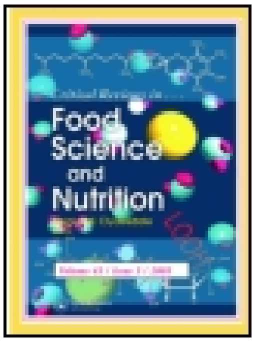

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REVIEW

Bactericidal effect of marinades on meats against different pathogens: a review

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ABSTRACT

Marinades are seasoned liquids used to improve tenderness, palatability, flavor, color and/or texture of different meats. In addition to contribute to the sensory characteristics, marinates can inactivate food microorganism as well. The purpose of this study was to assess the current state of knowledge regarding the effect of marinades on meats and important food pathogens. Using a systematic review of literature, different types of marinades were evaluated, identifying its ingredients, concentrations, temperature, marinating time and their effect on *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter* and *Vibrio*. Findings demonstrated that the use of marinades on meats not only prevents the growth of pathogens but also inactivates food pathogens. Most marinades were able to reduce < 3 log CFU/g of pathogens, and *Vibrio* populations demonstrated the highest reductions (> 4 log CFU/g). The pH was the most pronounced parameter influencing the pathogens inactivation, however, ingredients and storage temperature also affected pathogen reduction in marinades.

KEYWORDS
Food processing; gastronomy; marinating; marination

Introduction

Marinade is a seasoned liquid used to improve tenderness, palatability, flavor, color and/or texture of different meats, like beef, chicken, pork, fish and seafood. Marination or marinating is the process of incorporating or immersing meats in the marinade which can be a cooked or uncooked seasoned liquid (Lunde et al. 2008).

Originally, marinades were just water added of salt, sometimes sea water was used, which collaborate to preserve the meats and imparted flavor through the salt (Aitken 2013). Apparently, was sea water which led to the expression marinade, which originates from the Latin word "*mare*", meaning sea. However, some sources contest that the word may not have derived directly from the sea water association but via the Italian word *marinare* or the French word *mariner*. The *mariner* means "to pickle," and the *marinare* means "to marinate". Both these words refer to correspondent practices, whose purpose was to preserve or pickle food (Aitken 2013).

Over the years more ingredients have been added into marinades, and gradually, the picking or preserving process was changed to a flavoring and tenderizing process (Aitken 2013). Currently, the marinades usually contain water, salt, seasoning, spices, plant-derived additives, sugar, antimicrobial agents, aroma enhancers and acids such as vinegar, wine, lemon or lime juice, (Yusop et al. 2010). In general, the use of these ingredients in marinade formulation is important to achieve the sensory characteristics (texture and flavor) resulting in desirable final products (Birk et al. 2010). For example, the use of acid helps to break down the connective tissue of meats, contributing to tendering, while the use of seasoning and spices contribute to the flavor.

Beyond its contribution to the sensory characteristics, some acids, spices and seasoning can also have a significant impact on enzymes, growth, inhibition or even inactivation of spoilage and pathogenic microorganisms of meats. These effects can be attributed to compounds such as ethanol, organic acids, polyphenols and antimicrobial agents (Lunde et al. 2008; Vaquero et al. 2007). However, the bacteria behavior in marinades will depend on several factors such as the type of the acid, acid concentration in undissociated form, temperature, marinating and storage time, initial pathogen population, evolution of indigenous microbiota, food matrix etc. (Lytou et al. 2019).

Meat and meat products have traditionally been associated with foodborne diseases. In the United States, in 2017, the most outbreak associated illnesses were from turkey (609 illnesses), chicken (487) and pork (376), and the categories most commonly implicated were mollusks (41 outbreaks, 19%), fish (37, 17%), chicken (23, 11%), and beef (19, 9%) (CDC 2017). In Europe Union, *Salmonella* in meat and meat products was one the highest risk agent/food pairs, and Campylobacteriosis was the commonest reported zoonosis representing almost 70% of all the reported cases and with occurrence in fresh meats ranging from 37.4% to 31.5% in broilers and turkeys, respectively (EFSA 2018). In Brazil, beef, poultry, fish and pork represent 5.3%, 3.5%, 2.1% and

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Table 1. The main pathogens in meat products.

Chicken	Beef	Pork	Seafood
Salmonella	*Escherichia coli*	*Campylobacter*	*Vibrio*
Campylobacter		*Salmonella*	*Listeria monocytogenes*

2.0% respectively, totaling 12.9% of DTA outbreaks in the country (Brazil 2019).

Currently, due to the proprieties conferred to meats, marinades are not used only in homes but in industries and foodservices, as well. In homemade use, meats are simply immersed in marinades, allowing the passive penetration of components. In food industries and food services other marination techniques such as injection and vacuum tumbling, have been used (Birk and Knøchel 2009). After marination, meats can be packaged and stored, middle or well-cooked and even ready-to-eat with no heat treatment, which represents a high-value gastronomy product (Fuentes et al. 2010).

