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MARINATION: EFFECT ON MEAT SAFETY AND HUMAN HEALTH. A REVIEW

D. VLAHOVA-VANGELOVA and S. DRAGOEV
University of Food Technologies, Department of Meat and Fish Technology, Technological Faculty, BG - 4002 Plovdiv, Bulgaria

Abstract

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Marinating is commonly used method which involves injection, tumbling or immersion to disperse in the muscle tissue solutions contained water, salt and other ingredients. There are several types of marinating according to added solution ingredients. While alkaline marinade solutions contain phosphates, the acid solutions are prepared with organic acids or their salts. Water-oil emulsions are the third type marinade solutions. The ingredients are used to enhance yield by increasing water content, to improve color, flavor and tenderness and to increase shelf-life in final product. The addition of synthetic or natural food additives may affect on lipid oxidation process. This review paper will discuss the influence of acid, alkaline or water-oil marination on the development of spoilage microflora, pathogens and conditionally pathogenic microorganisms. The advantages of marinating on lipid oxidation in raw meat and content of polycyclic aromatic hydrocarbons (PAH) and heterocyclic aromatic amines (HAA) in cooked marinated meat and human health will be described.

Key words: meat, spoilage micloflora, HAA, PAH, ingredients

Abbreviations: polycyclic aromatic hydrocarbons (PAH); heterocyclic aromatic amines (HAA); modified atmosphere package (MAP); total viable counts (TVC); sodium chloride (NaCl); Lactic acid bacteria (LAB); psychrotrophic bacteria count (PTC); 2-thiobarbituric acid reactive substances (TBARS); butyl hydroxyanisole (BHA).

Introduction

Marination is commonly used to improve the functional and sensory properties of meat by soaking, injecting or tumbling with an aqueous solutions composed of different ingredients (Latif, 2001).

While alkaline marinade solutions contained salt-phosphate mixture, acid solutions contain organic acids or their salts. The third type is water-oil emulsions contain salt, sugar, vinegar or citric acid and other supplements.

The addition of phosphates such us sodium tripolyphosphate increases water holding capacity due to protein extraction and shifting of the pH from the muscles proteins isoelectric point (Barbut, 2002). Sodium carbonate and bicarbonate are known to be superior marinating agents, which reduce drip loss and improved yield (Bertram et al., 2008). Citric acid, a food acidulante, is not only often used in acid marinating to improve the water-holding capacity and tenderness of beef muscle but is also commonly used as a chelator to control the activity of pro-oxidant metals (Ke, 2006). Lactic acid is often used in the meat industry as an antimicrobial agent (Hinkle, 2010). Marinade solutions may also include natural or dried ingredients, spices, herbs and other extracts (Miller, 1998). Many studies have reported antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic, cardioprotective, and vasodilatory effects (Benhammou, 2009). Spices and herbs, added in marinades significantly enhance meat safety and controlled or minimized lipid oxidation (Gutierrez et al., 2009).

All data, in the available literature show that various changes occur in marinated meat due to lipid oxidation and microbial growth.

Accordingly, this review paper explores different types of marination and their effects on meat safety and human health.

E-mail: desislava_vangelova@abv.bg; logos2000lt@gmail.com

Changes in the microflora during marination

The development of bacteria in non-marinated meat constantly increases during refrigerated storage (Knöchel et al., 2009). This microbial growth in non-marinated beef is about 0.9 log cfu/cm^2 in total viable counts (TVCs) after 24h and greater than 9.5 log cfu/cm^2 after 8 days of refrigerated storage (Kargitou et al., 2011). Marination inhibits microbial growth in beef and the development of bacteria remains below 10^3 cfu/g (Knöchel et al., 2009). It was suggested (Silva et al., 1999) that the growth of microorganisms depends on the meat pH after marinating. Knöchel et al. (2007) confirmed these suggestions proving that the rate of microbial growth in DFD beef was greater than the growth in normal meat.

Lactic acid bacteria (LAB) are the most common microorganisms in the MAP packed marinated poultry meat, if proper cold storage is applied (Schirmer and Langsrud, 2010). Other types of microorganisms such as *Lactobacillus algidus, Lactobacillus sakei, Leuconostoc mesenteroides, Leuconostoc carnosum, Carnobacterium maltaromaticum, Carnobacterium divergens, Brochothrix thermosphacta* and *Serratia proteamaculans* was isolated by Björkroth (2005). *Pseudomonas spp., Enterobacteriaceae, Staphilococcus aureus, Salnonella spp.* and other microorganisms causing spoilage in marinated poultry (Smaoui et al., 2011) and during the storage of MAP marinated poultry *Brochothrix thermosphacta* (Borch et al., 1996) may also be the possible microorganism causing spoilage in meat.

