders, thiobarbituric acid reactive substances TBARS, total volatile nitrogen TVN, total phenolic compound TPC and antioxidant activity AOA were determined. Results indicated that powders showed high phenolic content and antioxidant activity especially pomegranate rind powder. The addition of different concentrations of PRP, OPP and TPP caused high storage stability and reduced values of TBA and TVN of prepared beef burger during frozen storage compared to control sample.

***Keywords*:** Phenolic, meat, lipid oxidation, natural antioxidants, pomegranate rind, orange peel and tomato pomace.

## Introduction

Meat and meat products are susceptible to quality deterioration due to their rich nutritional composition (Devatkal *et al*., 2014). The quality deterioration of meat and its products is due to chemical and microbial changes. The most common form of chemical deterioration is the oxidation of meat lipids. Lipid oxidation is a complex process and depends on chemical composition of meat, light, oxygen access and storage temperature (Kanner, 1994).

Lipid oxidation (apart from microbial spoilage) in meat and meat products is the main cause of their quality loss. A large number of compounds are generated during the oxidation processes which adversely affect texture, color, flavor, nutritive value and safety of meat products (Lahucky *et al*., 2010) and this limits the shelf-life of meat (Karakaya *et al*., 2011). Antioxidants agents can prevent lipid peroxidation using the following mechanisms: preventing chain inhibition by scavenging initiating radicals, breaking chain reaction, decomposing peroxides, decreasing localized oxygen concentrations and binding chain initiating catalysts, such as metal ions (Dorman *et al*., 2003).

Although synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate (PG) have been applied widely in meat, poultry and its products but in recent years their use has been discouraged because of their toxic effects and consumer interest in natural products (Jayathilakan *et al*., 2007). This has led the meat industry to search new economical and effective natural antioxidants that can replace synthetic antioxidants without adversely affecting the quality of finished products and consumer perceptions (Karre *et al*., 2013).

The natural antioxidants, especially from plant origin has increased in the recent years due to the growing concern among consumers about these synthetic antioxidants because of their potential toxicological effects (Juntachote *et al*., 2006; Naveena *et al*., 2008).

Plants are persistently the generous source to supply our foods with valuable bioactive substances (Tayel and El-Tras, 2012) and thus different plant products are being evaluated as natural antioxidants to preserve and improve the overall quality of meat and meat products. These natural antioxidants from plants, in the form of extracts, have been obtained from different sources such as fruits (grapes, pomegranate, orange, date and kinnow), vegetables, (tomato, broccoli, potato, drumstick, pumpkin, curry and nettle), herbs and spices (tea, rosemary, oregano, cinnamon, sage, thyme, mint, ginger, clove) were

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investigated to decrease the lipid oxidation (Kanatt *et al*., 2007; Akarpat *et al*., 2008; Shan *et al*., 2009; Devatkal *et al*., 2010; Huang *et al*., 2011; Wojciak, *et al*., 2011; Das *et al*., 2012; Nissen *et al*., 2004). Meanwhile, application of these extracts improved the overall sensory and nutritional quality of meat and meat products and hence their shelf life (Shah *et al*., 2014)

The effect of kinnow rind powder (KRP), pomegranate rind powder (PRP) and pomegranate seed powder (PSP) extracts were investigated in goat meat patties stored at 4±1 °C. The obtained results revealed that these extracts are rich in phenolic compounds and have free radical scavenging activity. And caused reduction in TBARS values (lipid oxidation) during storage of goat meat patties (Devatkal *et al*., 2010).

Turgut *et al*.(2016) found that the pomegranate peel extract (PE) contain high phenolic compounds and antioxidant activity. Addition of PE to meat products on thiobarbituric acid reactive substances (TBARS) value, peroxide formation, loss of sulfhydryl groups and formation of protein carbonyls decreased ($P$<0.01) this compound compared to control sample after 8 days of storage. Sensory evaluation with respect to color and rancid odor revealed that PE incorporation in meatballs prolonged the refrigerated storage up to 8 days.

Orange is an important source of phytochemicals which produced as secondary metabolites of plants also known as nutraceuticals or functional compounds recognized for their antioxidant activity and other properties (Gui-Fang *et al*., 2013 and Lee, 2013).

Escobedo-Avellaneda, *et al*., (2014) found that orange peel contain high amount of phytochemicals that effective in the prevention/treatment of chronic diseases. Phytochemical concentration, antioxidant activity (AOA) and their relationship were determined for comminuted orange, juice and peel such as vitamin, flavones and carotenoid which were found in flavedo. Meanwhile, albedo was the main source of phenolics, flavanones and AOA antioxidant activity linearly correlated with phenolic, hesperidin, and flavonoid concentrations.

Tomato by-products seed and peel contain a great variety of biologically active substances، principally lycopene, which have been demonstrated by in vitro and in vivo studies to possess antioxidant, hypolipidemic and anticarcinogenic activities (Viuda-Martos, *et al*; 2014).

Lycopene is mainly present in tomatoes and tomato products, and it has the highest antioxidant activities among the carotenoids due to its high number of conjugated double bonds. In addition, these compounds have a singlet-oxygen-quenching ability that is twice as high as that of β-carotene and 10 times higher than that of vitamin E (Sahin *et al*., 2014).

Therefore, the evaluation of its qualitative and quantitative compositions regarding its utilization as a possible functional ingredient is of particular interest.

## Materials and Methods

*Materials:*

*Agriculture wastes:*

*Orange waste:*

Orange waste (*Citrus sinensis*) Balady orange variety*:* were obtained from Kaha company for canned food, Kaha city, Kaliobia, Egypt.