The present review investigates scientific literature regarding different meats submitted to marinades. The review also evaluates the different types of marinades, including ingredients, concentration, temperature and marinating time, as well as the effect on bacterial reduction of some of the most important food pathogens.

Methodology

The systematic literature review approach has been adopted to explore the literature on behavior of the main food pathogens in meat marinades (Table 1). The sites used are PubMed and Web of Science and no date restrictions were applied. A search was carried out in April 2020 and used the terms “marinade” OR “marination” OR “marinated” OR “marinating” AND “*Salmonella*” OR “*Escherichia coli*” OR “*Listeria*” OR “*Campylobacter*” OR “*Vibrio*”. All articles found were checked for duplicates using the software Mendeley. The search focused on the inactivation *of Salmonella, E. coli, Listeria monocytogenes, Campylobacter* and *Vibrio* on artificially contaminated food matrices exposed to marinades. Articles were collected and included when they were published in English, Spanish or Portuguese, and relevant search terms appeared in the title, abstract, or key words. Publications were excluded if they were review or book chapters; had incomplete information on the content of marinade; did not use a food matrix or used natural contamination (Figure 1).

Results

Salmonella

The effect of marinades in food contaminated with *Salmonella spp.* was investigated in 16 studies. The majority of studies have used the beef as food matrix (62.5%), followed by chicken (25%) and seafood (12.5%). The studies demonstrated results ranging from no reduction to 4.4 log CFU/g reductions of *Salmonella.*

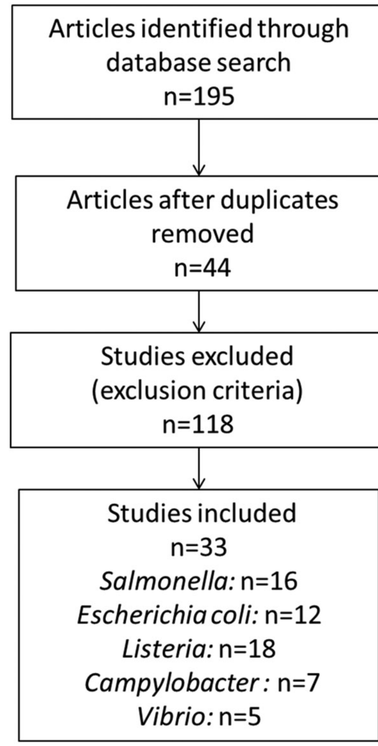

Figure 1. A flow chart of the exclusion process for the literature review.

Approximately half of the studies presented reductions lower than 3 log CFU/g and two of them presented no significant *Salmonella* reduction. Jofré et al. (2009) investigated the effect of sodium chloride, sodium tri-polyphosphate, sodium ascorbate and sodium nitrite marinating on beef loin inoculated with 3 log CFU/g of *Salmonella enterica* for 24 h at 4 °C. Marinated beefs presented a pH 6.3 and no significant reduction was observed ($p > 0.05$). Similarly, Moon et al. (2017) studied the bactericidal effect of teriyaki sauce marinade (soy sauce, wine, high fructose corn sirup, water, vinegar, salt, spices, onion powder, and garlic powder) on beef slices (1:1 w/v) inoculated with 3.5 log CFU/g of *S.* Typhimurium. The marinade occurred during 7 days at 4 °C and presented a pH 5.1. Teriyaki sauce did not significantly reduce *Salmonella* ($\alpha = 0.05$). However, in the study of (Pathania et al. (2010), teriyaki marinade (pH 4) was able to reduce 1.5 log CFU/g of *Salmonella* inoculated on chicken skin processed for 32 h at 4 °C or 25 °C.

Lower reductions (< 0.5 log CFU/strip) were obtained when marination was used in beef jerky process. Marinade containing spice blend (Lazy Jims Jerky Spice; York Springs, PA) with sterile water in contact with beef (proportion 1:0.65 – pH 6.6) for 24 h at 4 °C reduced only 0.28 log CFU/strip (Scheinberg, Svoboda, and Cutter 2014). Spice marinade (pH 5.3) used at 15% (w/w: 9.7% water and 5.3% dry ingredients) for 24 h at 5 °C resulted in approximately 0.4 log CFU/strip *Salmonella* reduction (Buege, Searls, and Ingham 2006). Similarly, marinade containing soy sauce, worcestershire sauce, pepper, garlic powder, onion powder and hickory-smoked salt (1:0.1 w/v beef-marinade, pH 4.5) in contact for 24 h at 4 °C reduced 0.51 log CFU/strip (Harrison et al. 2001). The use of spice marination was also investigated by Pathania et al. (2010), who demonstrated a

Salmonella reduction of 0.9 log CFU/g after the chicken skin (8 log CFU/g) was immersed in marinade at 4 °C or 25 °C for 16 hours.