The different composition of marinade solutions has different effects on meat microflora and gram-negative bacteria are more sensitive to acid conditions than gram-positive bacteria (Choi et al., 2009). Marination with salt-phosphate solutions increases pH and possibly stimulates the spoilage microflora, with optimal growth in alkaline pH (Jeremiah and Gibson, 2001). In contrast, acidic marinade solutions decrease pH and suppress microbial growth. The reason for this inhibition is the presence of weak organic acids (acetic, lactic) or their salts (lactates, acetates) and NaCl (Yusop et al., 2001).

Low temperatures during cold storage limit the growth of some types of spoilage microorganisms (Watada and Qi, 1999). The growth of gram-negative bacteria significantly decreases under aerobic conditions (Nychas et al., 2008) but the absence of oxygen favours the growth of gram-positive bacteria such as lactic acid bacteria (Abadias et al., 2008) and *Brochothrix thermosphacta* (Pin et al., 2002).

The antimicrobial effect of some acidic marinade solutions containing alcoholic drinks is due to the presence of compounds such as ethanol and phenolic derivatives (Rodríguez Vaquero et al., 2007). The addition of dry red wine, lime-tree honey, spices, garlic, marjoram, thyme and horseradish when marinating beef decreases the microbial growth of mesophilic aerobic bacteria (Istrati, 2011). Thymol, a major active component in thyme and oregano, inhibits the growth of *Escherichia coli* and *Salmonella typhimurium* (Zhou, 2007).

According to Rodríguez Vaquero (2007) the antimicrobial activity of wine is due to the garlic and vanilla acid contained, and to the flavonoids rutin and quercitin. Control over spoilage microflora can also be achieved through marinade solutions containing soya sauce (Kargitou et al., 2011).

The antimicrobial activity of honey against pathogen growth in marinated meat was confirmed (Mundo et al., 2004). Similarly to honey, herbs and spices added to the marinade solutions suppress bacterial growth because of specific compounds such as polyphenols (Hara-Kudo et al., 2004), allicin in garlic and piperine in black pepper (Dorman and Deans, 2000) etc. Cinnamaldehyde from cinnamon inhibits the growth of foodborne pathogens (Shan et al., 2007). Rosemary extracts (Valero and Salmeron, 2003) and grapefruit seed extract (Xu et al., 2007) have been used to inhibit bacterial growth, too.

The antimicrobial activity of pine and spruce knot wood extracts has also been studied. According to the author, the inhibition of the spoilage microorganisms was due to the polyphenols contained in the extract (Ariwo-ola, 2011).

The combinations of acids with other antimicrobial components like thymol + citric acid, grape fruit seed extract + citric acid and ascorbic acid + allyl isothiocyanate shows synergistic effects on the growth of lactic acid bacteria (Schirmer and Langsrud, 2010).

Development of spoilage microflora causing bacterial spoilage

Lactic Acid Bacteria (LAB)

LAB was identified as the causes of spoilage in fresh meat, tomato-marinated poultry and MAP packed marinated meat (Björkroth, 2005). The isolated microorganisms included LAB dominated by *Leuconostoc, Lactobacillus sakei, Lactobacillus curvatus*, and some others, phenotypically similar to the heterofermentative *Lactobacillus* types. Excessive gas formation was observed in this experiment, the reason probably being the decarboxylation of amino acid residues, according to the author. From MAP marinated poultry, *L. gasicomitatum, Carnobacterium divergens, L. sakei*, *L. curvatus* was isolated and suggested that most often microbial spoilage was caused by *L. gasicomitatum* (Björkroth, 2005).

Through the addition of thyme, oregano and horseradish to the marinade solution (Björkroth, 2005) successfully inhibited the LAB growth in poultry.

Pseudomonas and psychrotrophic bacteria

The spoilage in aerobically stored marinated poultry is mainly caused by gram-negative psychrotrophic bacteria (Carlos and Harrison, 1999) *Pseudomonas* in particular.