*Pomegranate fruits:*

Pomegranate fruits used in preparing pomegranate rind powder were purchased from local markets of Assiut, Egypt.

*Tomato pomace:*

Tomato pomace were collected after juice extraction from Heinz factory (6$^{th}$ of October city, Cairo, Egypt).

*Chemicals and Reagents:*

All chemicals and reagents used in the analytical methods (analytical grade) were produced by sigma chemical co. (St. Louis, M., USA) and purchased from EL. gamhouria trading chemicals and drugs co.

*Ingredients of beef burger:*

Fresh beef meat and back fat were obtained from a local retail market (Assiut, Egypt). After removal of visible fat and connective tissues, it was cut into chunks and frozen until work. Spices (black pepper, nutmeg, clove, thyme, celery, white pepper and rosemary) were obtained from a local market (Assiut, Egypt). All spices were fresh ground directly before used and sieved through mesh 60 (0.25 mm).

*Methods:*

*Preparation of pomegranate peels:*

The pomegranate was washed, then peeled and their edible portions were carefully separated. The peels were air-dried in a ventilated oven at 50 ºC for 48 h and grounded to a fine powder then packaged in polyethylene bags and stored at -18 ºC until used (El-Nashi, *et al* 2015).

*Preparation of orange peel:*

The peels were finely cut, and washed in tap water, loaded onto stainless trays, and dried at (50◦C, 24 h) in a convection oven. The dried peels were first grounded to a fine powder then packaged in polyethylene bags and stored at -18 ºC until used (Hosseini *et al*., 2016).

*Preparation of tomato pomace:*

The pomace was dehydrated at 50 ºC for 24 h in a ventilated oven and grounded to a fine powder then packaged in polyethylene bags and stored at -18 ºC until used (Namir *et al*., 2015).

*Beef burger preparation:*

Beef burger patties were formulated to contain the following ingredients 85% lean meat (less than 7% fat with 15% beef back fat, 0.022% seasoning (black pepper 22.73%, nutmeg 1.37 % and Clove 5.46%, salt 63.64% and 6.82% Sodium tripolyphosphate STPP). The resulting mixture was finally mixed with powdered peels, and the addition levels were 1%, 2% and 3% for pomegranate rind powder (PRP), 2%, 3% and 4% for orange peel powder (OPP) and 2%, 4% and 5% for tomato pomace powder (TPP) then the mixture were mixed and pushed through a plate with 8 mm holes. Burgers of 50 g ±1 with 9 cm diameter were formed by hand-press maker (italman, Italy). Beef burgers were stored at -18 °C ±1 for six months and analyzed every month in the storage period (Mitsumoto *et al.,* 2005).

*Chemical analysis:*

Moisture, ash, protein, fat and fiber content were determined according to AOAC (2005), while carbohydrate content was estimated by difference.

*The pH determination:*

Ten grams of raw beef burger samples were homogenized in 90 ml distilled water and one gram of powders were homogenized in 9 ml distilled water for 1 min in a blender (Bozkurt, 2006). The pH values were measured using a Jenway pH meter (Jenway 3010; Jenway Ltd., Essex, UK)

*Thiobarbituric acid (TBA):*

Lipid oxidation of the beef burger samples were determined using the distillation of 2-thiobarbituric acid (TBA) according to Tarladgis *et al*., (1960) and exposed as mg malonaldehyd/kg sample.

*Total volatile nitrogen (TVN):*

Total volatile nitrogen content of different beef burger samples was determined according to the method of Harold *et al*. (1987).

*Determination of total phenolic compounds in beef burger:*

One gram of beef burger sample and fifteen milligram of powders was extracted by 25 ml ethanol 50%, shacked for 2 hours, then centrifuged at 3000 rpm for 20 min. TPC was calculated in the ethanolic extracts, by using Folin–Ciocalteu method with slight modifications (Jaramillo-Flores *et al.,* 2003). A 100 µL aliquot of ethanolic extract was mixed with 900 µL of 10 fold Folin–Ciocalteu phenol reagent (diluted 1:10 with distilled water) and was allowed to stand for 5 min at room temperature; 0.75 µL of 7% sodium bicarbonate solution was added to the mixture and vortexed for 30 s, and allowed to stand for 90 min at room temperature. The absorbance was measured at 725 nm using a spectro-photometer (6505 UV/Vis, Jenway LTD., Felsted, Dunmow, UK). A calibration curve of gallic acid (ranging from 0 to 1.00 mg/mL) was prepared and tested under similar conditions. All values were expressed as mean (mg of Gallic acid equivalents/100g of fresh weight) ±SD for 3 replicat.

*Determination of antioxidant activity in beef burger sample:*

The DPPH method was used according to Lee *et al.*, (2003) with some modifications. Briefly, a 60-µmol/L DPPH solution was freshly made in 99% ethanol solution. The extract of the burger product (100 µL) was reacted with 3.9 ml of the DPPH solution for 60 min in dark place. The absorbance (A) at 515 nm was measured using a spectrophotometer against a blank of 95% ethanol. Antioxidant activity was calculated as follows: DPPH radical-scavenging activity (%)

= ((Acontrol-Asample)/ Acontrol) *100

Where, A is the absorbance at 515 nm. All DPPH tests were carried out in triplicate.

*Polyphenol fraction by HPLC analysis:*

Phenolic compounds in orange peel, pomegranate rind and tomato pomace powders were performed by HPLC analysis using the method described by Dragovic-Uzelac *et al*. (2005). Injection was carried out at wave lengths 282 nm for separation.