Reductions of approximately 2 log CFU/g were obtained when fruit juices were added in marinades. Mathur and Schaffner (2013) demonstrated this reduction when lime juice marination (pH 2.5) on Tilapia fillet pieces inoculated with 7 log CFU/g of *Salmonella* was maintained for 120 min at 25 °C and 4 °C. Ozturk and Sengun (2019) also demonstrated approximately 2 log CFU/g reduction when marinade was prepared with koruk juice (50% koruk juice plus 50% water, pH 3.2) and used on meat samples inoculated with 6 log CFU/g of *S.* Typhimurium and marinated at 4 °C for 48 h.

The effect of marinades containing soy sauce and wine on *Salmonella* reduction were also evaluated. Rhoades et al. (2013) tested different marinades: soy sauce base marinade without or with lactic acid and red wine base marinade without or with 0.5% v/v oregano essential oil on beef pieces inoculated with 6.5 log CFU/cm^2. After 18 h at 5 °C, soy sauce marinade base without lactic acid (pH 5.0) and red wine marinade without oregano essential oil (pH 5.0) were able to reduce 0.6 log CFU/cm^2, while soy sauce marinade base with lactic acid (pH 4.5) and red wine marinade with oregano essential oil (pH 5.0) reduced 2 log CFU/cm^2. However, in others studies pure red wine marinade (12.5% vol. alcohol, pH 4.8) was able to reduced 2 log CFU/g of *S.* Typhimurium in fresh beef after 96 h of marination (Nisiotou et al. 2013), and pure soy sauce marinade reduced 2.8 log CFU/g of *Salmonella* in raw crab after 28 days at 5 °C (Cho et al. 2016).

Largest reductions ranging from 3.5 log CFU/g to 4.4 log CFU/g were demonstrated by other authors. For example, Moon et al. (2017) tested teriyaki sauce marinade (soy sauce, wine, high fructose corn sirup, water, vinegar, salt, spices, onion powder, and garlic powder) in combination with carvacrol or thymol (0.5%) with pH 5.1 on meat for 7 days at 4 °C and reduced 3.5 log CFU/g of *Salmonella*. Fisher et al. (2016) tested a solution made with chitosan (5%), lauric arginate ester (4%) and organic acids (2% acetic, lactic and levulinic acid) on raw beef (1:0.8 v/w – pH 3.0) for 6 hours and reduced 3.5 log CFU/cm^2 of *Salmonella*. Sengun, Goztepe, and Ozturk (2019) tested pure koruk juice for 18 h at 4 °C on poultry meat and Nisiotou et al. (2013) tested red wine (12.5% vol. alcohol) supplemented with 0.3% thyme essential oil (pH 4.8) on beef after 96 h resulting in reductions of approximately 3.5 log CFU/g of *Salmonella*. Reductions above 4 log CFU/g were demonstrated by Yang et al. (2013) who investigated the efficacy of lemon juice or citric acid in reducing numbers of *S.* Enteritidis in the preparation kelaguen (beef) during marination process. Beef inoculated with 6 log CFU/g was marinated with lemon juice at 4 °C or citric acid (7.2%, pH 2.6) at 24 °C. Marinating beef at 4 °C and a ratio of lemon juice to beef of 5:1 (pH 2.75) required 6.8 h to achieve a 4 log reduction while citric acid required 3.6 h. Similarly, Cho et al. (2016) examined the survival of *S.* Typhimurium in raw crab marinated in soy sauce (salinity 15.6% and pH 4.6). After 14 days at 22 °C reductions of 4.4 log CFU/g were observed.

Escherichia coli

A total of twelve studies were focused on the effect of marinades on food contaminated with *Escherichia coli*. The majority of studies have used the beef as the food matrix (83.4%). Works demonstrated results ranging from no reduction to 4 log CFU/g reduction of *E. coli*.

Most of the researches (58%) presented reductions lower than 1.5 log CFU/g. Yoon et al. (2011) evaluated the effect of different marinades containing acetic acid, NaCl, calcium chloride, potassium lactate, flavoring agent and sodium tripolyphosphate (pH ∼5.5) on the inactivation of *E. coli* O157:H7 in a lean ground beef. The results showed that overnight acidified marination at 4 °C did not lead to significant reductions ($p > 0.05$) of *E. coli* O157:H7 populations in raw ground beef. Jofré et al. (2009) got the same reductions when investigated the effect of marinating for 24 h at 4 °C containing water, sodium chloride, sodium tri-polyphosphate, sodium ascorbate and sodium nitrite on beef loin inoculated with 3.8 log CFU/g of *E. coli*. Marinated beefs presented a pH of 6.3. Similar reductions were demonstrated by Buege, Searls, and Ingham (2006) who studied the survival of *E. coli* O157:H7 on beef strips marinated with spice marinade (pH 5.3) applied at 15% (w/w: 9.7% water and 5.3% dry ingredients) for 24 h at 5 °C. The researchers concluded that this marination step contributed little to lethality, resulting in approximately 0.3 log CFU/strip reductions.

