The Effect of Natural Antioxidants on Quality and Shelf Life of Beef and Beef Products

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SUMMARY

Oxidation processes are the major cause of deterioration of meat quality and shelf life of meat products, leading to negative changes in nutritive value and in sensory and physicochemical properties of meat. Until now, the synthetic antioxidants like butylated hydroxyl anisole (BHA) have been commonly used to detain oxidation processes, which however may cause potential human health risks and increase toxicity of the product. However, natural antioxidants can be the alternative solution of this problem since plants and plants materials are rich in bioactive compounds (as natural antioxidants) with potential health beneficial effects. Moreover, the interest of consumers in using natural products is still increasing. There is an expectation of replacing synthetic antioxidants and preservatives by natural ones. Therefore, the aim of the present review is to provide information on the effect of natural antioxidants vegetables and fruits like olives, pomegranate, grapes as well as herbs and spices e.g. rosemary, oregano, sage, black

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cumin or curcumin rich in bioactive compounds on quality and shelf life of beef and beef products.

**Key words**: natural antioxidants, beef, beef products, quality, shelf life

**INTRODUCTION**

Lipid and protein oxidation is the major cause of deterioration of meat quality and shelf life limitations of meat products. They lead to unfavourable changes in nutritive value, and in sensory and physicochemical properties of meat like colour, flavour or tenderness (1-6). The oxidative deterioration of meat can be reduced through different methods, i.e. the addition of synthetic or natural antioxidants. However the use of synthetic antioxidants like butylated hydroxyl toluene (BHT) or butylated hydroxyl anisole (BHA) to food (meat) products has been restricted because of possible health risks and toxicity (7). Thus, there is increased consumers’ interest in using natural products to preserve and improve meat, associated with the search of antioxidant, vitamins, minerals, fiber from plants and plant materials which are rich in bioactive compounds (e.g. with a high phenolic compounds content). Hydrocolloids could also be used in meat products to improve functional properties of meat in a way of salt reduction and freezing processing (8-9). Aside of antioxidants, plants also contain a range of other bioactive compounds with potential beneficial health effects (10-15). Plants rich in antioxidants include vegetables and fruits like olives, pomegranate, grapes, berries (16-21) in different forms, as well as herbs and spices like rosemary, oregano, broccoli, sage, black cumin, thyme or curcumin (22-24). Most of the plants possess relatively high nutrient contents (biological peptides, polyunsaturated fatty acids, calcium, iron or phosphorus) and have anti-microbial properties, which is overall very important for their human health benefits (25,26). It should be underlined that the concentration of antioxidant compounds in plant sources varies considerably and also their dosage and application form in diets and meat products is different between plants (27,28). High dosage may lead to unfavourable effects through pro-oxidative processes (29,30). Technological strategies involve the application of antioxidants directly into the meat and meat products or coating of packaging materials with plants in different form to improve the oxidative stability of the products. In this paper, we focus on the effect of selected plants and plant materials as natural antioxidants rich in bioactive molecules on quality and shelf life of beef and its meat products.
NATURAL ANTIOXIDANTS ADDITIVES

