Food Science and Quality Management
ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online)
Vol .11, 2013

Utilization of Fruit by-Product in Ground Meat Preservation

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Abstract

Meat is prone to both microbial and oxidative spoilage, therefore it is desirable to use a preservative with both antioxidant and antimicrobial properties. The use of fruit by-products such as Grapefruit rind powder (GRP), orange rind powder (ORP) and mandarin rind powder (MRP) with or without γ irradiation on microbial growth, lipid oxidation and color change of raw ground beef meat stored at $4 \pm 1\ ^0$C was evaluated. Also, the effect of these natural by-products on the survival of *Salmonella typhimurium*, *Escherichia coli* and *Bacillus cereus* inoculated into sterile ground beef meat was studied. All by-product additives significantly ($p< 0.05$) reduced total bacterial, lactic acid bacteria and total mold and yeast counts and extended the shelf-life of ground meat compared with the control. The control samples were microbiologically rejected on day 7 of storage at $4 \pm 1\ ^0$C. The counts of pathogenic bacteria inoculated into ground beef meat were significantly ($P< 0.05$) affected by the addition of additives. MRP showed high antimicrobial effect flowed by ORP then GRP, these results confirmed with the microbial tested of shelf-life of ground meat. The gram-positive bacteria (*Bacillus cereus*) were more resistance than gram-negative bacteria (*Salmonella typhimurium and Escherichia coli*) for tested treatments. It was also found that ORP had the highest scavenging effect (%) on DPPH followed by GRP then MRP. Concerning lipid oxidation, the control showed significantly ($p< 0.05$) higher malonaldehyde (MDA) content during all storage period and the color components were significantly ($p< 0.05$) affected by the additives used. The results of this study suggest the potential for developing natural food additives from fruit by-product for improving food stability, quality and safety.

Key words: By-product, ground meat, γ irradiation, antimicrobial and antioxidant

Introduction

Meat and meat products are important sources for protein, fat, essential amino acids, minerals, vitamins and other nutrients. Microbial growth and oxidative rancidity are the major problems causing shelf life quality deterioration, therefore, preservation technologies must be applied in order to preserve its safety and quality (**Aymerich *et al.*, 2008**). Irradiation is known to be the best method for the control of both spoilage and potentially pathogenic microorganisms in meat without affecting its physical state **(Kanatt *et al.*, 2005)**. Also, synthetic additives can reduce food spoilage, but consumers are concerned about chemical residues in food (**Ayala-Zavala & González-Aguilar, 2011**and **White & McFadden 2008**).

Regarding the food safety issues, one of the major emerging technologies is the application of natural additives. We have to consider that the high content of bioactive compounds present in fruit by-products can be used as natural food additives (antioxidants, antimicrobials, colorants, flavorings, and thickener agents). If this approach is realized, it would be feasible to fulfill the requirements of consumers for natural and preserved healthy food. In addition, the full utilization of fruits could lead the industry to a lower-waste agribusiness, increasing industrial profitability (**Ayala-Zavala & González-Aguilar, 2011**). The most common bioactive compounds present in fruits and fruit by-products are vitamins C, E, carotenoids, phenolic compounds and dietary fiber (**Gonzalez-Aguilar *et al.*, 2008**). As health related compounds, these have been attributed to lowering the risk of developing cancer, alzheimer, cataracts and Parkinson, among others. These beneficial effects have been attributed mainly to their antioxidant and radical scavenging activities which can delay or inhibit the oxidation of DNA, proteins and lipids. Indeed, these compounds have shown antimicrobial effects, playing an important role in fruits' protection against pathogenic agents, penetrating the cell membrane of microorganisms, causing lysis (**Ayala-Zavala & González-Aguilar, 2011**).

Citrus is the most abundant crop worldwide, its production is over 88×10^6 tons and one-third of the crop is processed. Oranges, lemons, grapefruits and mandarins represent approximately 98% of the entire industrialized crop. Citrus fruits are processed, mainly to obtain juice, but also, in the canning industry, to produce jam and segments of mandarin (**Izquierdo & Sendra 2003**). Worldwide industrial citrus wastes may be estimated at more than 15×10^6 tons, as the amount of residues obtained from the fruits accounts for 50% of the original whole fruit mass, which are exploited by the chemical industry to extract flavonoids and essential oils (**Mar ín *et al.*, 2007**). Flavonoids are polyphenols with diphenylpropane (C6C3C6) skeletons (**Alothman *et al.*,**

2009). Among these compounds, mirecitine, mangiferin, gallic acid and hydrolysable tannins, which are most likely gallotannins, constitute the major antioxidant polyphenolics found in some citrus by-products (**Gonzalez-Aguilar *et al.*, 2008**).

Thus citrus by-products, are promising new sources of phenolic antimicrobial and antioxidant compounds offering new commercial opportunities to food industry. However, to date there are very scarce information and studies on by-products and their applications in meat, which is an important area of research. Therefore the purpose of this paper is to use combination treatments of irradiation with citrus by-products, as a source of functional compounds, and their application to preserve ground meat.

Materials and Methods

2.1. Preparation of natural additives

Mature and healthy grapefruit, mandarin and orange fruits were purchased from retail fruit market, washed thoroughly, cut manually and peeled off. The rind (peel) obtained was cut into small pieces using a sharp knife and dried in an air circulatory tray drier (Narang Scientific Works, New Delhi, India) at 60 ^{0}C for 48 h. Dried pieces were cooled and powdered in a heavy duty kitchen grinder. The obtained powder (grapefruit rind powder (GRP), orange rind Powder (ORP) and mandarin rind Powder (MRP), respectively) was sieved using a sieve (1.651 mm, ASTM No. 10) then packed in polyethylene bags individually and stored at room temperature until further use.