*Statistical analysis:*

All treatments and determinations were carried out in triplicates and the data are presented as means ±SD. They were subjected to analysis of variance (ANOVA) accompanied with Duncan test using SPSS software (version 16.0 for Windows, SPSS Inc., Chicago) to identify the significance ($p< 0.05$) among the treatments (Erukainure *et al*., 2016).

## Results and Discussion

### Chemical constitutes and antioxidant activity of raw powders

From data presented in table (1) it could be observed that tomato pomace powder had the highest value of moisture content 10.03% while pomegranate rind powder had the lowest value 5.04% compared with 6.82% for orange peel powder, the same trend was observed in case of ash content which were 7.38, 4.35 and 4.55%. These values indicated that TPP is a good source of ash.

Concerning protein content PRP is poor in protein content 5.51% this value reached to 6.68% in OPP while in case of TPP the protein content was 20.85%. The highest value of fat content was 10.92% for TPP while the lowest value was 2.93% for PRP and it was 5.37% for OPP. On the other hand, TPP was a good source of fiber content 33.27% compared with 13.37% and 12.76% for both OPP and PRP, respectively. In case of carbohydrate PRP had the higher value 82.53% followed by 76.58% for OPP and 50.82% for TPP.

Regarding to the pH value results in table (1) indicated that OPP and TPP had the highest pH value 4.72 and 4.47 while PRP had the lowest of pH value 3.44. These values indicated that PRP had higher acidity than others.

Concerning total phenolic compound and antioxidant activity, data in table (1) showed that PRP is a good source of TPC and AOA with 259.94 mg/g and 90.05% followed by OPP which had 59.07 mg/g and 15.55% while TPP had the lowest values (12.31 mg/g and 7.37%, respectively). These results agree with that reported by El-Nashi *et al.,* (2015) and Al-Rawahi, *et al*., (2014).

**Table 1:** Chemical constitutes and antioxidant activity of raw powders (Mean ±S. D)

| Parameter | | Orange peel powder | Pomegranate rind powder | Tomato pomace powder |
|---|---|---|---|---|
| Chemical composition | Moisture | 6.82±0.010$^{b}$ | 5.04±0.030$^{c}$ | 10.03±0.015$^{a}$ |
| | Ash | 4.55±0.010$^{a}$ | 4.35±0.010$^{c}$ | 7.38±0.020$^{a}$ |
| | Protein | 6.68±0.026$^{b}$ | 5.15±0.035$^{c}$ | 20.85±0.030$^{a}$ |
| | Fat | 5.37±0.020$^{b}$ | 2.93±0.030$^{c}$ | 10.92±0.030$^{a}$ |
| | Fiber | 13.37±0.025$^{b}$ | 12.76±0.020$^{c}$ | 33.27±0.020$^{a}$ |
| | *Carbohydrates | 76.58±0.026$^{b}$ | 82.53±0.060$^{a}$ | 50.82±0.058$^{c}$ |
| Antioxidant activity | TPC (mg/gm DW) | 59.07±0.030$^{b}$ | 259.94±0.040$^{a}$ | 12.31±0.015$^{c}$ |
| | DPPH % | 15.55±0.040$^{b}$ | 90.05±0.047$^{a}$ | 7.37±0.035$^{c}$ |
| The pH values | | 4.72±0.010$^{a}$ | 3.44±0.020$^{c}$ | 4.47±0.010$^{b}$ |

**Carbohydrates by difference including fiber , TPC=total phenolic compound, DPPH= antioxidant activity assayed by diphenylpicrylhydrazyl free radical (Sample weight of powders was fifteen milligram)*
*Means with different letter in the same row indicate significant differences between the raw powders ($p < 0.05$)*

**Fractionation of phenolic compound (ppm) from tested powder:**

Polyphenols are secondary metabolites that are widespread throughout the plant kingdom with more than 8000 phenolic compounds, and it is known for their pharmacological properties, for example as antioxidants, anti-inflammatory, anti-mutagenic, anti-carcinogenic, and antimicrobial (Gropper *et al*., 2009 and Ismail *et al*., 2012).

Fractionation, identification and content of phenolic compound, extraction from OPP, PRP and TPP are presented in table (2) from these table it could be stated that, 24 compounds were found for all tested powder. Pomegranate rind powder contained higher amount of phenolic compound than other sample (except compound coumarin, salycilic, cinnamic and e-vanillic which were higher in OPP). Compounds pyrogallol, 4-amino-benzoic, protocatchuic, catechein and gallic had the higher values it were 22759.05, 5243.64, 1319,04, 1182.65 and 1020.27 ppm/g respectively.

On the other side, TPP had the lower numbers of phenolic compound especially coumarin, cinnamic and 4-amino-benzoic. These results agree with that reported by (Oikeh *et al.* (2013) and Al-Rawahi *et al.* (2014).

**Proximate chemical composition of beef burger prepared:**

Proximate chemical composition of different beef burger samples formulated by addition of 2%,3% and 4%orange peel powder(OPP), 1%, 2% and 3% of pomegranate rind powder (PRP) and tomato pomace powder (TPP)are given in Table (3).

A significant ($p \leq 0.05$) decrease in moisture content was observed of the beef burger with increasing the level of incorporation of orange peel powder, pomegranate rind powder and tomato pomace powder which may be attributed to a lower moisture content of this powders compared to the control sample. Similar results were also obtained by Hayes *et al*. (2013) and Verma *et al*. (2013).