Additives obtained from fruits and vegetables

Pomegranate aryl's mesocarp contain a high concentration of antioxidants as compared to blueberry, grapes and green tea (31). The peel and seeds are the proper sources of especially tannins and anthocyanins (31,32). Hydrolysable tannins cover mainly bioactive molecules like ellagitannins, gallotannins and punicalgin, whereas anthocyanins are including delphinindin, cyaniding and pelargonidin (32). El-Nashi et al. (33) assessed the impact of pomegranate peels powder (1, 2 or 3 %) on stability of quality of beef sausage during storage at 4 °C for 12 days. The addition of various concentrations of pomegranate peels powder increased storage stability and reduced values of tert-butyl alcohol (TBA) of beef sausage during refrigerated storage and microbiological parameters. This could be the effect of phenolic compounds presented in pomegranate peels. Antioxidant effect of pomegranate peel extract (PE) to delay lipid and protein oxidation in beef meat balls was also investigated during frozen storage at -18 °C by Turgut et al. (34). It is interesting that addition of PE up to 1.0 % concentration into the meatballs limited the oxidation processes more than a synthetic antioxidant like BHT, probably due to the high content of polyphenols in PE (34,35). Also, the 1.0 % PE led to maintaining of colour intensity. The results from sensory analyses revealed that PE addition to meat balls was effective on preventing rancid odour formation. The effect of dose or concentration, storage temperature and time of exposure of the various natural antioxidants on lipid and protein oxidation in beef and beef products is shown in the Table 1 (35-48). The effect of skin wine pomace, rich in phenolics and stilbenes like resveratrol (49), on protein oxidation in beef patties during high oxygen atmosphere storage was studied by Garcia-Lomillo et al. (36). For gaining wider perspective, the ability of red (9.9 mg phenols) and white (4.0 mg phenols) skin grape wine pomaces were tested. The results showed that red skin grape pomace protected against protein radical formation, as opposed to white skin pomace, thus it could be added as an alternative of antioxidants, e.g. the sulfite used in meat products. In a later study the researchers (50) assessed the ability of wine pomace (used as seasonings in three different variants) to inhibit lipid oxidation in raw and cooked beef patties. All types of seasonings showed different effectiveness to inhibit lipid oxidation, thus the seedless red wine pomace was the most effective - significantly inhibiting the formation of volatile organic compounds (e.g. hexanal, 1-pentanol or 1-hexanol). This study also showed the potential capacity of wine pomace to delay the formation of rancid odors during storage - suggesting that it could be used as a natural alternative to extend the shelf-life of meat products.
Decreased lipid oxidation in precooked roast beef when treated with fresh plum juice concentrate and dried plum juice concentrate was reported by Nunez de Gonzalez et al. (37). The authors showed that the 5% addition of the fresh plum juice concentrate treatment induced the lowest level of TBARS (thiobarbituric acid reactive substances) to the 0.16 mg malonyl dialdehyde (MDA)/kg as compared to the control group (0.62 mg MDA/kg). There was no significant impact on colour or flavor from plum juice addition, which is important since differences noted in color of meat may lead consumers to believe that the product is not fully cooked or even is rotten. The shelf life and oxidative stability of refrigerated raw ground beef enriched with omega-3 fatty acids with grape seed extract used as inhibitor of lipid oxidation in ground beef were studied by Gómez et al. (51). Grape seed extract addition prevented rancidity in raw ground beef enriched with omega-3 fatty acids, decreasing the oxidation value with no differences in color and odor standards among the types of beef during demonstration. The results suggest that grape seed extract can be a technologically viable alternative for stabilizing the lipid oxidation in new fresh or functional meat products (50).

The impact of grape seed extract (GSE) on sensory characteristics and on inhibition of lipid oxidation and bacterial growth in raw minced beef during refrigerated storage at 4 °C for 10 days was studied by Amin et al. (38). The authors applied different levels of GSE and compared it with the synthetic antioxidant BHT. The results showed that GSE is efficient and its action is concentration-dependent. GSE treated minced beef samples showed significantly lower values of pH, TBARS and total bacterial counts (TBC) than the control samples and BHT samples during refrigerated storage. Grape seed extract confers proper protection against lipid oxidation and microbial spoilage, thus, it could be used as an alternative to synthetic antioxidants, e.g. BHT. In this line, the authors concluded that grape seed extract can be used as both natural antioxidant and antibacterial during refrigerated storage of meat. Also Banon et al. (39) evaluated the antioxidant and antimicrobial activities of grape seed extracts and green tea in raw beef patties. Their work showed that either green tea or grape seed extracts had preservative effects on beef patties, especially protecting against meat oxidation, and had also had positive effect on sensory properties.

DeJong et al. (52) assessed the antioxidant activity of extracts from olive oil in beef and compared results with green tea and red wine. It should be noted that main component of virgin olive oil and olive waste is hydroxytyrosol which is a type of phenolic phytochemical with strong antioxidant properties (53). Further, important nutrient of olive oil are free fatty acids, aliphatic alcohols, tocopherols, hydrocarbons, sterols, triterpenic compounds and pigments (54). This study showed that polyphenolic extracts from the olive oil
reduced the TBARS value in pre-cooked beef (63-83 %). When comparing this results with the other natural antioxidants, the highest antioxidant potential has tea, followed by olive oil, and the lowest antioxidant potential had red wine. Therefore, the olive oil extract has an excellent potential and significantly inhibits lipid oxidation in ground beef.