2.2. Sample preparation, packaging and irradiation

Beef meat was obtained from local butcher in Giza governorate then transported in an ice-box to the lab in the National Center for Radiaton Research and Technology (NCRRT), Nasr city, Cairo, Egypt, where it was ground twice (10 mm plate followed by 8 mm plates) using a meat blender (Sirman, Italy). Ground meat samples were divided into 4 groups, the first group served as control without any additives, while the other 3 groups were sub-divided into 3 sub-groups each, for the addition of additives (GRP, MRP and ORP) in different concentrations with or without irradiation. Totally, ground meat samples were assigned to ten different treatments: Control (ground meat without any additive); GRP1 (ground meat with 1% salt and 1 % GRP); GRP2 (ground meat with 2 % salt and 2 % GRP); GRP+ γ (ground meat with 1% salt, 1 % GRP and irradiation at 2 kGy); MRP1 (ground meat with 1% salt and 1% MRP); MRP2 (ground meat with 2 % salt and 2 % MRP); MRP+ γ (ground meat with 1% salt, 1% MRP and irradiation at 2 kGy); ORP1 (ground meat with 1 % salt and 1 % ORP); ORP2 (ground meat with 2 % salt and 2 % ORP) and ORP+ γ (ground meat with 1 % salt, 1 % ORP 1 % and irradiation at 2 kGy) Immediately after adding all ingredients, samples were thoroughly mixed, packaged in polyethylene bags (25 g for microbiological analysis, 20 g for lipid oxidation and 50 g for instrumental color). Samples subjected to irradiation (GRP+ γ, MRP+ γ and ORP+ γ) were then transferred to the irradiation facility in the NCRRT which were irradiated with 2 kGy gamma irradiation at dose rate 3.49269 kGy/ h using the "Indian Gamma Chamber 4000 A" with a ^{60}Co source. After irradiation, all samples were transferred to a refrigerator and stored at 4 ± 1 ^{0}C for 21 days. Three packages from each treatment were analyzed immediately after irradiation and at regular intervals. During storage microbiological analysis (for shelf-life and inoculation test), lipid oxidation and instrumental color were evaluated at 7 days interval.

2.3. Microbiological analysis

Total bacterial counts (TBC) **(APHA, 2001)**, Lactic acid bacteria (LAB) **(Oxoid Manual**, **1982)** and total mold and yeast **(Oxoid Manual, 1998)**, were enumerated on plate count agar, MRS and oxytetracycline glucose yeast extract agar medium, respectively, by pour plate technique.

2.4. Artificial inoculation test

Bacterial strains used in artificial inoculation (*Escherichia coli*, *Salmonella typhimurium* and *Bacillus cereus*) were obtained from Microbiology Department in the NCRRT which were stored in 20 % glycerol (v/v) at 20 ^{0}C. Before the beginning of the experiment, the cultures were grown on nutrient agar and the isolates were subculture twice before inoculation. The cultures were then serially diluted in sterile saline (0.85% NaCl) for standardization by pour plate assay in duplicate using nutrient agar plates incubated at 35^{o}C for 18 h.

Packaged ground beef meat samples (25g) used for artificial inoculation test were first sterilized by accelerated electrons at 20 kGy in the NCRRT using electron beam accelerator (energy: 1.5 Mev and current: 0.9 m A). Additives were then added according to the previously mentioned scheme, stock cultures of all test bacteria were grown in nutrient broth for 18 h and then 1ml of each organism (10^5) separately was inoculated in each pack (3 packages for each organism for each treatment). Survivors of the studied organisms were

enumerated on plate count agar (PCA) medium **(APHA, 2001)** using pour plate technique after incubation at 35 ^{o}C for 24 h, immediately after irradiation and at 7 days interval.

2.5. Scavenging of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical

Measurement of free radical scavenging activity on DPPH radical was determined according to the method described by **(Yamaguchi *et al.*, 1998)**. Briefly, 1.5ml of DPPH solution (0.1mM, in 95% ethanol or methanol) was incubated with varying concentrations of GRP, ORP and MRP. The reaction mixture was shaken well and incubated for 15 min at room temperature and the absorbance of the resulting solution was read at 517 nm against a blank (control). The radical scavenging effect was measured as a decrease in the absorbance of DPPH and can be calculated using the following equation:

Scavenging effect (%) = [1 - (A samples 517 nm / A control 517 nm)] x 100

2.6. Lipid oxidation

A common method used for quantitating malonaldehyde (MDA), a major lipid peroxidation product, was performed in triplicates according to **Vyncke (1970**), using trichloroacetic acid (TCA 7.5%), freshly prepared 0.02M thiobarbituric acid solution (TBA) and the absorbance (A) of the developed red color was measured at wavelength 538nm. The results were expressed as mg malonaldehyde/ kg sample.

2.7. Instrumental color measurements

Instrumental color determinations were made by a micro color unit attached to a data station (Brano Lange –Germany) using the standard CIE LAB color system as follows: a-value (redness/green), b-value (yellowness/blue) and L-value (lightness/darkness,). Color measurements were determined in triplicate on each treatment group. All samples were measured in polyethylene bags. Six readings were taken at various points on each sample **(CIE, 1978)**.

2.8. Statistical analysis

Results obtained were subjected to statistical analysis using one way analysis of variance **(Rao & Blane 1985).** All data were the average of three replicates.

Results and Discussion

Effect of additives on the shelf-life of ground beef meat

The growth of microbes, such as bacteria, molds and yeast deteriorate the safety and quality of meat products and cause significant economic losses (**Asefa *et al.*, 2010**). Fig (1 A, B and C) represents that the control of ground meat at had initial counts of 3.34, 2.64 and 1.28 log CFU/g for total bacterial counts (TBC), lactic acid bacteria (LAB) and mold and yeast, respectively. TBC were found to be in the acceptable range according to the Egyptian Organization for Standardization (EOS) for ground meat (1694/ 2005).