Other four studies tested a typical marinade in the production of beef jerky. Yoon et al. (2005) evaluated beefs contaminated with *E. coli* (4 log CFU/cm^2 and 7 log CFU/cm^2) exposed to a traditional marinade (soy sauce, Worcestershire sauce, black pepper, garlic powder, onion powder and old hickory- smoked salt) for 24 h at 4 °C, or exposed to a 5% acetic acid solution (pH 2.5 for 10 min) followed by traditional marinating for 24 h at 4 °C. After the marination, counts of *E.coli were* reduced approximately 0.2 log CFU/cm^2 when submitted to a traditional marinade (beefs presented a pH of 5.3) and reduced approximately 1.5 log cfu/cm^2 when acetic acid solution + traditional marinade was used (beefs presented a pH of 4.2), regardless the initial contamination. Harrison et al. (2001) examined the survival of *E. coli* O157:H7 in beef loin marinated in a solution containing soy sauce, worcestershire sauce, pepper, garlic powder, onion powder and hickory-smoked salt (1:0.1 w/v beef-marinade, pH 4.5). After marinating for 24 h at 4 °C, *E. coli* counts reduce only 0.5 log CFU/strip. Similar results were obtained by Scheinberg, Svoboda, and Cutter (2014) who evaluated the reduction of *E. coli* O157:H7 in beef jerky after marination. Marinade was prepared with sterile water and a spice blend (Lazy Jims Jerky Spice; York Springs, PA), in contact with beef (1:0.65 – pH 6.60) for 24 h at 4 °C and results demonstrated reduction of only 0.82 log CFU/strip. The reduction of *E. coli* O157:H7 after the marination process during beef jerky processing also was studied by

Calicioglu et al. (2002). Acid-adapted and non-acid-adapted *E. coli* O157:H7 were used. Different marinades were tested: (i) traditional marinade (soy sauce, Worcestershire sauce, black pepper, garlic powder, onion powder, hickory-smoked salt, sodium-L-lactate and acetic acid (pH 4.3); (ii) double-strength traditional marinade and modified (pH 3); (iii) dipping into 5% acetic acid for 10 min followed by application of traditional marinade (pH 2.5); and (iv) dipping into 1% Tween 20 (pH 6.6) for 15 min and then into 5% acetic acid for 10 min followed by traditional marinade. Beefs were inoculated with 6.5 log CFU/cm^2 and marinated for 24 h at 4 °C. Results indicated that bacterial populations decreased approximately 0.1, 0.5, 1 and 1.1 log CFU/cm^2, regardless it was acid-adapted or non-acid-adapted.

Reductions ranging from 3 to 4 log CFU/g were found by other researchers. Two of them with soy sauce marinade. Cho et al. (2016) investigated the survival of *E. coli* O157:H7 in raw crab marinated in soy sauce (salinity 15.6% and pH 4.6). Inoculated crabs (4.4 log CFU/g) were immersed in soy sauce and then stored at refrigeration (5 °C) or room temperature (22 °C) for up to 28 days. At 5 °C, after 28 days, *E. coli* reduced 3 log CFU/g, while at 22 °C *E. coli* was completely reduced in 14 days. Moon et al. (2017) verified the bactericidal effect of teriyaki sauce marinade (soy sauce, wine, high fructose corn sirup, water, vinegar, salt, spices, onion powder, and garlic powder) alone or in combination with carvacrol or thymol (0.3-0.5%). Beef slices inoculated with *E. coli* O157:H7 (3.5 log CFU/g) were marinated (1:1 w/v) for 7 days at 4 °C and presented a pH 5.1. Teriyaki sauce alone did not reduce *E. coli*, while marinade with 0.5% of carvacrol or thymol containing teriyaki sauce inactivated all bacteria without recovery within 7 days.

Greater reductions were also verified when fruit juices and acids were used in marinade. Yang et al. (2013) investigated the efficacy of lemon juice or citric acid in reducing numbers of *E. coli* O157:H7 in the preparation kelaguen (beef) during marination. Beef inoculated with 6 log CFU/g was marinated with lemon juice at 4 °C or citric acid (7.2%, pH 2.6) at 24 °C. Marinating beef at 4 °C and a ratio of lemon juice to beef of 5:1 (pH 2.7) required 22 hours to achieve a 4 log CFU/g reduction while citric acid required 4 h. Similar results were found by Ozturk and Sengun (2019) who studied the effects of marination prepared with koruk juice in reducing *E. coli* O157:H7 on meat samples. Meat was inoculated with two different levels (≅3 log CFU/g and ≅6 log CFU/g) of *E. coli* O157:H7 and marinating at 4 °C for different times (2 h, 24 h and 48 h) in liquids containing koruk juice (25% and 50%) or dried koruk pomace (1% and 2%) with or without ingredients (1% salt and 0.1% thyme). The most effective treatment in reducing the counts of *E. coli* O157:H7 was achieved by marination with 50% koruk juice plus 50% water for 48 h (pH 4.1), reaching 3.4 log CFU/g of reduction. Fisher et al. (2016) evaluated the efficacy of a solution made with chitosan (5%), lauric arginate ester (4%) and organic acids (2% acetic, lactic and levulinic acid) on *E. coli* O157:H7 to be used as a marinade for raw beef (1:0.8 v/w – pH 3.0). Fresh beef top round steaks were inoculated with 4.5 log CFU/cm^2. After 6 h, the marination reduced *E. coli* to levels below the limit of detection (< 1 log CFU/cm^2), resulting in a 3.5 log CFU/cm^2 reduction.