Aouidi et al. (41) investigated the antioxidative potential of olive leaves added to minced beef. The results showed that olive leaves have an ability to inhibit (p<0.05) lipid oxidation (TBARS values were reduced by 25-65 %) and myoglobin oxidation. It was also observed that olive leaves decreased the weight loss of minced beef after the defrosting process and during the storage. Addition of olive leaves has no impact on sensory properties. The authors concluded that the addition of olive leaves could enhance the stability of the beef products and extend storage time.

Hayes et al. (42) investigated the effect of olive leaves extract on the quality and shelf-life stability of packaged raw minced beef patties. The olive leaves extract reduced TBARS values in raw patties in packaging and it reduced significantly oxymyoglobin oxidation. Moreover, olive leaves extract increased antibacterial and antifungal activities against gram-positive bacteria (e.g. Helicobacter pylori, Staphylococcus aureus), Gram-negative bacteria ( e.g. Campylobacter jejuni) and fungi, as shown by Sudjana et al. (54).

The effects of garlic extracts on color, lipid oxidation, and oxidative breakdown products in raw ground beef during refrigerated storage was studied by Zhang et al. (43). Allicin is a thiosulfinate extract of garlic, and recognized as a very strong antioxidant, also exhibiting a range of other interesting bioactivities (56). Also, garlic is rich in selenium, allyl cysteine, and allyl disulfide. It was demonstrated that garlic extracts protected phospholipids, fatty acids and polypeptides from oxidation. Moreover, garlic extracts could stabilize the redness in raw ground beef during refrigerated storage due to antioxidant properties preventing the oxidation of oxymyoglobin. In next investigations, Zhang et al. (57) investigated the impact of mulberry leaves extracts (MLE) on the colour, lipid oxidation, antioxidant activity of enzymes on raw ground beef during storage. Mulberry leaves are rich in quercetin 3-(-malonylglucoside), rutin, and isoquercitin. It was observed that MLE can be used as natural antioxidant to maintain the meat quality, e.g. colour, to extend shelf-life and to prevent economic loss for food processing industry (58). Moreover, the results showed that it is better to combine the mulberry leaves extracts with vitamin E to maintain the quality of meat during storage in the greater scale.
Additives obtained from herbs and spices

The antioxidative activity of ground beef and pork meat homogenized with oregano and sage (3 % essential oils), during 12 days of storage, was determined by Fasseas et al. (44). Sage and rosemary include phenolic compounds and are rich in carnosic acid, carnosol and rosmarinic acid. Sage contains high amounts of compounds with diverse bioactivities, such as as flavonoids, diterpenoids triterpenes and steroids including rosmarinic acid or picein, 6-O-(E)-feruloyl-(α and β)-glucopyranoside (58,59). Before storage both types of ground meat samples were stored at 4 °C in raw for 30 min and cooked at 85 °C for 30 min. The results demonstrated that the sage and oregano significantly reduced the oxidation (44). The reported data demonstrated that oregano is more effective as antioxidant in both types of meat than the sage, as demonstrated by TBA assay. Moreover, much higher TBA values were noted for the cooked meat samples as compared to the raw ones. According to the authors, the higher antioxidants effect was observed in cooked meat than in raw and the meat proteins greatly affected the antioxidant activity. Oregano may be used as a natural antioxidant in meat products, but current research shows that, e.g. grape seed extract, cranberry, and sage extracts are more powerful natural antioxidants, that exhibit a more significant reduction in TBARS values in a variety of products (44).

Rosemary contains a very high amounts of antioxidants, including phenolic compounds (e.g. carnosic acid (60), carnosol and rosmarinic acid, caffeic acid, and flavonoids) which have been associated with the high antioxidant activity (61-64). The influence of rosemary extract in combination with modified atmosphere packaging on protein and lipid oxidation in beef patties during storage up to 6 days at 4 °C was investigated by Lund et al. (65). They demonstrated that in high oxygen atmospheres this antioxidant protected the natural red colour in beef.