Fig 1 A, B and C shows the survivors of TBC, LAB and mold and yeast in ground meat in the presence of MRP, ORP and GRP, respectively. The results revealed that tested additives caused a significant *($P< 0.05$)* decrease in all microbial counts compared with the control. This finding was similar to that reported by **Fernandez-Lopez *et al*. (2005)** in beef meat-balls. After 7 days, GRP, ORP and MRP reduced TBC by 1.8, 2.8 and 3.94 log cycles, LAB by 0.98, 1.58 and 2.03 log cycles and mold and yeast by 0.6, 1.1 and 1.76 log cycles, respectively. In the present study, the control samples were rejected on day 7 as the TBC reached log 7 CFU/g, which is the acceptable limit as defined by **ICMFS (1986)**. GRP extended the shelf-life of samples to 14 day. However, the samples treated with MRP and ORP extended the shelf-life for more than 21 days. Contrasting with our results, **Mexis *et al*. (2012)** found that the addition of citrus extract had a small preservative effect on fresh ground chicken meat. Although the combination between additives and γ irradiation (2 kGy) (MRP+ γ, ORP+ γ and GRP+ γ) was more effective in reducing all microbial counts, but this reduction was not significant *($p> 0.05$)*. Irradiation is a simple feasible technique to reduce the load of microbes, avoid post packaging recontamination and so extend the shelf life **(Kanatt *et al.*, 2005)**. **Mattar & Abd-Eldaiem (2008)** reported that the shelf-life of chicken burger treated by 2 % ethanol extract of propolis and 4.5 kGy gamma irradiation increased up to 27 days and this combined treatment was more effective as antimicrobial.

The antimicrobial activity of citrus fruit rinds depended on their volatile oils present in the rinds. Citrus essential oils (EOs) contain 85–99% volatile and 1–15% non-volatile components (**Fisher & Phillips 2008**). Limonene is the major chemical component of citrus EOs, ranging from 32 to 98% (**Svoboda & Greenaway 2003**). Others like flavonoids, present three phenolic rings with several hydroxyl groups. The site(s) and number

of hydroxyl groups on the phenol group are thought to be related to their antioxidant and antimicrobial capacity and relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased microbial toxicity (**Cowan 1999**). Citrus EOs have been industrially applied in many products, including foods and beverages, cosmetics and medicines (**Abd EI-Aziz & Abd EI-Khalek 2010** and **Uysal *et al.*, 2011**).

The results of this study showed significant differences among the antimicrobial activity of the tested citrus fruit rinds. In fact, MRP was the most effective followed by ORP then GRP. Since limonene was present at very high and similar concentration in the three citrus peels, the greater antimicrobial activity of mandarin EO might not be attributed to limonene, but it might be related to the presence of other EO constituents. **Burt (2004)** reported that the chemical characterization of the three EO demonstrated the presence of a significantly higher proportion of oxygenated monoterpenes in mandarin EO (13.6%) in contrast to 5.7% and 5.2% of lemon and orange EOs, respectively. Therefore, oxygenated monoterpenes might be involved in the higher antimicrobial activity of mandarin EO.

Effect of additives on artificially inoculated pathogenic bacteria

The effect of the three dried rind powders on *Salmonella typhimurium*, *Escherichia coli* and *Bacillus cereus* inoculated into sterile ground beef meat were tested and represented in Figs. 2 A, B and C. It is well known that these pathogenic and food poisoning bacteria are frequently isolated from meat products (**Aymerich *et al.*, 2008**).

The results revealed that the counts of all the tested organisms were significantly *(P< 0.05)* affected by all treatments. By using GRP1, there was a 1.82, 1.21 and 0.65 log reduction in the counts of *Salmonella typhimurium, Escherichia coli* and *Bacillus cereus*, respectively, after 21 days of storage compared with the control. In samples treated with ORP1, this reduction was 2.73, 2.04 and 1.24 log, respectively. While, treating the samples with MRP1 resulted in 3.1, 3.2 and 2.38 log reduction in the previously mentioned organisms, respectively, at the end of storage (21 days) compared with the control. Although there was an increase in the log reduction in counts of the previously mentioned organisms in samples treated with 2% rind powders or a combination of rind powders with γ- irradiation, but this increase in the reduction was non significant (*p> 0.05*). The reduction in the population of *Salmonella* spp. and *Escherichia coli* O157:H7 by citrus essential oils was reported by several investigators **(Fisher & Phillips 2006** and **Fisher *et al.*, 2007)**. **Benelli *et al.* (2012)** found that orange pomace had strong antimicrobial activity against *Staphylococcus aureuis* and moderated activity against *Escherichia coli*, while **Kanatt *et al.* (2010)** reported good antimicrobial activity of Pomegranate peel extract against *Staphylococcus aureus* and *Bacillus cereus.*

From the previous results, MRP showed the highest antimicrobial effect flowed by ORP then GRP which was in agreement with our microbiological results of the shelf-life. This finding was similar to that reported by **Espina *et al.* (2011)**, who found that Gram-positive bacteria (*Bacillus cereus*) was more resistant than Gram-negative bacteria (*Salmonella typhimurium* and *Escherichia coli*) for mandarin, orange and lemon essential oil.

Scavenging effect on DPPH

A simple method developed to determine the antioxidant activity of foods. DPPH radical scavenging assay is one of the most extensively used for antioxidant assays, for assessment of free radical scavenging potential of an antioxidant molecule and considered as one of the standard and easy colorimetric methods for the evaluation of antioxidant properties of pure compounds. DPPH is a stable radical in solution and appears purple color absorbing at 517 nm in ethanol **(Yamaguchi *et al.*, 1998)**.