The development of psychrotrophic bacteria and *Pseudomonas* depends on the substances contained in acid solutions. The addition of 1% lactic acid in the marinade solution decreases significantly the psychrotrophic bacteria count (PTC) and *Pseudomonas* (Smaoui et al., 2011). It has been suggested that the growth of *Pseudomonas* spp. is highly dependent on the presence of lactic acid bacteria (Gerez et al., 2009). The growth of H_2S synthesising bacteria, like *Pseudomonas spp.,* is largely inhibited by the presence of lactic acid - a product from the *LAB* (Gonzalez-Fandos et al., 2009). As a result, the amount of released hydrogen sulphide and other sulphur containing compounds decreases and storage time increases.

The addition of 1 and 3% sodium lactate to poultry leads to a reduction of 0.2-log and 0.4-log respectively in the psychrotrophic bacteria count (PTC) (Smaoui et al., 2011).

The antibacterial effect of a marinade solution with 1% acetic acid can be increased by the addition of 1 unit of LPS (Lactoperoxidase system: 1μg / ml LP, 5.9 KSCN ml and 2.5 ml H_2O_2) and contribute to a significant decrease in total microbial counts and psychrotrophs (Tan and Ockerman, 2006).

Some spices and their extracts exhibit antimicrobial activity against *Pseudomonas.* For instance, 0.5% pimento leaf oil or 0.5% clove oleoresin added to the marinade solution inhibits *Pseudomonas* growth (Carlos and Harrison, 1999).

There are evidences that 0.2 % pimento leaf oil was also effective against yeast although yeast was not among the major causes of meat spoilage.

Influence of marination on pathogens and conditionally pathogenic microorganisms

It was considered that gram-negative bacteria are more sensitive to acid conditions than gram-negative bacteria (Choi et al., 2009). Therefore *L. monocytogenes* survived longer in acidic conditions than *E. coli O157: H7* or *S. enterica* (Lin et al., 2000) and were more resistant than *Salmonella* strains at low pH (Tan and Ockerman, 2006).

Salmonella

The use of sodium lactate in acid marination of poultry significantly inhibits *Salmonella* development (Smaoui et al., 2011). The strong antimicrobial potential against *Salmonella* of the teriyaki sauce (soya, rice wine, sugar and spices) added to a marinade solution was also examined (Pathaniaet al., 2010). To inhibit the growth of *Salmonella*, the combination of nisin with EDTA, sodium lactate or potassium sorbate was used (Ukuku and Fett, 2004). According to the results obtained, only the combinations were effective, whereas nisin used on its own did not show antimicrobial activity.

Listeria monocytogenes

At pH different from the ultimate value, the growth and development of *L. monocytogenes* in acidic conditions was significantly delayed and reached a stationary phase (Buchanan et al., 1993). Greater inhibition in *L. monocytogenes* growth was reached with the use of weak organic acids compared to strong hydrochloric acid (Vasseur et al., 1999). In this aspect, acetic and lactic acids proved to be more effective against *L. monocytogenes*, while critic acids had the weakest effect (Alvarado and McKee, 2007).

Escherichia coli

The combination of organic acid (having antimicrobial action) and sodium chloride in marinade solutions may effectively inactivate *E. coli O157: H7* in beef (Mukherjee et al., 2008). Maintaining at low pH during 24h ageing, and significant inhibition of the microbial growth of *E. coli O157:H7* were achieved when the meat were marinated by a solution containing 0.2 % citric and 0.3 % acetic acid was used.

Other ingredient of the marinade solutions that displayed antibacterial action against *Escherichia coli* was sodium lactate (Lee et al., 2007).

The growth of *Listeria monocytogenes, Salmonella typhimurium* and *Escherichia coli O157:H7 was* inhibited using supercritical carbon dioxide (SC-CO_2) in marinade solutions with soya sauce and hot-pepper paste (Choi et al., 2009).

Data published in available literature demonstrate the antimicrobial effect of other spices on the microbial growth of *E. coli* and that marinating in garlic juice could decrease total bacteria and total coli forms (Nurwantoro et al., 2011). The antimicrobial effect of isothiocyanate and allyl isothiocyanate from horseradish on the development of *Escherichia coli, Staphilococcus aureus* and *Vibrato parahaemolyticus* has also been discussed (Istrati, 2011).