Listeria monocytogenes

Eighteen studies were found which investigated the effect of marinades on food contaminated with *Listeria monocytogenes*. The majority of studies have used the beef as food matrix (55.6%), followed by seafood (27.8%), chicken (11.1%) and pork (5.5%). Works demonstrated results ranging from no reduction to 6 log CFU/g reduction of *L. monocytogenes*.

Most of the studies (61%) presented reductions lower than 3 log UFC/g and three of them presented no significant inhibition of *L. monocytogenes* ($p > 0.05$). Carlos and Harrison (1999) studied the inhibition of *L. monocytogenes* in marinated chicken by pimento leaf oil and clove oleoresin. The marinade contained 5.0% sodium tripolyphosphate, 2.5% sodium chloride in water, clove oleoresin and pimento leaf (0%, 0.2%, and 0.5% levels) (pH 6.0). Chicken was marinated for 1 h and no reduction was detected. In addition, bacteria presented growth during the storage at 4 °C and 12 °C. No bacterial reduction was also found in marinated seafood studied by Jemmi and Keusch (1992) who inoculated *L. monocytogenes* in trout and submitted to salt marinade containing 10% NaCl and 0.7% spices (pH 6.4) for 12 h and Mejlholm, Devitt, and Dalgaard (2012) inoculated *L. monocytogenes* in shrimp *(Pandalus borealis)* marinated with benzoic, citric and sorbic acids (pH 6) for 4 h at 3 °C, both presented no significant changes in the concentration of *L. monocytogenes* was observed.

Low L. *monocytogenes* reductions were demonstrated by Scheinberg, Svoboda, and Cutter (2014) and Jofré et al. (2009). The first study evaluated the reduction of *L. monocytogenes* after the marination process containing spice blend with sterile water in contact with beef (1:0.65 – pH 6.6) for 24 h at 4 °C and found reduction of 0.2 log CFU/strip. The second study investigated the effect of marinating during the same time and temperature conditions but in marinade containing water, sodium chloride, sodium tri-polyphosphate, sodium ascorbate and sodium nitrite on beef loin (pH 6.3) and found reduction of 0.2 log CFU/g.

Reductions ranging from 1 to 3 log CFU/g were observed in six studies. Among them, two studies tested red wine marinades. Vasilijević et al. (2019) evaluated the activity of essential oils (*Juniperus communis* and *Satureja Montana*) in the red wine (13.5% vol. alcohol) marinated beef against *L. monocytogenes*. Beef was inoculated with 5 log CFU/g and marinated in red wine, salt, pepper, garlic powder in combination or not with 0.25% of *J. communis* and 0.125% of *S. montana* for 24 h at 4 °C. Results demonstrated a reduction of 1.5 log CFU/g for all marinades tested. Similar results were found by Birk and Knøchel (2009) who tested red wine and yogurt marination in pork medallions *(Longissimus thoracis)*. The immersion of the pork meat in 42 °C red wine (pH 3.7) for 15 min and subsequent storage at 4 °C, still submerged in red wine, promoted approximately 2 log

CFU/piece reduction of *L. monocytogenes*, after 3 days. When pork meat was immerged in 4 °C red wine during the entire experiment or in natural yogurt (pH 4.4) at 4 °C the reduction after 3 days was approximately 1 log. Other two studies investigated marinades containing soy sauce. Inoculated raw crabs (4.4 log CFU/g) were immersed in soy sauce (salinity 15.6% and pH 4.6) and then stored at 5 °C or 22 °C, for up to 28 days. At 5 °C, after 28 days, *L. monocytogenes* populations were reduced 1.1 log CFU/g, while at 22 °C the reduction was higher, i.e. 2.6 log CFU/g (Cho et al. 2016). Harrison et al. (2001) examined beef loin marinated in a solution containing soy sauce, worcestershire sauce, pepper, garlic powder, onion powder and hickory-smoked salt (1:0.1 w/v beef-marinade, pH 4.5). After marinating for 24 h at 4 °C, *L. monocytogenes* counts were reduced 1.8 log CFU/strip. Reductions around 2 log CFU/g were also found by Naidoo and Lindsay (2010) and Ozturk and Sengun (2019) who tested apple cider vinegar and koruk juice plus 0.1% thyme, respectively, in marinating process at 4 °C for 24 h.