Figure (3) shows the scavenging effect (%) of GRP, ORP and MRP on DPPH radical. In general, the DPPH radical scavenging effect (%) increased as the concentration of the previously mentioned additives increased. The DPPH scavenging effect (%) of GRP, MRP and ORP at a concentration of 1 mg/ml was 48.72, 52.30 and 33.82 (%), respectively, which became 62.18, 78.82 and 56.13 (%), respectively at a concentration of 2 mg/ml. So, the ORP had the highest scavenging effect (%) followed by GRP then MRP. Also, it was found that the 50% inhibition concentration (IC_{50}) of DPPH radical scavenging ability of GRP, ORP and MRP was obtained at 1.18, 0.97 and 1.82 mg/ml, respectively.

Lipid oxidation

The antioxidant effect of treatments, as measured by MDA content, over 21 days of refrigerated storage are shown in figure (4). On day 0, MDA content for all treatments were significantly ($p< 0.05$) lower than those for the control. This result indicates that lipid oxidation was effectively retarded by GRP1, GRP2, GRP1 + γ, ORP1, ORP2, ORP1 + γ, MRP1, MRP2, MRP1 + γ. Compared to the control, all treatments had significantly *(p< 0.05)* lower TBARS values at each day of analysis throughout storage.

MDA content of the control rapidly increased ($p< 0.05$) with increasing storage time. This increase over time was due to deteriorative reactions (microbiological and enzymatic) **(Brannan, 2008** and **Selani *et al.*, 2011)**. At the end of the experiment (21 days) ORP1, ORP2, ORP1 + γ, MRP1, MRP2, MRP1 + γ treated sample showed the lowest degree of oxidation ($p< 0.05$) of all the samples (was the most effective at reducing the formation of MDA), while the control showed the highest values for this parameter. The decrease in MDA content at day 14, was attributed to its metabolism by spoilage bacteria as **Lebepe *et al.* (1990)** reported.

The antioxidant activity of by-products obtained from industrial manipulation of citrus fruit has been widely demonstrated in meat products, whether fresh (**Aleson-Carbonell *et al.*, 2005**), cooked (**Viuda-Martos *et al.*, 2009**) or dry cured (**Fernandez-Lopez *et al.*, 2008**). Such activity is basically due to their composition: mainly to phenolic compounds and flavonoids. The solubility of flavonoids in fats and oils is very low and their role in the oxidation of oil is not significant; however, they can contribute to decreasing the oxidation of fat in food emulsions (**Zhou *et al.*, 2005**).

Flavonoids act as antioxidants (i) or pro-oxidants (ii) and synergist (iii) depending on: (i) their structural features (catechol-stuctured flavonoids scavenge lipid peroxy radicals by donating hydrogen and become more stable phenoxy radicals) (**Choe & Min 2009**) (ii) concentration, temperature, light, type of substrate, physical state of the system as well as micro components acting (**Yanishlieva-Maslarova, 2001**) or (iii) a combination of two or more different free radical scavengers in which one antioxidant is generated by others, a sacrificial oxidation of an antioxidant to protect another antioxidant, and a combination of two or more antioxidants whose antioxidant mechanisms are different (**Decker, 2002**).

Verma & Sahoo (2000) indicated MDA concentration between 1000-2000 µg/ Kg as threshold values for rancidity, while **Greene & Cumuze (1982)** considered a TBARS range of 600-2000 µg/ Kg to be the minimum detectable level for oxidized flavour in ground beef by an un-experienced panel. Therefore, MDA content of the control in this study was higher than the threshold level after 7 days of refrigerated storage and should be rejected

Instrumental color

The treatment effects on color parameters (L*, a* and b*) of ground beef meat during refrigerated storage are shown in figures (5A, B and C). All treatments increased L* values (lightness) significantly *($p< 0.05$)* compared to the control over the 21 days of storage. Lightness (L*) and yellowness (b*) were significantly *($p< 0.05$)* affected by fiber content (**Viuda-Martos *et al.*, 2010**). Lightness in food is related with many factors, including the concentration and type of pigments present (**Lindahl *et al*., 2001**), water content (**Aleson-Carbonell *et al.*, 2005**) and fiber content and type (**Fernandez-Gines *et al.*, 2003**). Lightness increases when citrus fiber and spice essential oils were added (**Viuda-Martos *et al.*, 2010**). This increase is probably due to the fact that fiber, structurally, is composed of macromolecules that are rehydrated and remain outside the meat matrix, thus affecting the color coordinates such as lightness. These results are consistent with those obtained by **Fernandez-Gines *et al*. (2003)**.

For redness (a*), at day 0, the treatments GRP1+ γ, ORP2, ORP1+ γ, MRP1, MRP2 and MRP1+ γ caused a significant ($p< 0.05$) reduction in a* value of ground meat compared to other treatments (fig. 5B). The use of GRP1, GRP2 and ORP1 led to a decrease in this parameter (except GRP2), but with no significant difference ($p> 0.05$) with respect to the control. Treatment with GRP2 and control had the highest a* value and gave greater stability to the samples with regards to red discoloration. Significant changes in a* values were also observed in chicken meat (**Brannan, 2009)**, however, this author reported an increase in a* values of samples with grape seed extract, which was different from what **Selani *et al*. (2011)** observed. This variation in results may be due to different colorations of the grape extracts used, which may have interfered with the meat color in different ways. This coordinate is affected by the structural integrity of the food, the pigment content and disposition (water or lipid soluble) and surface water availability (**Fernandez-Lopez *et al.*, 2005**). As regards, the composition of the food, the water/ oil relations of the product also play an important role. This coordinate could have a linear relationship with the concentration of pigment (**Viuda-Martos *et al*, 2009**).

