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(Heloísa Carneiro de Rezende)

AÇÃO DESCONTAMINANTE DO ÁCIDO PERACÉTICO EM FRANGO DE CORTE

Patos de Minas – MG

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Ação Descontaminante do Ácido Peracético em Frango de Corte

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SUMÁRIO

1. INTRODUÇÃO GERAL

O agronegócio no Brasil é responsável por grande parte do desenvolvimento econômico do país se destacando nesse cenário, o setor da avicultura. A produção de frango de corte tem impressionado pelo dinamismo e pela competência conquistada nas últimas décadas, atingindo números expressivos em toneladas/ano 14,329 milhões, onde 68% se concentram no mercado interno e 33% em exportações (ABPA, 2022). Nesse contexto, o abate de aves em larga escala é um processo rápido e altamente automatizado o que possibilita uma contaminação cruzada no decorrer do processo, caso os parâmetros de controle não sejam rigorosamente controlados, e ainda assim, é desafiador assegurar o controle microbiológico de animais vivos dentro dos processos produtivos (BOUBENDIR et al., 2021; CHEN et al., 2020). Sabe-se que os impactos na saúde pública devido a doenças de origem alimentar são enormes, além de significativos encargos sociais e econômicos somados aos esforços da indústria avícola desde o início da análise de riscos e do ponto crítico de controle para reduzir a carga de doenças transmitidas por alimentos (DTA's) (EFSA, 2021).

Sendo assim, a avicultura traz consigo desafios diários devido à complexidade ao longo da cadeia produtiva na qual as indústrias abatedoras de aves possuem grande responsabilidade para controlar e reduzir significativamente os riscos de contaminação dentro dos processos. Porquanto, os programas de autocontrole dentro dos processos fabris, auxiliam na garantia das boas práticas de fabricação e somado a utilização de barreiras tecnológicas no decorrer do processo são cada vez mais necessárias para reduzir significativamente as contaminações entéricas prováveis de acontecer nos estabelecimentos avícolas, a exemplo do uso de ácidos orgânicos para a descontaminação em carnes de aves (FDA, 2022).

No Brasil, ainda não há a aprovação para o uso de coadjuvante de tecnologia que possam auxiliar no controle e disseminação da carga de DTA's pelo MAPA (MAPA, 1998), encontrando-se atrás de países desenvolvidos tais como, Canadá, USA, Japão e China e suas regulamentações, que preveem o uso de intervenções antimicrobianas, a exemplo do uso do ácido peracético (PAA), o qual possui embasamento científico comprovado quanto a sua eficácia para o controle de patógenos (EFSA, 2008; FDA, 2020; PEYRAT et al., 2008).

O ácido peracético (PAA), também conhecido como ácido peroxiacético ou peroxiácido, é uma mistura de ácido peroxiacético, ácido acético, peróxido de hidrogênio e ácido 1-hidroxietilideno-1,1-difosfônico (HEDP), opcionalmente ácido octanóico e ácido sulfúrico. Seu uso em produtos avícolas crus foi aprovado pela Food and Drug Administration (FDA) com concentração máxima de 2.000 ppm de peroxiácidos (USDA-FSIS, 2021).

2. ESTRUTURAÇÃO DA PESQUISA

É possível dividir este trabalho em duas partes, Capítulo 1 e Capítulo 2.

A primeira (capítulo 1) é relativa ao artigo de revisão que contempla uma abordagem holística sobre a aplicação do ácido peracético como antimicrobiano e seus resíduos, em especial o ácido etidrônico (HEDP).

A segunda parte (capítulo 2) consiste nos ensaios microbiológicos e físico químicos realizados através do Delineamento Composto Central Rotacional (DCCR) para determinar as melhores condições das variáveis concentração e tempo de aplicação do ácido peracético nos cortes de peito e coxa de frango.

3. OBJETIVOS ESPECÍFICOS

Os objetivos específicos desse trabalho são:

1. Capítulo 1 → (i) Realizar uma abordagem regulatória quanto à segurança toxicológica do antimicrobiano PAA, em especial do estabilizante HEDP, sugerindo a possibilidade de pesquisar o HEDP residual em carnes através de sua estequiometria básica, corroborando com as recomendações das autoridades sanitárias; (ii) Esta revisão também fornece conhecimento detalhado sobre a prevalência de bactérias presentes na microbiota avícola em cada etapa da linha de processamento de aves, o uso de PAA e sua eficácia como coadjuvante tecnológico.

2. Capítulo 2 → (i) Avaliar a eficácia do antimicrobiano ácido peracético, por meio da redução de bactérias indicadoras de relevância no processo avícola; (ii) Selecionar através do Delineamento Composto Central rotacional (DCCR) as melhores condições das variáveis concentração e

tempo de aplicação do ácido peracético nos cortes de peito e coxa de frango; (iii) Avaliar o impacto do antimicrobiano nos aspectos tecnológicos da carne de frango.