Largest reductions ranging from 3 to 6 log UFC/g were cited by other authors in 7 studies. Most of them (5) used acids in the marinades. Vergara et al. (2003) evaluated the behavior of *L. monocytogenes* in anchovies during marination. Anchovies were inoculated with 1.2×10^7 CFU/g. The marinade solution (pH 3.1) was prepared by mixing water and vinegar (6% alcohol) and adding 8.06% NaCl (w/v) for 24 h at 20 °C. At the end of marinating process, a marked decrease of the inoculum was observed resulting in a reduction of 4.6 log CFU/g. Similar, Bremer and Osborne (1995) showed that the concentration of *L. monocytogenes* was reduced by approx. 3 log CFU/g in green shell mussels (Perna canaliculus) following marination in 3% (w/v) acetic acid or 1.5% (w/v) acetic acid + 1.5% (w/v) lactic acid for 10 to 15 days at 5 °C (pH 4.0). Rhoades et al. (2013) investigated the effect of marination for 18 h at 5 °C on beef pieces inoculated with 6,5 log CFU/cm^2. Soy sauce base marinade added with lactic acid (pH 4.5) presented the higher reduction, decreasing 3.4 log CFU/cm^2. Similar decrease was observed (3.5 log CFU/cm^2) when acids were used was also found by Fisher et al. (2016) in marinade made with chitosan (5%), lauric arginate ester (4%) and organic acids (2% acetic, lactic and levulinic acid) (pH 3.0) after 6 h of marination and by Moon et al. (2017) in teriyaki sauce marinade (soy sauce, wine, high fructose corn sirup, water, vinegar, salt, spices, onion powder, and garlic powder) in combination with carvacrol or thymol (0.5%) on beef slices for 7 days at 4 °C and (pH 5.1).

Yang et al. (2013) investigated the efficacy of lemon juice or citric acid in the reduction of *L. monocytogenes* during the preparation of kelaguen (beef). Beef inoculated at 6 log CFU/g was marinated with lemon juice at 4 °C or citric acid (7.2%, pH 2.6) at 24 °C. Marinated beef at 4 °C and a ratio of lemon juice to beef of 5:1 (pH 2.7) required 16 hours to achieve a 4 log UFC/g reduction while citric acid required 2.6 h. Higher reductions were observed by Shukla et al. (2017) who investigated the inhibitory effects of *Adhatoda vasica* ethanolic leaf extract (AVELE) against *L. monocytogenes* on raw chicken breast meat inoculated with 6.0 log CFU/g. The solution was used at concentrations of 5%, 10%, and 20% (pH 6.0). The solution was maintained in contact with chicken for 5, 10, 15, 30, 60, and 90 min. AVELE at 200 mg/mL for 90 min completely inactivated *L. monocytogenes*.

Campylobacter

The *Campylobacter* species inoculated into food matrices of identified studies were *C. jejuni* and *C. coli*, which are associated with poultry meat (Kaakoush et al. 2015; Berthenet et al. 2019; García-Sánchez et al. 2018).

Among the included studies, 71.4% were related to chicken meat followed by pork (14.3%) and beef (14.3%). Studies demonstrated *Campylobacter* reductions ranging from 0.5 to 6 log CFU/g. The studies investigated the use of many types of marinades in different chicken cuts (chicken breast, chicken breast fillets and chicken wings). The highest reduction for chicken meat was obtained by Thanissery and Smith (2014), who evaluates the effect of salt-phosphate marinade solution containing 0.5% of thyme orange essential oil on breast fillets and whole wings inoculated with approximately 6 log CFU/mL of *Campylobacter coli*. In this study, meats were marinated for 20 min in vacuum with 10% (vol/wt) of a prechilled (4 °C) marination solution reducing numbers of viable *C. coli by* approximately 3.0 log CFU/mL on breast fillets and whole wings.

Except for the study cited above, in general, the reductions of *Campylobacter* in marinated chicken meat were low. Park, Hong, and Yoon (2014) investigated the effects of 3% cultured sugar/vinegar blended with 0.6% polish rub seasoning containing 32% of herbs, refined salt, garlic flake, brown sugar, α-corn, crust breading, soybean oil and oleoresin paprika on chicken breasts inoculated with *C. jejuni* and obtained approximately 1.8 log CFU/g reduction. In this study the breasts were vacuum-packaged and stored at 4 °C and 10 °C for 30 days that is a longer period when compared to others included studies. In shorter marinade times, regardless of chicken cuts and the ingredients used in the marinades (wines, vinegar, pomegranate sirup, lemon juice) the reductions varying between 0.5 and 1.3 log CFU/g (Birk et al. 2010; Zakarienė et al. 2015; Isohanni et al. 2010).

Birk and Knøchel (2009) tested the effect on *C. jejuni* of a marinade containing red wine and yogurt marination in pork medallions *(Longissimus thoracis)*. The most effective antimicrobial procedure was the immersion of the pork meat in 42 °C red wine (pH 3.7) for 15 min and subsequent storage at 4 °C, still submerged in red wine. After 3 days, approximately 6 log CFU/g of *C. jejuni* were reduced. When pork meat was submerged in 4 °C red wine during the entire experiment or in natural yogurt (pH 4.4) at 4 °C the reduction was 3.5 log and 2 log, respectively, after 3 days.