Regarding, the fat content a significant ($p \leq 0.05$) declining in fat content was observed of the beef burger prepared with the addition of OPP, PRP and TPP in comparison to the control sample. This decrease in fat content may be due to a comparatively lower fat content of this powders compared to beef meat. Mehta *et al*. (2013a, 2013b) reported a decrease in the fat content of psyllium husk-added patties. This is in conjugation with the study of Verma *et al*. (2013) who observed a decrease in the fat content of sheep meat nuggets incorporated with guava powder. Suradkar *et al*. (2013) also reported a decrease in the fat content of chicken nuggets containing bread crumbs.

On the other hand; the protein content of prepared beef burger was significantly ($p \leq 0.05$) decreased at the all levels of incorporation of the pomegranate rind powder and orange peel powder compared to control sample. This may be attributed to the dilution effect caused by the incorporation of PRP and OPP which is particularly low in protein content in comparison to the control. Similar findings were reported by Bhaskar *et al*. (2012). Suradkar *et al*. (2013) also reported a decrease in the protein content of chicken nuggets containing bread crumbs. Verma *et al*. (2013) also observed a decrease in the protein content of sheep meat

nuggets incorporated with guava powder. Taludkar and Sharma (2009) observed a decrease in the protein content of chicken meat patties incorporated with wheat and oat bran. While the protein content incorporated with tomato pomace powder showed a non-significant ($p \geq 0.05$) increase with an increasing level of incorporation in comparison to the control which might be attributed to the high protein content of tomato powder as shown in Table (1) compared with other powders. These results are in agreement with the study of Suk *et al*. (2011), who reported a slight increase in the protein content of low fat pork sausages prepared with tomato powder.

**Table 2:** Identification and contents (ppm) of phenolic compound extracts from the tested powders

| Phenolic compounds | The tested powders | | |
|---|---|---|---|
| | Pomegranate rind powder | orange peel powder | tomato pomace powder |
| Gallic | 1020.27 | 9.86 | 10.84 |
| Pyrogallol | 22759.05 | 301.17 | 129.42 |
| 4-Amino-benzoic | 5243.64 | 15.41 | 3.31 |
| Protocatchuic | 1319.04 | 136.97 | 18.78 |
| Catechein | 1182.65 | 74.11 | 55.45 |
| Chlorogenic | 184.83 | 11.33 | 13.55 |
| Catechol | 636.02 | 88.32 | 9.33 |
| Epi-Catachin | 1024.8 | 90.67 | 19 |
| Caffeine | 100.28 | 53.82 | 4.05 |
| P-OH-benzoic | 228.88 | 44.62 | 30.57 |
| Caffeic | 69.69 | 69 | 2.59 |
| Vanillic | 269.71 | 49.42 | 19.59 |
| P-coumaric | 129.81 | 53.69 | 2.86 |
| Ferulic | 29.46 | 25.2 | 3.12 |
| Iso-ferulic | 294.35 | 22.17 | 16.04 |
| Reversetrol | 14.08 | 16.94 | 2.25 |
| Ellagic | 211.73 | 136.81 | 42.17 |
| e-vanillic | 528.55 | 1369.55 | 237.18 |
| Alpha-coumaric | 49.35 | 15.8 | 2.78 |
| Benzoic | 219.47 | 202.79 | 89.03 |
| 3,4,5-methoxy-cinnamic | 23.56 | 35.77 | 2.25 |
| Coumarin | 7.13 | 21.84 | 1.08 |
| Salycilic | 28.41 | 1446.31 | 20.37 |
| Cinnamic | 2.9 | 10.68 | 2.21 |

**Table 3:** Chemical composition of prepared beef burger treated with different levels of tested powder (means ± S.D)

| Treatment | | Moisture | Fat | Protein | Ash | Fiber | *Carbohydrate |
|---|---|---|---|---|---|---|---|
| Control | | 60.15±0.046$^{a}$ | 21.29±0.095$^{a}$ | 55.83±0.071$^{b}$ | 2.73±0.015$^{g}$ | 0.79±0.100$^{h}$ | 19.36±0.280$^{b}$ |
| Orange peel powder | 2% | 59.61±0.010$^{b}$ | 19.78±0.015$^{d}$ | 55.52±0.015$^{c}$ | 4.65±0.055$^{e}$ | 1.80±0.200$^{f}$ | 18.24±0.140$^{d}$ |
| | 3% | 58.67±0.030$^{c}$ | 19.69±0.015$^{e}$ | 55.24±0.100$^{d}$ | 4.96±0.060$^{d}$ | 2.40±0.050$^{e}$ | 17.71±0.095$^{e}$ |
| | 4% | 57.43±0.020$^{e}$ | 19.65±0.005$^{e}$ | 54.99±0.346$^{e}$ | 5.12±0.060$^{c}$ | 4.30±0.090$^{c}$ | 16.13±0.480$^{f}$ |
| Pomegranate rind powder | 1% | 59.99±0.030$^{a}$ | 19.74±0.100$^{de}$ | 55.42±0.020$^{cd}$ | 3.72±0.025$^{f}$ | 1.22±0.070$^{g}$ | 19.90±0.175$^{a}$ |
| | 2% | 58.89±0.015$^{c}$ | 19.65±0.050$^{e}$ | 55.26±0.000$^{d}$ | 4.62±0.080$^{e}$ | 1.77±0.020$^{f}$ | 18.70±0.050$^{c}$ |
| | 3% | 58.04±0.051$^{d}$ | 19.52±0.030$^{f}$ | 54.90±0.060$^{e}$ | 5.17±0.040$^{bc}$ | 1.89±0.070$^{f}$ | 18.52±0.080$^{cd}$ |
| Tomato pomace powder | 2% | 58.90±0.015$^{c}$ | 19.90±0.011$^{c}$ | 55.88±0.080$^{b}$ | 5.26±0.035$^{b}$ | 4.10±0.110$^{d}$ | 14.87±0.215$^{g}$ |
| | 4% | 57.81±0.571$^{d}$ | 19.93±0.025$^{c}$ | 56.02±0.090$^{ab}$ | 5.38±0.035$^{a}$ | 5.66±0.070$^{b}$ | 13.01±0.030$^{h}$ |
| | 5% | 57.05±0.050$^{f}$ | 20.03±0.058$^{b}$ | 56.15±0.040$^{a}$ | 5.42±0.075$^{a}$ | 6.51±0.090$^{a}$ | 11.88±0.112$^{i}$ |