Enterobacteriaceae

To suppress the growth of *Enterobacteriaceae*, marinade solutions containing lactic acid or sodium lactate was used (Smaoui et al., 2011). The authors proved that 1% lactic acid was a stronger inhibitor (1.31 log cfu/g), than 3% sodium lactate (1.74 log cfu/g).

Staphylococcus aureus

Only some *S. aureus* strains produced enterotoxin and could cause food poisoning (HPA, 2009). *S. aureus* growth was slower than that of other microorganisms and never exceeded the limit of 4 log cfu / g in non-marinated meat (Smaoui et al., 2011). The addition of lactic acid to poultry marinade solutions significantly inhibited the growth of *Staphylococ-*

cus aureus until the 15th day of storage. Similar results were achieved when using sodium lactate.

It has been suggested (Koutsoumanis et al., 2006) that the inhibiting effect of lactic acid on Staphylococcus aureus is mainly due to the low pH.

Campylobacter

The hypothesis that marinating increases meat safety is accepted by the scientific community. Surprisingly, Perko-Mäkelä (2000) did not discover any difference in the growth of *Campylobacter jejuni* in marinated and non-marinated meat. Their results demonstrated that the marinating could not be an effective method for suppressing the development of enteric pathogens. As a probable reason, Björkroth (2005) pointed at the buffering capacity of meat causing fast pH neutralisation during acid marination. According to the same author, changes in pH to a neutral level probably led to dissociation of lipophilic acids and their antimicrobial effect decreased quickly.

Clostridium perfringens

Clostridium perfringens is a food pathogen causing 248 000 food poisonings annually in the US (Sánchez-Plata et al., 2005). The *C. perfringens* spores are widely spread in the soil and water and the possibility for *post-mortem* contamination exists. *C. perfringens* may also be found in processed food after inappropriate thermal processing. The sodium salts of organic acids used in marinade solutions reduced the risk of *Clostridium perfringens.*

Inhibition of lipid oxidation during marination

Natural antioxidants from thyme, oregano or wine are used for inhibiting lipid oxidation in marinated meat. The similar antioxidants decrease peroxide value (POV) in marinade solutions (Istrati, 2011). The researchers explain this fact with the rich content of phenolic compounds. There is plenty of evidence for antioxidative effect of the honey components (Mundo et al., 2004). The key role of sulphureted organic compounds and their precursors (allicin, diallyl sulphide and diallyl trisulphide) in garlic antioxidant activity is studied (Kumar and Berwal, 1998). Horseradish contains isothiocyanate and allyl isothiocyanate which inhibits lipid oxidation (Istrati, 2011).

Marinade solutions rich in phenolic compounds suppress the secondary products of lipid oxidation and keep 2-thiobarbituric acid reactive substances (TBARS) almost unchanged during storage (Gray and Pearson, 1987). In meat marinated with wine, honey, garlic, black pepper and salt, the TBARS value reached after 14-day storage is low. Thyme, oregano and horseradish have high antioxidant activity and decrease the formation of TBARS. After red meat marinating with red wine, a 20% decrease in the formed conjugated dienes after cooking was reached (Blackhurst et al., 2011).

The addition of berry powders to marinade solutions with citric acid slowed down the linoleic acid oxidation. The authors discovered that berries were rich in polyphenolic antioxidants, with activity increasing in the following order: lingonberry < sea buckthorn < black chokeberry < bilberry < black currant (Püssa et al., 2008).

Meat food is the major source of cholesterol. There is a theory that some cholesterol oxides are atherogenic agents and they have mutagenic, carcinogenic and cytotoxic effect. Cholesterol oxides can replace cholesterol molecules in the membranes causing imbalance in permeability, stability and other properties (Shozen et al., 1995). Cholesterol oxidation products also cause cancer and coronary heart disease. After examination of seven cholesterol oxidation products: 7α-hydroxycholesterol, 7β-hydroxycholesterol, 5,6α-epoxycholesterol, 5,6β-epoxycholesterol, 5α-cholestane-3β, 5,6β-triol, 5-cholesten-3β-25-diol, and 7-ketocholesterol, was discovered (Lee et al., 2007) that marination with soya sauce and sugar reduced their formation. The addition of vitamin C, B, butyl hydroxyanisole (BHA) and trolox to marinade solutions decreased the amount of cholesterol oxidation products (Lee et al., 2007). Vitamin C was most effective for eggs; for pork BHA and trolox have the greatest effect. The inhibition effect of vitamins C and B, BHA and trolox increases with the increase in these substances in the marinade solution. In contrast, vitamin C and E are most effective at low concentrations, which is probably due to their pro-oxidant activity. In meat marinated with garlic/onion, the TBARS value is lower compared to non-marinated meat (Kim et al., 2010).