Regarding storage time, a significant decline ($p< 0.05$) was verified only in relation to the a* value of the samples treated with GRP1+ γ, ORP1+ γ, MRP2 and MRP1+ γ . However, the reduction in the intensity of red color during storage could be explained due to the interdependence between lipid oxidation and color oxidation in meats (**Lynch & Faustman 2000**). The pigment oxidation may catalyze lipid oxidation, and free radicals produced during oxidation may oxidize the iron atoms or denature the myoglobin molecules, negatively changing the color of the products. Thus, because the TBARS values of treated samples in this study increased

slightly throughout the storage time, this trend of decreasing a* values may be due to interference with the lipid oxidation in the myoglobin oxidation. (**Selani *et al.*, 2011**)

For the yellowness (b*), see figure 5C, the results show that at day 0 different treatments caused a significant ($p< 0.05$) increases in the value of this coordinate over the control value, with no statistically significant differences between the control, ORP1+ γ and MRP2. According to **Fernandez-Gines *et al.* (2003)**, this increase could be due to the carotenoids present in the orange fiber, which were not eliminated by washing.

By storage, b* value, increased significantly ($p< 0.05$) in the control, MRP1, and MRP2. This finding was similar to that reported by **Viuda-Martos *et al.* (2009)**. The behavior of b* depends to a great extent on the food matrix, and it is recognized that changes (pH, oxidation extent, water activity, etc.) in the matrix have the greatest influence on this coordinate in many foods (**Cofrades *et al.*, 2004**).

Conclusion

Addition of dry citrus by-products in food increases the nutritive value of this food, because its contain about 22 % sugars, 7.5 % protein, 1.6 % fat, 16 % pectin and 80 % fibe (**Izquierdo & Sendra 2003**). Results obtained herein, may suggest that the Citrus by-products combined with NaCl or γ irradiation preserved ground meat and extended its shelf-life for more than about 21 days and therefore, they can be used in biotechnological fields as natural preservative in food industry.

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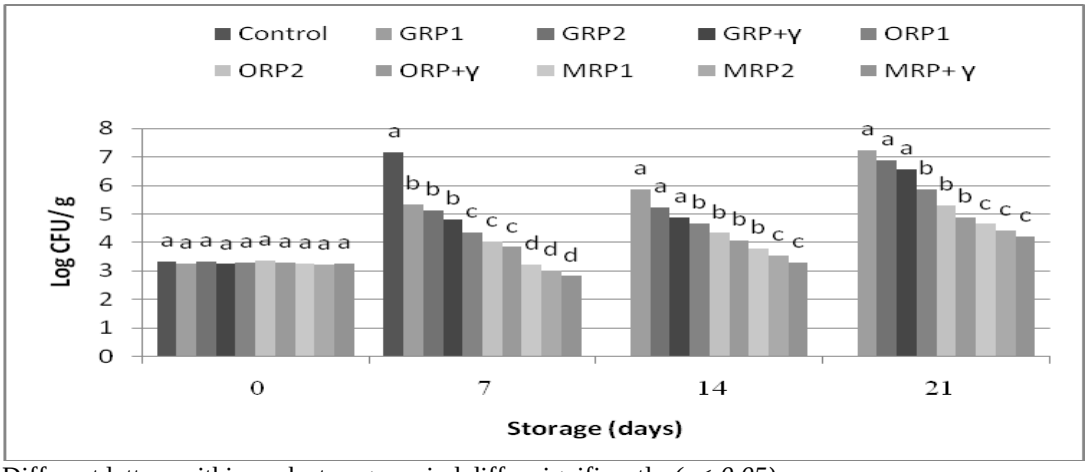
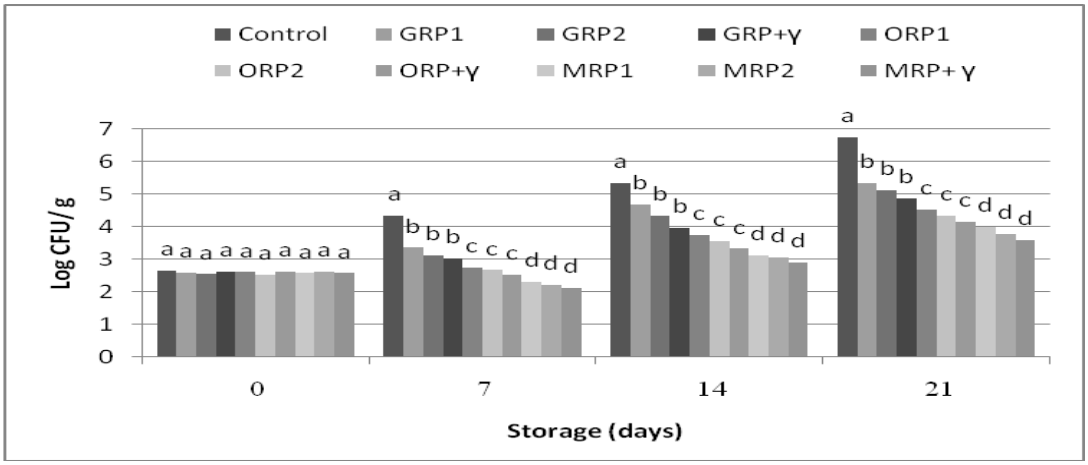


Different letters within each storage period differ significantly *(p< 0.05)*.
Fig (1A) Effect of additives on the total bacterial counts of ground beef meat
Grapefruit rind powder GRP1 (1%), GRP2 (2%), GRP+ γ (GRP %1+ 2 kGy), orange rind powder ORP1 (1%), ORP2 (2%), ORP+ γ (ORP 1% + 2 kGy), mandarin rind powder MRP1 (1%), MRP2 (2%) & MRP + γ (MRP 1% + 2 kGy).