*Control = Meat mixture without any additives,*Carbohydrates by difference*
*Means with different letter in the same column indicate significant differences between treatments ($p < 0.05$).*

Ash percent increased with the addition of pomegranate rind powder. orange peel powder and tomato pomace powder and was significantly ($p \geq 0.05$) higher at the 4% and 5% level of incorporation with TPP in comparison to the control. This may be attributed to the higher mineral content or ash content of this powders in compared to beef meat. Similar results were reported Salem (2013), who also reported a similar finding in beef sausages incorporated with tomato peel.

A significant ($p \geq 0.05$) increase was observed in fiber content in beef burger with an increase in the level of incorporation of orange peel powder, pomegranate rind powder, as well as tomato powder, which may be attributed to their higher fiber content in this powders as shown in table (1). Similar increase in the

fiber content was also observed by Verma *et al*. (2013), Das *et al*. (2013) and Bhaskar *et al*. (2012) in different meat products.

The increasing fiber content could constitute an additional nutritional benefit for the consumer and permits a reduction of the rate of meat incorporation (Attia *et al*., 2008). The high level of fiber in the diet can be useful in decreasing the cholesterol level in humans (Marlett, 2001).

**Changes of total phenolic compounds of prepared beef burger during frozen storage:**

The levels of total phenolic compounds (TPC) in the control formula and treated samples of beef burger are listed in Table (4). Results showed that, the addition of orange peel powder (OPP2,3 and 4%), pomegranate rind powder (PRP1,2and 3%), and tomato pomace powder (2,4and5%) caused significant increase in TPC contents in the freshly prepared beef burgers mixtures. Control formula contained (49.07) mg/100g TPC while samples treated with orange peel powder, pomegranate rind powder and tomato peel powder had higher levels of TPC it ranged between 161.19 to 151.06 mg/100g.

**Table 4:** Changes in phenolic compounds (mg/100 g*) of prepared beef burger during frozen storage at -18ºC±1 (Means± S.D)

| Treatment | | Frozen Storage period (months) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Control | | 49.07±0.555$^{h}$ | 48.35±0.396$^{h}$ | 45.69±0.725$^{j}$ | 40.41±0.626$^{i}$ | 33.84±0.611$^{i}$ | 28.40±0.305$^{h}$ | 22.29±0.305$^{j}$ |
| Orange peel powder | 2% | 91.21±0.335$^{e}$ | 75.42±0.594$^{d}$ | 68.56±0.170$^{f}$ | 67.48±0.305$^{e}$ | 58.37±0.628$^{e}$ | 53.74±0.610$^{e}$ | 45.69±0.402$^{e}$ |
| | 3% | 121.63±0.495$^{c}$ | 93.74±0.578$^{c}$ | 84.91±0.698$^{d}$ | 78.72±0.665$^{d}$ | 74.25±0.680$^{c}$ | 67.67±0.440$^{c}$ | 62.56±0.444$^{c}$ |
| | 4% | 151.06±0.600$^{b}$ | 105.12±0.897$^{b}$ | 94.65±0.610$^{b}$ | 91.29±0.305$^{b}$ | 76.64±0.305$^{b}$ | 70.84±0.615$^{b}$ | 66.87±0.305$^{b}$ |
| Pomegranate rind powder | 1% | 92.90±0.190$^{d}$ | 64.73±0.610$^{f}$ | 62.43±0.594$^{g}$ | 59.82±0.551$^{g}$ | 48.19±0.617$^{f}$ | 42.97±0.910$^{f}$ | 40.89±0.507$^{f}$ |
| | 2% | 121.14±0.636$^{c}$ | 93.32±0.731$^{c}$ | 93.07±0.360$^{c}$ | 84.08±0.698$^{c}$ | 68.14±0.745$^{d}$ | 62.29±0.610$^{d}$ | 59.84±0.610$^{d}$ |
| | 3% | 161.19±0.680$^{a}$ | 129.02±0.587$^{a}$ | 119.94±0.360$^{a}$ | 113.33±0.586$^{a}$ | 98.90±0.551$^{a}$ | 90.87±0.402$^{a}$ | 88.57±0.503$^{a}$ |
| Tomato pomace powder | 2% | 61.76±0.530$^{g}$ | 61.01±0.731$^{g}$ | 56.79±0.610$^{i}$ | 56.18±0.610$^{h}$ | 46.08±0.308$^{gh}$ | 39.39±0.305$^{g}$ | 30.73±0.290$^{i}$ |
| | 4% | 62.95±0.407$^{f}$ | 61.37±0.310$^{g}$ | 60.26±0.551$^{h}$ | 56.49±0.305$^{h}$ | 45.80±0.610$^{h}$ | 40.30±0.610$^{g}$ | 33.78±0.286$^{h}$ |
| | 5% | 91.07±0.869$^{e}$ | 71.75±0.410$^{e}$ | 69.64±0.503$^{e}$ | 62.87±0.503$^{f}$ | 47.10±0.869$^{g}$ | 43.36±0.610$^{f}$ | 38.05±0.292$^{g}$ |