In turn, Fernandez-Lopez et al. (66) studied the antioxidant and antibacterial effects of rosemary, lemon, and orange extracts in beef meatballs. The authors have shown the positive effect of addition of rosemary and citrus extract to beef meatballs without negative impact on the acceptability of the product. Sensory analysis especially aroma and acceptability indicated the significant advantages of application of rosemary and citrus extracts in beef products (67). Green tea extract is studied as a source of antioxidant and antimicrobial compounds that have the potential to improve the overall quality and extend the shelf life of beef. Supplementation of tea catechins at levels of 200-400 mg/kg can be used as inhibitor on lipid oxidation with significant effect on beef, reaching much greater effect than vitamin C (68). Moreover, tea catechins and vitamin C treatments were having important effect on lipid oxidation in cooked beef (0.32
mg MDA/kg of meat) compared to beef controls without these supplements (>1.1 mg MDA/kg meat).

According to the author of the study green tea extract could be a proper antioxidant solution for the meat producers, comparable to synthetic alternatives.

The influence of rosemary oil, pine bark extract, grape seed extract and butylated hydroxyanisole/butylated hydroxytoluene (BHA/BHT) on microbial growth, colour change, lipid oxidation were analyzed in ground beef by Ahn et al. (45). The study demonstrated that grape seed extract and pine bark extract, at 1.0 % levels, rapidly reduced the numbers of E. coli in the first 3 days of storage whereas 1.0% pine bark extract most effectively inhibited the increase of E. coli or L. monocytogenes after 9 days of storage, followed by the grape seed extract and rosemary extract (45). It should be stressed that, pine bark extract is a proper source of phenolic compounds like procyanidins or condensed tannins, which have been shown to possess antioxidant activity (68,69). Among the natural extracts used in this study, rosemary oil showed lower inhibitory effects against the pathogens than did grape seed extract and pine bark extract (45). Moreover, the retention of the red colour of cooked beef treated with pine bark and grape seed extracts was observed, and it was concluded that it may originate from their antioxidative effects and their contribution of pigments.

The effect of rosemary and oregano extracts, added individually or in combination and butylated hydroxyanisole/butylated hydroxytoluene (BHT/BHA), on lipid oxidation and fatty acid composition on frozen beef burgers was investigated by Trindade et al. (70). The results of the experiment showed that rosemary extract, applied alone and in combination with either BHT/BHA or oregano extract, was more effective in maintaining a low oxidation level in the samples compared to oregano extract used individually or in combination with BHT/BHA and the antioxidant capacity of natural extracts decreased and lipid oxidation increased with storage time (70). Among the natural additives studied, the highest antioxidant capacity was obtained with rosemary extract.

In turn, Rojas and Brewer (71) evaluated the potential of oregano essence due to oxidative stability of cooked and refrigerated beef. Oregano has antibiotic and antioxidative effects, along with other biological activities (57,72). The authors demonstrated that addition of 0.02 % oregano essence was effective at decreasing the lipid oxidation in the meat.

Jayathilakan et al. (46) compared the effect of natural antioxidants like cinnamon (cinnamaldehyde and eugenol in cinnamon oil were identified as the most active antibacterial components) and cloves with synthetic one like butylated hydroxyanisole (BHA) in beef ground meat samples purchased in local store, packed in polypropylene bags, cooked in a water bath under atmospheric pressure for 35 min and then
stored at 5 °C for 6 days. Clove is a source of eugenol which is reported to have antifungal activity (73), and also β-caryophyllene, α-humulene (2.1 %), and eugenyl acetate (74). The authors reported that either cinnamon or cloves had positive effect (p>0.05) manifested as decreasing of TBARS in cooked ground beef. No difference was observed (p>0.05) between samples treated with cinnamon as compared to (BHA) at 0.02 % in ground beef. It is interesting that cloves showed higher antioxidant potential (p > 0.05) than BHA. The authors stated that cloves had antioxidant potential similar to the synthetic antioxidant BHA and could be applied as a natural substitute to increase shelf life of beef products.

The combined effect of sage (0.3 and 0.6 %) and high pressure processing (HPP) on the antimicrobial and antioxidant quality of beef burgers, during prolonged storage (up to 60 days), was investigated by Mizi et al. (75). The results showed that lipid oxidation was higher in all samples during storage but sage powder can be recognized as an effective antioxidant, retarding lipid oxidation in HPP treated beef burgers over 60 days of chilled storage. Moreover, the microbial quality of the burgers was pointed as acceptable. The application of both treatments to receive burgers with better oxidative and microbiological stability due to prolonged storage without any effect on sensory attributes could be considered as alternative way.