Different letters within each storage period differ significantly *(p< 0.05)*.
Fig (1B) Effect of additives on the total lactic acid bacteria of ground beef meat
Legend as in Fig 1A

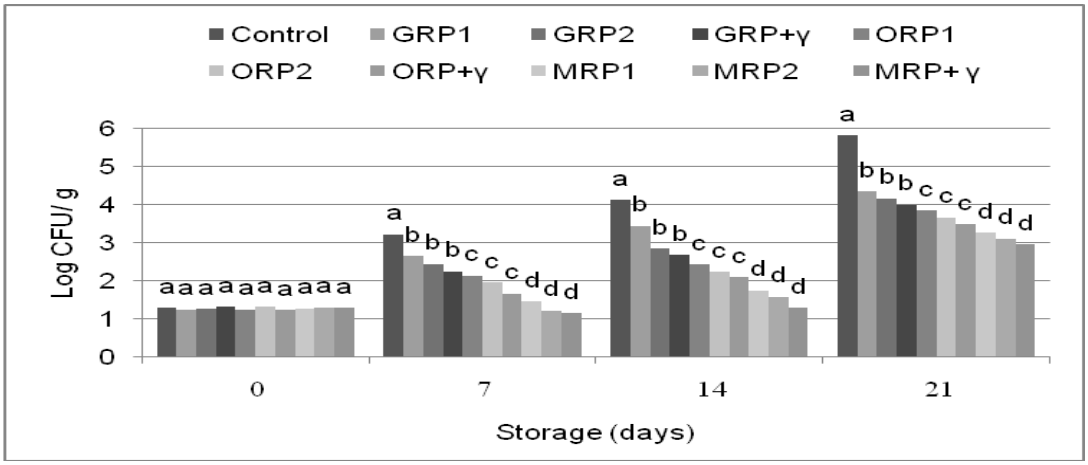


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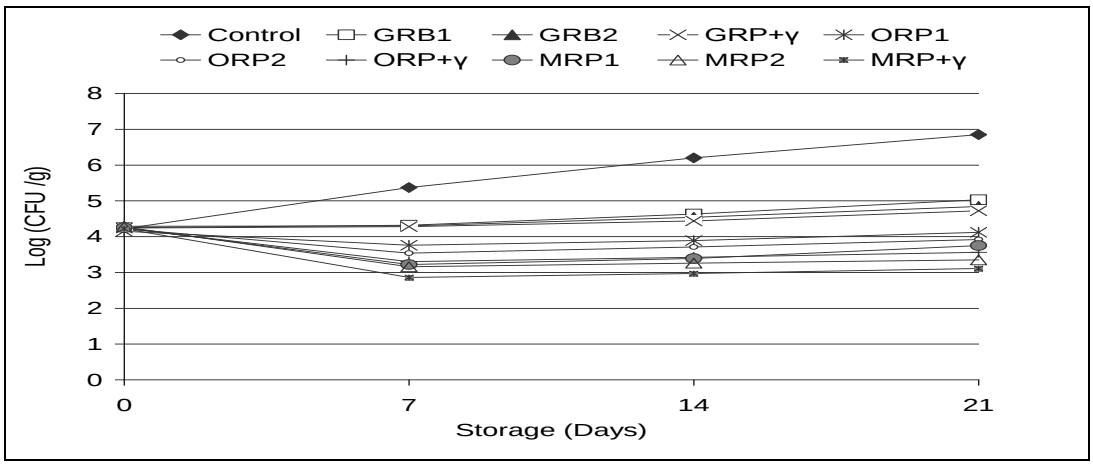
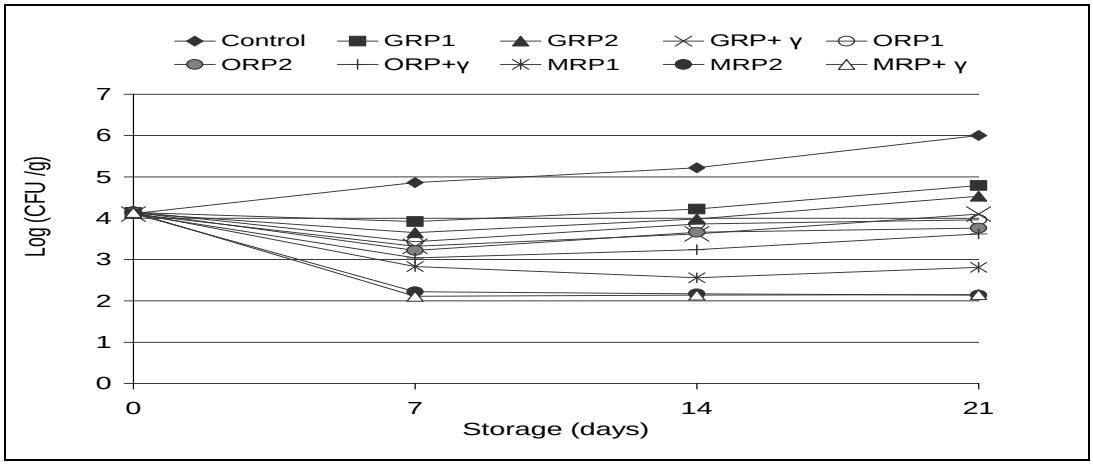
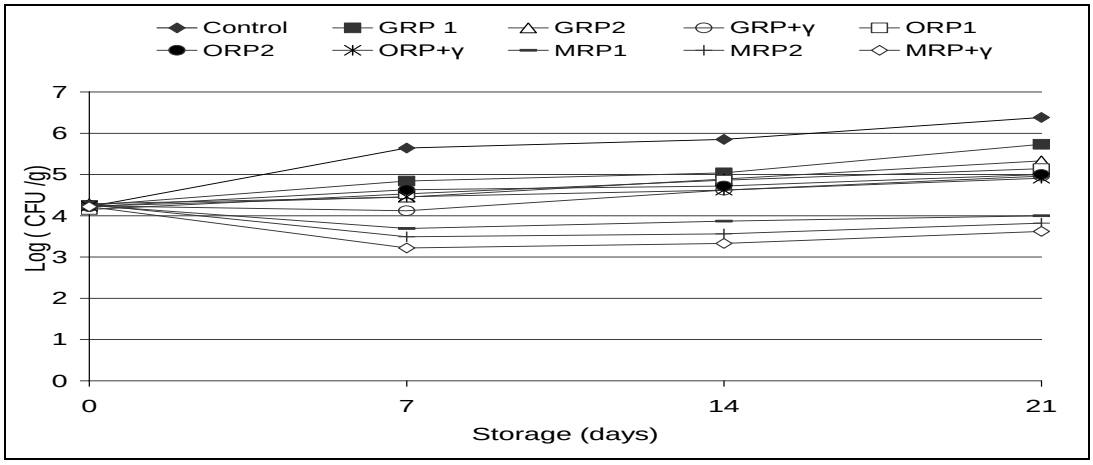
Fig (1C) Effect of additives on the total mold and yeast of ground beef meat
Legend as in Fig 1A