*Control = meat mixture without any additives *mg/100gm fresh weight*
*Means with different letter in the same column indicate significant differences between treatments ($p < 0.05$).*

Beef burger formulated with PRP had the highest phenolic content 92.90, 121.14 and 161.19 mg/100g (for the concentration 1, 2 and 3%, respectively) followed by samples treated with OPP 91.21, 121.63 and 151.06 mg/100g (for the concentration 2, 3 and 4%, respectively), while samples treated with TPP had the lowest values 61.76, 62.95 and 91.07mg/100g (for the concentration 2, 4 and 5%, respectively).

This may be attributed to the higher levels of TPC in pomegranate rind powder and orange peel powder when compared to the tomato pomace powder as shown in Table (1). Moreover, the levels of TPCs were proportional with the addition levels of pomegranate rind, orange peel and tomato pomace powders in beef burgers. The present results are in a good agreement with those reported for cookies fortified with pomegranate rind (Naveena *et al.,* 2008) and (Kanatt *et al*., 2010).

From the same table, it could be observed that total phenol content was decreased in all sample at the time of frozen storage increased but in low rate compound with control sample which decreased from 49.07 to 22.29 mg/100g at the end of storage period whiles sample treated with 3% PRP decreased from 161.19 to 88.57 mg/100g at the end of storage period.

Data presented in table (5) showed the changes of antioxidant activity of beef burger during frozen stage for 6 months. A significant increase ($p \leq 0.05$) was observed in the antioxidant activity (DPPH) in all treatments when compared with control sample. Also, the gradient increase in the amounts of all added powders caused a proportional increase in the antioxidant activity. Thus, the highest levels were observed in pomegranate rind powder and orange peel powder exhibited higher antioxidant activities (79.90%). These results were related to the composition of the raw powders which had high levels of antioxidant activity as shown in (Table 1).

On the other hand, tomato pomace powder (TPP2%) exhibited lower antioxidant activities (28.62%). The antioxidant properties of phenolic compounds were very well documented and a significant relation between phenolic content and antioxidant activity. Thus, the high level of antioxidant activity in beef burgers containing OPP, PRP and TPP powders was attributed to the high level of phenolic compounds found in these powders. The present results agreed with those reported by El-Gharably and Ashoush, 2011.

Total phenolic compounds (TPC) and antioxidant activity were gradually decreased in all treatments and the lowest levels were observed at 6 months of storage. The decrease in TPC may be attributed to the

decomposition of TPC during the storage time. However, some treatments still had high level of antioxidant activity at 6 months, such as PRP3% (43.37). These values of antioxidant scavenging activities indicate that orange peel powder, pomegranate rind powder and tomato pomace powder could be effectively used to retard the oxidative process in beef products.

**Table 5:** Changes in antioxidant activity (DPPH %) of prepared beef burger during frozen storage at -18ºC±1 (Means± S.D)

| Treatment | | Frozen Storage period (months) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Control | | 24.82±0.185$^{i}$ | 20.39±0.960$^{g}$ | 17.48±0.000$^{g}$ | 17.21±0.305$^{f}$ | 16.27±0.960$^{f}$ | 14.85±0.225$^{h}$ | 13.83±0.115$^{h}$ |
| Orange peel powder | 2% | 31.31±0.060$^{f}$ | 25.31±0.565$^{e}$ | 23.13±0.535$^{e}$ | 21.90±0.000$^{d}$ | 20.98±0.345$^{d}$ | 19.56±0.055$^{e}$ | 18.54±0.055$^{e}$ |
| | 3% | 34.13±0.180$^{e}$ | 29.16±0.594$^{c}$ | 25.33±0.595$^{d}$ | 24.34±0.855$^{c}$ | 22.73±0.345$^{c}$ | 21.60±0.510$^{d}$ | 21.20±0.225$^{d}$ |
| | 4% | 38.11±0.615$^{d}$ | 29.95±0.340$^{c}$ | 26.28±0.000$^{d}$ | 24.83±0.245$^{c}$ | 23.70±0.225$^{c}$ | 22.90±0.115$^{c}$ | 22.00±0.115$^{c}$ |
| Pomegranate rind powder | 1% | 47.34±0.695$^{c}$ | 29.56±0.395$^{c}$ | 28.67±0.928$^{c}$ | 24.68±0.548$^{c}$ | 23.12±0.594$^{c}$ | 22.39±0.625$^{c}$ | 21.83±0.055$^{c}$ |
| | 2% | 56.80±0.550$^{b}$ | 39.01±0.115$^{b}$ | 38.74±0.869$^{b}$ | 36.86±0.141$^{b}$ | 31.63±0.680$^{b}$ | 30.84±0.110$^{b}$ | 29.99±0.170$^{b}$ |
| | 3% | 79.90±0.245$^{a}$ | 55.45±0.500$^{a}$ | 53.34±0.691$^{a}$ | 51.62±0.055$^{a}$ | 44.56±0.565$^{a}$ | 43.66±0.335$^{a}$ | 43.37±0.055$^{a}$ |
| Tomato pomace powder | 2% | 28.62±0.065$^{h}$ | 23.62±0.000$^{f}$ | 20.87±0.535$^{f}$ | 20.44±0.370$^{e}$ | 19.16±0.110$^{e}$ | 18.03±0.115$^{g}$ | 17.06±0.285$^{g}$ |
| | 4% | 29.53±0.245$^{g}$ | 25.03±0.170$^{e}$ | 20.51±0.180$^{f}$ | 20.20±0.000$^{e}$ | 19.39±0.565$^{e}$ | 18.65±0.170$^{f}$ | 17.91±0.225$^{f}$ |
| | 5% | 30.54±0.954$^{f}$ | 27.53±0.595$^{d}$ | 25.86±0.005$^{d}$ | 21.42±0.485$^{d}$ | 19.27±0.340$^{e}$ | 18.82±0.340$^{f}$ | 18.54±0.055$^{e}$ |