Vibrio spp

Vibrio parahaemolyticus and *Vibrio cholera* were the most investigated microorganisms in studies concerning the

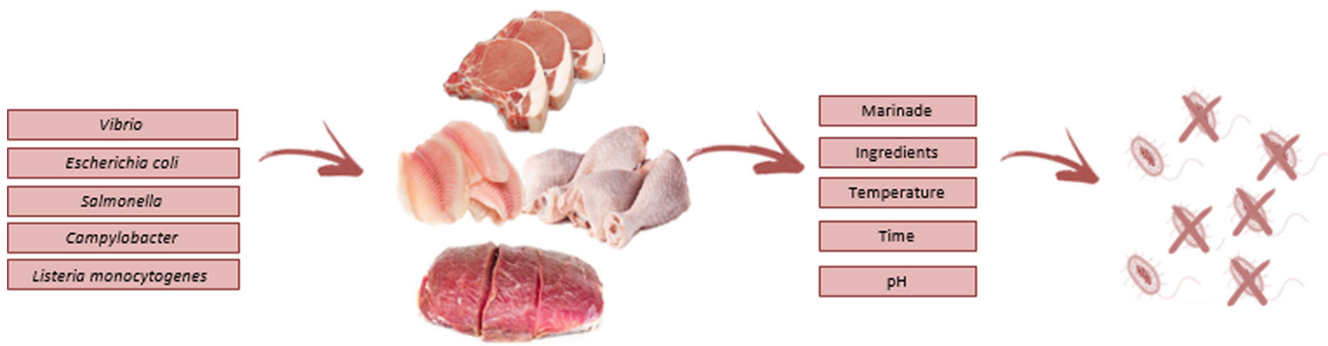

Figure 2. A scheme of the effect of marinades on meat products.

bactericidal effect of marinades on seafood. Therefore, in general the foodborne illnesses associated with this bacterium are the consumption of raw or undercooked contaminated seafood (Scallan et al. 2011; Nishibuchi and DePaola 2005; Elbashir et al. 2018). Works demonstrated of *Vibrio* reductions ranging from 0.1 to 9 log CFU/g.

From five relevant studies identified, three investigated ceviche marinade in lime/lemon juice. Ceviche is a food with marinated raw fish, and is a typical meal in Peru and other Latin American countries. Studies demonstrated *Vibrio* reductions ranging from 4 log CFU/g to 9 log CFU/g. Mathur and Schaffner (2013) evaluated the effects of lime juice marination (pH 2.5) on Tilapia (Oreochromis niloticus) fillet pieces (ratio 1.5:1 (w/w) with a final pH 3.6) inoculated with *V. parahaemolyticus* (7.0 log CFU/g). The marination occurred at 25 °C and 4 °C for 30 or 120 min resulting in *Vibrio* inactivation of 5 log CFU/g in all conditions studied. Similar result was found by Mata, Vives, and Vicente (1994) when fish (*Coryphaena hippurus*) inoculated with *V. cholerae* (10^9 CFU/g) was marinated in lime and lemon juice mixture (pH 3) at room temperature and resulted in elimination of vibrios after 30 min of exposure. Significant reduction ($p < 0.05$) was also obtained in a study with other seafood. Crab (*Portunus trituberculatus*) inoculated with approximately 4 log CFU/g of *V. parahaemolyticus*. The preparation was marinated with soy sauce (alcohol 2%, salinity 15.6% and pH 4.6) in the ratio 1:5 (w/w) presented a completely inactivation in one day of marination conducted at 5 °C and 22 °C (Cho et al. 2016).

However, contradictory with these studies, Herrera et al. (2010) found a reduction of approximately 0.1 log of *V. parahaemolyticus* when fish was marinated with lime juice, cilantro, garlic, hot peppers, sweet potatoes and corn (pH 5.2) for 30 min. Similar, shrimp *(Pandalus borealis)* marinated with benzoic, citric and sorbic acids (pH 4.9) reduce 0.9 log CFU/g of *V. parahaemolyticus* reduction, after 24 hours of marination, at 3 °C (Mejlholm, Devitt, and Dalgaard 2012).

Discussion

The use of marinades on meats not only prevents the growth of bacterial pathogens but also promotes their inactivation (Figure 2). Only one of the thirty-three studies identified in the presented review demonstrated the growth of a pathogen in their most advantageous marinade formulation. The highest reductions found in all identified studies were 4.4 log CFU/g for *Salmonella*, 4 log CFU/g for *E. coli*, 6 log CFU/g for *L. monocytogene*s, 6 log CFU/g for *Campylobacter* and 9 log CFU/g for *Vibrio*. However, in general, the marinade promotes lower pathogen reductions with approximately 3 log CFU/g or less, except for *Vibrio* (reductions > 4 log CFU/g). Considering that these reductions may not be sufficient to completely eliminate all pathogens present in meats, the use of high-quality meats, coming from industries with Good Hygiene Practices, is very important. Furthermore, aiming at food safety purposes, when possible, it is also recommended the use of an effective heat treatment before the consumption of marinated meats.