Different letters within each storage period differ significantly *(p< 0.05)*.
Fig (2A) Effect of additives on the survival of *Salmonella typhimurium* inoculated in ground meat.
Legend as in Fig 1A



Different letters within each storage period differ significantly *(p< 0.05)*.
Fig (2B) Effect of additives on the survival of *Escherichia coli* O157:H7 inoculated in ground meat.
Legend as in Fig 1A



Different letters within each storage period differ significantly *(p< 0.05)*.
Fig (2C) Effect of additives on the survival of *Bacillus cereus* inoculated in ground meat
Legend as in Fig 1A

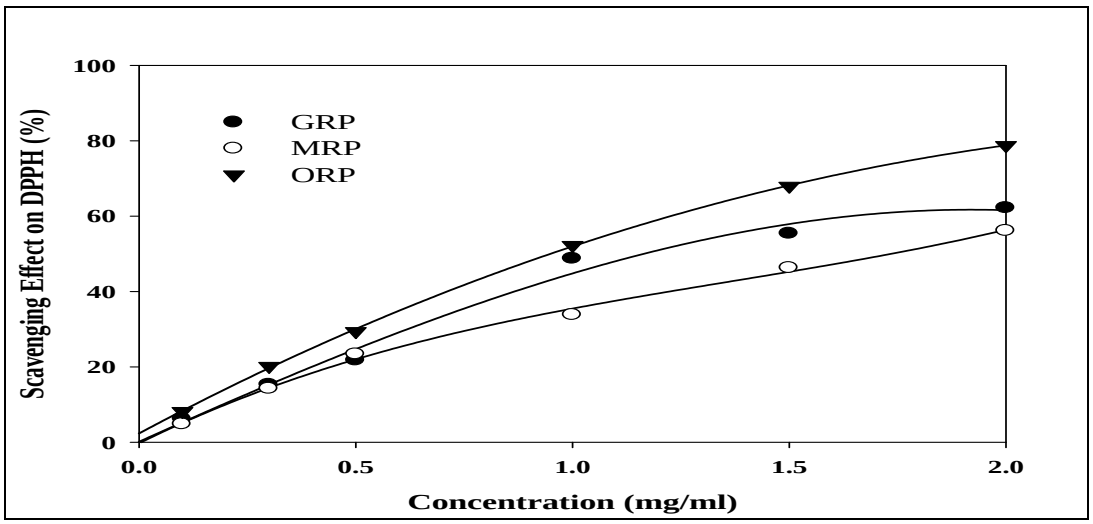
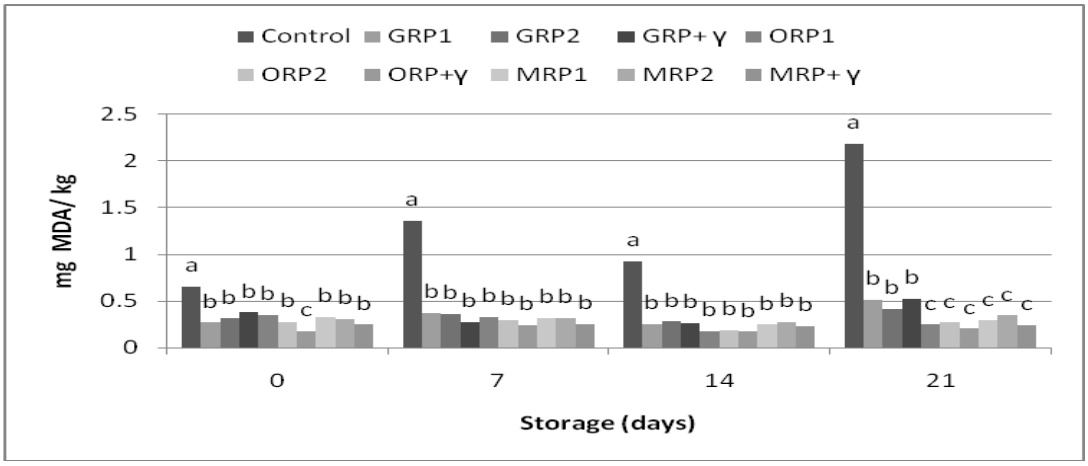
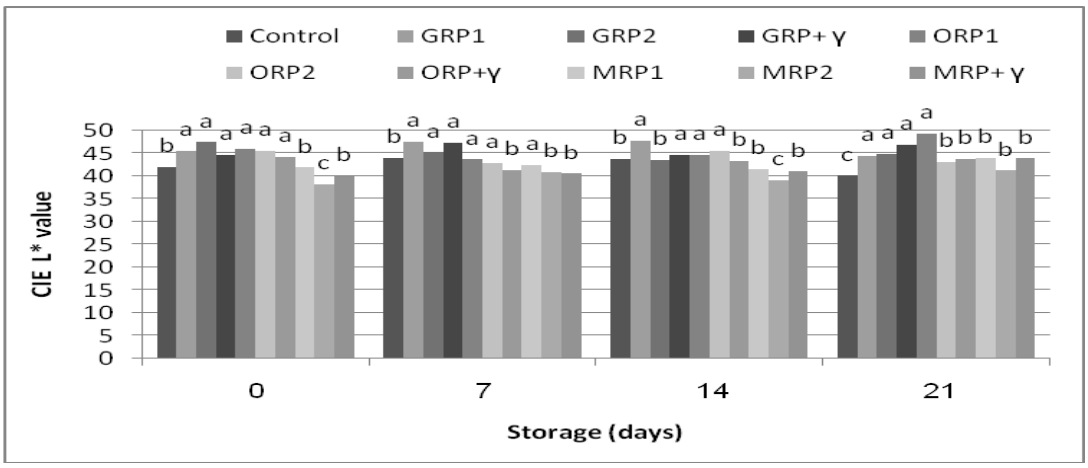