*Control = meat mixture without any additives*
*Means with different letter in the same column indicate significant differences between treatments (p < 0.05)*

**Changes of lipid oxidation in beef burger samples:**

Thiobarbituric acid (TBA) values (mg of malonaldhyde/kg sample) as a criterion of lipid oxidation of meat products are presented in Table (6). Positive effect (p≤0.05) was noticed of addition different concentration of orange peel powder (OPP), pomegranate rind powder(PRP) and tomato pomace powder(TPP) as a natural antioxidant compared to control sample. Lipid oxidation inhibition effect was the highest with adding pomegranate rind powder and orange peel powder especially at concentration of 3%and4% at all of storage times. TBA values significantly (P< 0.05) increased in control sample as the time of storage period increased. On the other hand, the increase in TBA values in treated samples was slow and remained lower than of control sample up to end of storage period. This result could be correlated to the presence of phenolic compounds in pomegranate peels powder. The large amount of phenolics contained in rind powder extract may cause its strong antioxidant ability (Li *et al.,* 2006).

El-Nashi *et al.,* (2015) reported that the positive effect of addition of pomegranate peels powder as a natural antioxidant was noticed with significant differences in TBA values of prepared beef sausage samples.

Our results are in agreement with the results of Naveena *et al.,* (2008) and Devatkal *et al.,* (2010) as well as El-Gharably and Ashoush (2011).

**Table 6:** Effect of some agriculture waste as natural antioxidants on thiobarbituric acid values (mg MDA/kg meat) during frozen storage at-18°C ±1of beef burger (means ±S. D)

| Treatment | | Frozen Storage period (months) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Control | | 0.218±0.008$^{a}$ | 0.374±0.007$^{a}$ | 0.566±0.019$^{a}$ | 0.757±0.015$^{a}$ | 0.894±0.023$^{a}$ | 1.002±0.011$^{a}$ | 1.154±0.023$^{a}$ |
| Orange peel powder | 2% | 0.168±0.004$^{b}$ | 0.242±0.023$^{d}$ | 0.328±0.031$^{d}$ | 0.456±0.011$^{e}$ | 0.601±0.015$^{d}$ | 0.737±0.035$^{d}$ | 0.835±0.015$^{d}$ |
| | 3% | 0.121±0.004$^{d}$ | 0.195±0.008$^{ef}$ | 0.285±0.004$^{e}$ | 0.410±0.019$^{f}$ | 0.577±0.008$^{e}$ | 0.679±0.015$^{e}$ | 0.768±0.004$^{e}$ |
| | 4% | 0.105±0.004$^{e}$ | 0.176±0.003$^{f}$ | 0.246±0.004$^{f}$ | 0.367±0.015$^{g}$ | 0.437±0.008$^{h}$ | 0.554±0.015$^{g}$ | 0.647±0.023$^{fg}$ |
| Pomegranate rind powder | 1% | 0.148±0.024$^{c}$ | 0.207±0.011$^{e}$ | 0.332±0.003$^{d}$ | 0.437±0.008$^{e}$ | 0.546±0.008$^{g}$ | 0.620±0.004$^{f}$ | 0.659±0.043$^{f}$ |
| | 2% | 0.125±0.008$^{d}$ | 0.156±0.000$^{g}$ | 0.293±0.003$^{e}$ | 0.406±0.015$^{f}$ | 0.480±0.011$^{f}$ | 0.527±0.011$^{h}$ | 0.612±0.011$^{g}$ |
| | 3% | 0.074±0.004$^{f}$ | 0.257±0.007$^{cd}$ | 0.273±0.008$^{e}$ | 0.339±0.011$^{h}$ | 0.437±0.008$^{h}$ | 0.464±0.004$^{i}$ | 0.562±0.023$^{h}$ |
| Tomato pomace powder | 2% | 0.207±0.004$^{a}$ | 0.265±0.000$^{c}$ | 0.363±0.011$^{c}$ | 0.644±0.019$^{c}$ | 0.749±0.008$^{b}$ | 0.905±0.008$^{b}$ | 1.057±0.012$^{b}$ |
| | 4% | 0.179±0.008$^{b}$ | 0.246±0.004$^{cd}$ | 0.382±0.008$^{c}$ | 0.612±0.004$^{d}$ | 0.675±0.011$^{c}$ | 0.803±0.015$^{c}$ | 0.913±0.015$^{c}$ |
| | 5% | 0.168±0.004$^{b}$ | 0.304±0.015$^{b}$ | 0.445±0.015$^{b}$ | 0.710±0.008$^{b}$ | 0.671±0.023$^{c}$ | 0.780±0.008$^{c}$ | 0.885±0.019$^{c}$ |

*Control = meat mixture without any additives , MDA = malondialdehyde.*
*Means with different letter in the same column indicate significant differences between treatments (p < 0.05)*

**Changes of total volatile nitrogen (TVN) in prepared beef burger:**

Total volatile nitrogen (TVN) content is widely used as an indicator for protein decomposition caused by microorganisms as well as protein breakdown caused by tissue proteolytic enzymes during storage (Gibriel *et al*., 2007). The obtained data in Table (7) indicate that, TVN content was gradually increased (p ≤ 0.05) during storage of different formulated beef burger samples. Results also revealed that, the control

sample had highest TVN content at all storage period, being 9.90 mg TVN/100 g sample at the beginning of the storage period, and increased to 23.29 mg TVN/100 g sample after 6 months.