Mansour et al. (47) evaluated the antioxidant activity of ginger, potato peels, fenugreek seeds extracts in ground beef patties. Ginger is rich in compounds as gingerols (e.g. 6-gingerdiol) and has antimicrobial and antioxidative potential, along with diverse additional bioactivities (76-78), whereas fenugreek seeds are rich in flavonoids, e.g. vitexin, tricin, naringenin, quercetin and tricin 7-O-β-D-glucopyranoside (79). In the referenced work (47), the authors showed that ginger rhizome extract exhibited the highest antioxidant activity and had an activity comparable to commercial antioxidants. Moreover, the extracts from ginger rhizomes and fenugreek seeds added to beef patties were more effective than potato peel extract in controlling lipid oxidation and color changes during the storage (47,79).

Falowo et al. (48) examined the effect of leaves extracts of *Moringa oleifera* and *Bidens pilosa* on lipid oxidation and pH value of fresh beef during the storage time. The results showed that application of both extracts can protects beef against oxidation during the storage. The *B. pilosa* extract showed higher antioxidant activity (at 0.5 and 1 g/kg) than the *M. oleifera*, and it is mainly pointed out because *B. pilosa* leaves contained higher amounts of bioactive substances (e.g. tetradecanoic acid or DL-alpha-tocopherol) and has higher antioxidant content (p<0.05). Furthermore, this study showed that both extracts are rich in phytochemicals with significant free radical scavenging activity (48).
Interesting research was also conducted by Van Hecke et al. (79), who assessed the antioxidant capacity of typical herbs e.g. rosemary and spices, like curcuma and garlic during cooking of beef product and during in vitro digestion. The additions to the meat product were made separately either before or after heating, as a seasoning. Addition of the herbs and spices before heating of the meat was more effective for reduction of oxidation during digestion, as compared to the after heating addition. Herbs and curcuma showed higher antioxidant activity compared to other investigated additives (74,79).

This study demonstrated the potential of commonly utilized herbs and spices to reduce the lipid oxidation in meat and meat products. A reduction of the lipid oxidation products formation in meat is considered to be beneficial for human health (79,80,81,83), while the prevention of creation of lipid oxidation products can cause reduction of the risk of developing civilization diseases related to a common diet rich in processed red meat (84,85).

CONCLUSIONS

Natural antioxidants, including variety of fruits, vegetables, herbs and spices added to meat and its products have multiple functions. They may play an anti-oxidative, anti-microbial and preservative role in beef and beef products during processing and storage, since plants and plant materials are rich in bioactive compounds, therefore can be used as source of natural, effective antioxidants for meat products. These compounds have the potential of inhibiting or delaying oxidation, which can be widely used by the food industry. Among culinary herbs and spices the best protection capacity of the beef and beef products was found when applying sage, rosemary, especially for oxidative and colour stability. In turn, grape seeds, olive oil and leaves, characterized by the high amount of polyphenols, also proved the similar positive antioxidant effect on beef and beef products during the storage. In light of this the mentioned antioxidants could be considered as a natural alternative to synthetic preservatives of the meat products.

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Table 1. Effect of dose/concentration, storage temperature, and time of exposure of the natural antioxidants on lipid and protein oxidation in beef and beef meat products.