Regarding the influence of marinades temperatures on the bacterial effect, it was possible to observe that studies which evaluated the same scenario at different marinade temperatures showed different results. In general, the comparison was between refrigerate (4 °C − 5 °C) and room temperature (20 °C − 25 °C). The studies of Cho et al. (2016), Mathur and Schaffner (2013), Pathania et al. (2010) and Yang et al. (2013) demonstrated the same or greater reductions when marinades were carried out at room temperature. In the literature many studies have reported this same bacteria behavior on different foods and this can be explained because bacterial metabolism is more active at higher temperatures, facilitating the entry of toxic compounds into cells, resulting in bactericidal effect (Eklund et al. 2004; Grounta et al. 2013; Kim, Kim, et al. 2014; Kim, Park, et al. 2014; Rhee et al. 2003; Tsegaye, Ephraim, and Ashenafi 2004).

The exposure times of meats in marinades ranged from 20 min to 30 days. The most common time used was 24 h. In general, marinating times lower than 12 h presented no significant changes in the bacteria concentration. However, some studies demonstrated that a few minutes can promote significant reductions (3 log CFU/cm^2, 3 log CFU/mL; $p < 0.05$) (Fisher et al. 2016; Thanissery and Smith 2014). Thus, apparently the time of exposure is not one of the main factors that influence the inactivation of bacterial pathogens and this factor seems that have to be associated to other parameters of the marinade such as pH and ingredients.

Marination pH was one of the most important parameters responsible for bacterial inactivation, considering all pathogens studied. In general, greater reductions were observed when marinades presented pH < 4.5 were used, regardless of the other ingredients containing in the marinade and the food matrix (Birk and Knøchel 2009; Fisher et al. 2016; Rhoades et al. 2013; Vergara et al. 2003; Yoon et al. 2005). These results can indicate that decreasing pH values can also increase the sensitivity of pathogens to others ingredients of marinade. For example, low pH is important to keep acids in an undissociated form, and organic acids exhibit their maximum antimicrobial effect in this form. This pH influence was also observed in non-marinade studies when inactivation of pathogens by chemical compounds were evaluated (Periago and Moezelaar 2001; Saliani, Jalal, and Goharshadi 2015).

However, even at higher pH, high reductions can be obtained when natural and antibacterial extracts were used in the marinade (Moon et al. 2017; Shukla et al. 2017; Ozturk and Sengun 2019). These reductions can be attributed to phenolic, flavonoid and alkaloid contents of these extracts (Shukla et al. 2017). In these cases, the concentration of the extracts and the synergy of combinations were important parameters and its effectiveness varies according to the pathogen tested. This fact was observed, for example, in the study of Ozturk and Sengun (2019) which evaluated koruk juice. When 50% koruk juice plus 50% water (pH 3.2) were used in marinade, the most efficient reduction of *S. Typhimurium* and *E. coli* O157:H7 were obtained, while when koruk juice plus 0.1% thyme was used, the most efficient reduction for *L. monocytogenes* was observed.

The use of wine in marinades is very common and the included studies demonstrated different results in bacterial reduction. Wine associated with essential oils demonstrated major reductions when compared with pure wine (Moon et al. 2017; Rhoades et al. 2013). Moreover, the efficacies of pure wine varied according to the inoculated pathogen and the wine type used, which may be associated with the content of phenolic compounds specific to each wine. Moreover, food matrix played an important role in the different reductions obtained by pure wine marinades (Birk and Knøchel 2009).

According to the literature not related with marinades, alcohol content in the beverages can improve the inactivation of pathogens and there is a correlation between alcohol and pH in the survival of bacteria (Marimón et al. 1998; Gaglio et al. 2017; Lopes and Tondo 2020). In the studies included for this review the only alcohol beverage used was wine which had approximately 13% of alcohol content. For further marinade studies, the use of beverages with higher alcohol content associated with a low pH must be taken into consideration to try to improve the pathogen reductions.

Conclusion

The present review was able to find many researches concerning meat marinades and their effectiveness against pathogens. The information found in these investigations demonstrated that the use of marinades can prevent the pathogen growth and promotes their inactivation as well. Most of marinade studies promoted lower pathogen reductions of < 3 log CFU/g, which demonstrates the importance of using quality meats and adopt heat treatment in marinated meats, when possible. The low pH was identified as the most pronounced parameter, affecting the inactivation of pathogens in marinades, however, some effects of the ingredients and storage temperature cannot be excluded. These factors must be taken into consideration in future studies, as well as the performance of sensorial analysis in order to assess the consumer perception and acceptance.

Disclosure statement

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