Reduction in the content of polycyclic aromatic hydrocarbons and heterocyclic aromatic amines in roasted marinated meat

Polycyclic aromatic hydrocarbons (PAH)

PAH are potentially carcinogenic compounds with chemical structure of condensed benzene rings without heteroatoms (Kuhnle and Bingham, 2010). They are formed during incomplete combustion and are present in most foods.

After marinating beef with acidic marinade solution containing 1.2% lemon juice and a basic solution of sugar, water, onions, turmeric and cinnamon, (Farhadian et al., 2012) found out that PAH formation decreased up to 70%. The activity of the marinade solution in relation to PAH decreased in the following sequence: basic + lemon juice > basic + oil+ lemon juice > basic + oil. The same study proved that the marinating time did not influence the quantity of the PAH formed.

Heterocyclic aromatic amines (HAA)

In 1977 the presence of HAA in the culinary processed meat products was proved and more than 20 HAA have been detected so far (Sugimura et al., 2004).

HAA are formed in the presence of creatine, free amino acids formed during meat ageing, and sugar which is often used in marinating mixtures (Emamgholizadeh, 2008). These substances react with each other during heating according to the Maillard reaction and form free radicals. However, the excess of glucose and lactose restores the amount of creatine, and as a result less creatinine is formed and mutagenic activity decreases.

In roasted meat, mutagen concentration can be 10 times as high as that in boiled meat (Emamgholizadeh, 2008).

In the 90s, Edenharder et al. found that the chlorophyll in green beans, broccoli and spinach significantly decreased the IQ and MeIQx mutagen effect. The intermediate products in HAA formation were eliminated and stabilised with the addition of BHA, sesamol or epigallocatechin gallate (Kikugawa et al., 1999). The reduction in HAA formation was explained with the stabilising effect of the added antioxidants on the intermediate products from the Maillard reaction and creatinine (Verdin, 2002). Marinade solutions containing soya sauce, sesame oil, sugar, garlic and onion powder decreased mutagenicity in roasted beef (Emamgholizadeh, 2008). Green tea catechins, epigallocatechin gallate, luteolin, quercetin and caffeic acid had an inhibitory effect on MeIQx and PhIP formation (Guo et al., 1995). With the addition of 1.0% grape seed extract and pine bark extract, a decrease in the norharmane content by 60% - 100% was achieved (Dashwood, 2002). Phenolic antioxidants contained in rosemary and thyme effectively inhibited the formation of IQ type HAA (Murkovic et al., 1998). Marinade solutions with basil are rich in eugenol and marinade solutions with allspice are rich in eugenol and methyl eugenol – effective polyphenolic antioxidants. The addition of herbs like rosemary, thyme, salvia and garlic successfully decreased the HAA formation below 60% (Murkovic et al., 1998). Sprinkling the meat with 1% red pepper decreased HAA formation by 75 - 100% (Oz and Kaya, 2011). Marinade solution containing sugar, olive oil, vinegar, garlic, mustard, lemon juice and salt, and decreased the HAA content by 92% (Salmon et al., 1996).

Conclusion

Results of the present study indicate that type of marinating impact differently on the meat safety.

After marinating, the growth of microorganisms depends on the meat pH. Gram-negative bacteria are more sensitive to acid conditions than gram-negative bacteria. Therefore, while salt-phosphate solutions increases pH and possibly stimulates the spoilage microflora, acidic marinade solutions decrease pH and suppress microbial growth. This antimicrobial effect is due to the present of weak organic acids. On the other hand, the addition of some spices, herbs or their extracts, wine, honey, soya souse suppress microbial growth too, because of specific compounds such as polyphenols, ethanol and phenolic derivatives, garlic and vanilla or flavonoids like rutin and quercetin.

Marinade solutions, rich in phenolic compounds decrease POV, suppress the secondary products of lipid oxidation and keep TBARS almost unchanged during storage. Red wine decreases with 20% the formed conjugated dienes after cooking.

The presence of weak organic acids (lemon juice for example) decreased PAH formation in meat up to 70 % and the additions of polyphenolic antioxidants, inhibit HAA formation up to 75-100% and this is positive effect of marinating on human health.

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