<table>
<thead>
<tr>
<th>Item</th>
<th>Dosage</th>
<th>Meat</th>
<th>Storage temperature/ °C</th>
<th>t (storage/days)</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pomegranate peel extract</td>
<td>1 %</td>
<td>beef meat balls</td>
<td>4±1</td>
<td>0, 2, 4, 6, 8</td>
<td>effective prevention on rancid odour formation, noticeable antioxidant activity</td>
<td>(35)</td>
</tr>
<tr>
<td>wine pomace</td>
<td>2 g/100 g</td>
<td>refrigerated beef patties</td>
<td>4</td>
<td>0, 4, 8, 12,15</td>
<td>inhibition of lipid oxidation capacity to delay the formation of rancid odors during storage</td>
<td>(36)</td>
</tr>
<tr>
<td>plum juice concentrate</td>
<td>2.5 %, 5 %</td>
<td>roast beef</td>
<td>&lt;4</td>
<td>0, 2, 4, 6, 8, 10</td>
<td>lower level of TBARS reduction of lipid oxidation significantly lower values of pH, TBARS and TBC than those of control and BHT treated samples</td>
<td>(37)</td>
</tr>
<tr>
<td>grape seed extract</td>
<td>50 mg/kg</td>
<td>minced beef</td>
<td>4±1</td>
<td>0, 2, 4, 8, 10</td>
<td>enhance of shelf life inhibition of lipid oxidation</td>
<td>(38)</td>
</tr>
<tr>
<td>green tea extract</td>
<td>300 mg/100 g</td>
<td>raw beef patties</td>
<td>4</td>
<td>0, 3, 6, 9</td>
<td>lower microbial spoilage, extension of redness decreasing storage loss and defrosting loss, inhibition of lipid oxidation and myoglobin oxidation reduction of TBARS value in raw beef patties in packaging systems, reduction of oxymyoglobin oxidation protection of phospholipids, fatty acids and polypeptides from oxidation, stabilization the redness in raw meat extension of shelf-life, maintaining color, reduction of peroxide and thiobarbituric acid reactive substances values during storage</td>
<td>(39)</td>
</tr>
<tr>
<td>olive leaves extract and powder</td>
<td>100/150 µg/g</td>
<td>minced beef</td>
<td>4</td>
<td>0, 3, 6, 9</td>
<td>antioxidant effect/reduced the oxidation</td>
<td>(40)</td>
</tr>
<tr>
<td>olive leaves extracts</td>
<td>100 and 200 µg/g</td>
<td>minced beef patties</td>
<td>4</td>
<td>9, 12</td>
<td>antioxidant effect/reduced the oxidation</td>
<td>(41)</td>
</tr>
<tr>
<td>garlic extract</td>
<td>50 mg/100g</td>
<td>ground beef</td>
<td>4</td>
<td>1, 3, 5, 7, 9, 11, 13</td>
<td>antioxidant effect/reduced the oxidation</td>
<td>(42)</td>
</tr>
<tr>
<td>mulberry extract</td>
<td>0, 100, 500, 1000 µg/g</td>
<td>raw ground beef</td>
<td>4</td>
<td>0, 6 h, 12 h and 1, 2, 3, 4, 7, 10, 13</td>
<td>antioxidant effect/reduced the oxidation</td>
<td>(43)</td>
</tr>
<tr>
<td>oregano/ sage essential oils</td>
<td>3 %</td>
<td>raw beef</td>
<td>4</td>
<td>12</td>
<td>highly antioxidant and antimicrobial activity</td>
<td>(44)</td>
</tr>
<tr>
<td>pine bark, grape seed, rosemary extracts</td>
<td>1 %</td>
<td>cooked beef</td>
<td>4</td>
<td>3, 9</td>
<td>highly antioxidant and antimicrobial activity</td>
<td>(45)</td>
</tr>
<tr>
<td>Ingredient</td>
<td>Treatment</td>
<td>Food Type</td>
<td>Storage Time</td>
<td>Notes</td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cinnamon / cloves</td>
<td>250 mg/100 g</td>
<td>ground beef</td>
<td>5</td>
<td>showed higher antioxidant potential than BHA, positive effect on decreasing TBARS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ginger rhizomes / extract / fenugreek seeds</td>
<td>0.5 mg/1 g</td>
<td>ground beef patties</td>
<td>5, 25, 37</td>
<td>effective in retarding rancid odor, TBA, and color change, control of lipid oxidation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moringa oleifera / Bidens pilosa leaves extract</td>
<td>0.5 g and 1 g/kg</td>
<td>raw ground beef</td>
<td>4</td>
<td>protection against lipid oxidation during the storage, lower the pH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(46) cloves showed higher antioxidant potential than BHA, positive effect on decreasing TBARS.

(47) effective in retarding rancid odor, TBA, and color change, control of lipid oxidation.

(48) protection against lipid oxidation during the storage, lower the pH.