On the other hand, the corresponding TVN value for different prepared beef burger samples containing different concentrations of pomegranate rind powder, orange peel powder and tomato pomace powder had lower TVN content at all of storage times especially PRP3% at the initial of storage period (6.97 mg/100 g,), and at the end of the storage period after 6 months (14.50 mg/100 g). These results indicated the significant ($p \geq 0.05$) positive effect of addition of pomegranate peels powder on the inhibition of microbial growth especially proteolytic microorganisms that cause the breakdown of protein resulting in volatile nitrogen compounds.

The increase in TVN during cold storage of prepared beef burger might be attributed to the breakdown of nitrogenous substances by microbial activity. These results are in agreement with those of Madkour *et al*. (2000) and Gibriel *et al*. (2007).

**Table 7:** Effect of some natural antioxidants on Total volatile nitrogen TVN (mg /100 g sample) prepared of beef burger during frozen storage at-18°C ± (means ±S. D)

| Treatment | | Frozen Storage period (months) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Control | | 9.90±0.700$^{a}$ | 13.25±0.695$^{a}$ | 16.59±0.140$^{a}$ | 17.29±0.280$^{a}$ | 17.29±0.220$^{b}$ | 21.20±0.275$^{a}$ | 23.29±0.420$^{a}$ |
| Orange peel powder | 2% | 7.95±0.140$^{cd}$ | 11.29±0.135$^{cd}$ | 13.25±0.420$^{ef}$ | 15.06±0.280$^{cd}$ | 17.99±0.140$^{a}$ | 19.10±0.140$^{c}$ | 19.52±0.280$^{cd}$ |
| | 3% | 7.81±0.000$^{cd}$ | 11.16±0.000$^{cd}$ | 12.69±0.140$^{f}$ | 14.64±0.140$^{de}$ | 16.45±0.275$^{c}$ | 17.15±0.420$^{d}$ | 18.96±0.275$^{d}$ |
| | 4% | 7.25±0.560$^{de}$ | 10.60±0.560$^{de}$ | 11.57±0.140$^{g}$ | 14.08±0.140$^{ef}$ | 15.75±0.420$^{d}$ | 16.18±0.275$^{e}$ | 17.15±0.700$^{e}$ |
| Pomegranate rind powder | 1% | 7.95±0.140$^{cd}$ | 11.29±0.135$^{cd}$ | 12.41±0.140$^{f}$ | 14.64±0.140$^{de}$ | 16.87±0.140$^{bc}$ | 17.57±0.280$^{d}$ | 18.69±0.555$^{d}$ |
| | 2% | 7.25±0.560$^{de}$ | 10.60±0.560$^{de}$ | 11.29±0.135$^{gh}$ | 13.94±0.275$^{f}$ | 15.76±0.695$^{d}$ | 16.45±0.000$^{e}$ | 17.29±0.840$^{e}$ |
| | 3% | 6.97±0.560$^{e}$ | 10.32±0.560$^{e}$ | 11.02±0.140$^{h}$ | 11.99±0.560$^{g}$ | 13.25±0.695$^{e}$ | 13.67±0.555$^{f}$ | 14.50±0.835$^{f}$ |
| Tomato pomace powder | 2% | 8.92±0.275$^{b}$ | 12.27±0.280$^{b}$ | 15.48±0.140$^{b}$ | 16.04±0.695$^{b}$ | 18.13±0.280$^{a}$ | 20.08±0.560$^{b}$ | 21.20±0.275$^{b}$ |
| | 4% | 8.37±0.280$^{bc}$ | 11.71±0.280$^{bc}$ | 14.64±0.140$^{c}$ | 15.62±0.280$^{bc}$ | 17.99±0.140$^{a}$ | 19.10±0.130$^{c}$ | 20.36±0.280$^{bc}$ |
| | 5% | 8.09±0.280$^{c}$ | 11.43±0.275$^{c}$ | 13.80±0.135$^{d}$ | 15.34±0.280$^{c}$ | 16.59±0.140$^{c}$ | 17.15±0.140$^{d}$ | 18.69±0.505$^{d}$ |

*Control = Meat mixture without any additives*
*Means with different letter in the same column indicate significant differences between treatments ($p < 0.05$).*

## Conclusion

It could be concluded that, addition of different concentration of pomegranate rind powder, orange peel powder and tomato pomace powder improved the quality criteria and significantly ($p \leq 0.05$) increased the values of total phenolic compound, antioxidant activity and fiber content of beef burger during frozen storage. The application of different concentrations of this powders had decreased the value of TBA and TVN as well as increase of shelf life. In the same time, utilization of the investigated pomegranate rind powder, orange peel powder and tomato pomace powder could be useful to achieve high stability of beef burger during storage without any negative effects on the product.

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