Figure (3) The scavenging effect (%) on DPPH radicals of (●) grapefruit rind powder (GRP), (○) mandarin rind powder (MRP) and (▼) orange rind powder (ORP).



Different letters within each storage period differ significantly *(p< 0.05)*.
Figure (4) Effect of additives on lipid oxidation (mg MDA/ kg) of ground meat during storage
Legend as in Fig 1A



Different letters within each storage period differ significantly *(p< 0.05)*.
Figure (5A) Effect of additives on L*-value of ground beef meat during storage
Legend as in Fig 1A

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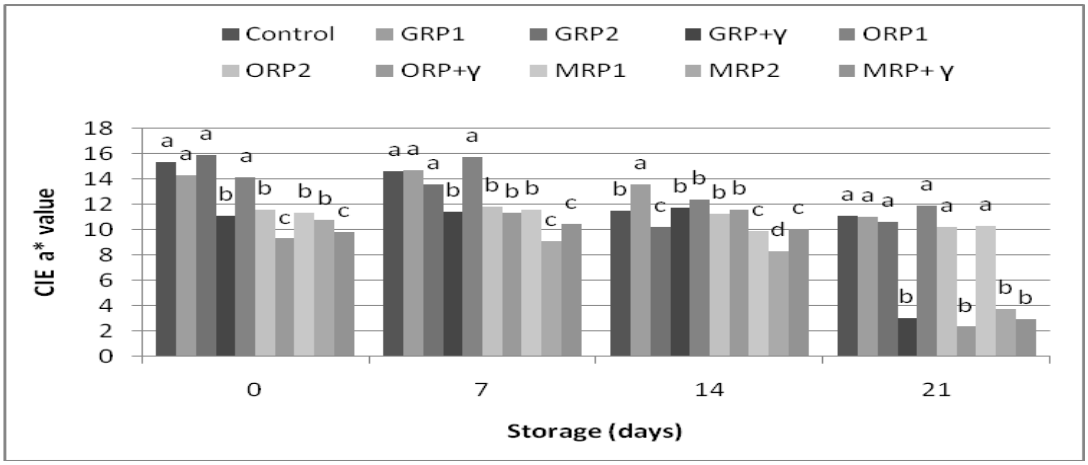
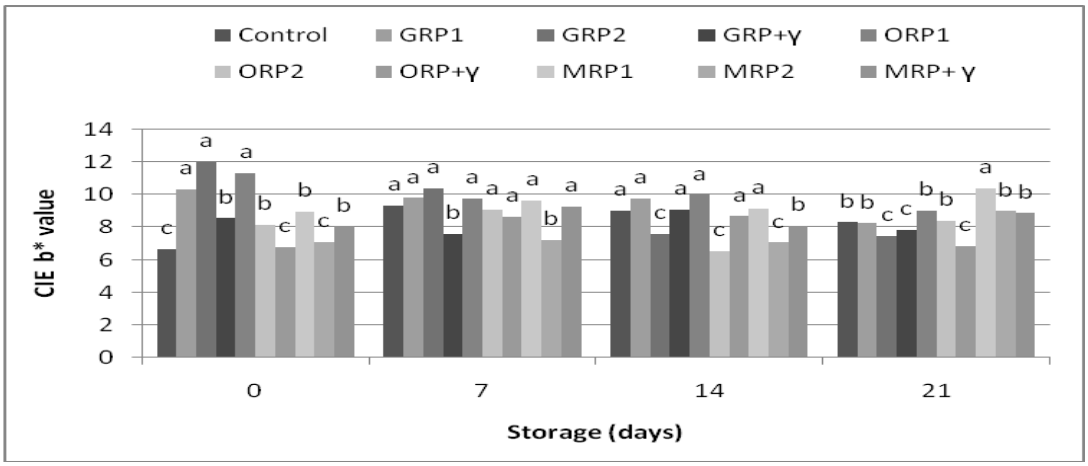


Different letters within each storage period differ significantly *(p< 0.05)*.
Figure (5B) Effect of additives on a*-value of ground beef meat during storage.
Legend as in Fig 1A



Different letters within each storage period differ significantly *(p< 0.05)*.
Figure (5C) Effect of additives on b*-value of ground beef meat during storage.
Legend as in Fig 1A