1 CAPÍTULO 1 - PERACETIC ACID APPLICATION AS AN ANTIMICROBIAL AND ITS RESIDUAL (HEDP): A HOLISTIC APPROACH ON THE TECHNOLOGICAL CHARACTERISTICS OF CHICKEN MEAT

PERACETIC ACID APPLICATION AS AN ANTIMICROBIAL AND ITS RESIDUAL (HEDP): A HOLISTIC APPROACH ON THE TECHNOLOGICAL CHARACTERISTICS OF CHICKEN MEAT

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Abstract

The most significant occurrence of food-borne diseases is due to *Campylobacter* and Salmonella contamination from chicken meat, and for this reason, strict regulations about strategies to improve the control of food pathogens are imposed by food safety authorities. Despite the efforts of poultry industry since the beginning of risk analysis and critical control point to reduce the burden of food-borne illness, technological barriers along the way are increasingly necessary to ensure safe food. The aim of this review was to carry out a scientific approach to the influence of Peracetic acid (PAA) as an antimicrobial and its toxicological safety, in particular the stabilizer used in the formulation of PAA, 1-hydroxyethylidene 1,1-diphosphonic acid (HEDP), suggesting the possibility of researching the residual HEDP in meat, which would allow the approval of the PAA by the health authorities of several countries that still restrict it. This review also aims to ascertain the effectiveness of peracetic acid (PAA), in different cuts and carcasses, by different application methods, comparing the effectiveness of this antimicrobial with other antimicrobials, and its exclusive or combined use, for the decontamination of poultry carcasses and raw parts. The literature results support the popularity of PAA as an effective intervention against pathogenic bacteria during poultry processing.

Keywords: Food safety; microbial intervention; poultry process; peracetic acid; HEDP.

1.1 Introduction

The public health impacts due to food-borne illness are enormous, in addition to significant social and economic burdens. Therefore, the safe production and distribution of food from field to table is crucial to ensure that consumers receive healthy food products. The World Health Organization (WHO) reports that annually, nearly 1 in 10 people worldwide fall ill after eating contaminated food, leading to more than 420,000 deaths. Children are disproportionately affected, accounting 125,000 deaths per year in people under the age of 5. Most of these cases are caused by diarrheal diseases. Other serious consequences of food-borne illnesses include kidney and liver failure, brain and nerve disorders, reactive arthritis, cancer, and death (WHO, 2022).

In this context, evaluating the United States' history, a country that is efficient in food-borne illnesses reporting, the U.S. federal government estimates that there are about 48 million cases of food-borne diseases annually - the equivalent of 1 in 6 Americans becoming ill each year, these diseases result in 128,000 hospitalizations and 3,000 deaths annually. Although the American food supply is among the safest in the world, it reinforces the relevance of adopting procedures and control measures that corroborate with food safety (FDA, 2022).

In Brazil, 7,674 outbreaks were notified to the National Health Surveillance Agency (ANVISA) from 2009 to 2019, with 109 recorded deaths. The most commonly identified etiologic agents in ATD outbreaks were *Escherichia coli bacteria,* accounting for 29% of the total, followed by Salmonella and *Staphylococcus aureus*, with 17% and 16%, respectively (MARIA et al., 2021).

Poultry slaughter and processing establishments regulated by official agencies must determine the "food safety hazards that may occur before, during, and after animals enter the

establishment" through a farm-to-fork approach in their Hazard Analysis and Critical Control Point (HACCP) plan risk analysis. These interventions and procedures are necessary, as they are an integrated part of reducing the impact of Salmonella and other pathogens on public health. These pathogens are a danger that establishments producing raw poultry products must control using a HACCP plan or prevent in the processing environment employing a sanitary operational program (HOP), or other prerequisite program corroborating the HACCP plan for the control of carcass and poultry contamination by enteric pathogens such as Salmonella and *Campylobacter* spp (RIISPOA, 2017; USDA- FSIS, 2015). In the same context, the European Union states that the main elements in poultry meat inspection are the accurate analysis of pre-slaughter batch information, *ante-mortem* examination of animals, *post-mortem* examination of carcasses and organs, and programs that monitor the hygiene of the process. The control of these steps over the years can be seen by the reduction of *Campylobacter* counts in European and imported products compared to previous study periods. The last Annual Report of the Scientific Network on microbiological risk assessment, however, presented studies of resistant bacteria found within the poultry slaughter processes, such as E.coli. Out of the samples analyzed, 12.5% were tested positive, which is higher than expected in the literature (5- 10%), reinforcing the constant challenge of the poultry segment to control pathogens (EFSA, 2021).

In 2019, the WHO reiterated the role of governments in treating food safety as a public health priority, as crucial play a key role in developing policy and regulatory frameworks and establishing and implementing effective food safety systems(WHO, 2022). USDA-FSIS(2015), reports that enteric contaminations are reasonably likely hazards to occur in poultry slaughterhouses, and that these should be controlled and monitored in the HACCP system through the critical control points of the establishment, and to identify described effective measures to reduce Salmonella and *Campylobacter* in raw poultry products.

For the United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS), preventing food-borne diseases with the use of antimicrobial interventions in poultry meat is a priority for the industry. Concerned about meat safety, USDA-FSIS has developed a strategic plan for 2011 to 2016, formulating strict regulations and including goals that address strategies to improve food-borne pathogen control every year (KATARIA et al., 2020; USDA- FSIS, 2015).

Although the American food supply is among the safest in the world, reinforcing the relevance of adopting procedures and control measures that corroborate with the safety of the food, the annual ATD report still brings worrying numbers of hospitalizations and deaths caused by these diseases (FDA, 2022). Therefore, the recommendation for use of microbial interventions are a possibility of adopting microbiological barriers for the control of alimentary pathogens (KATARIA et al., 2020; USDA- FSIS, 2015). The Ministry of Agriculture and Supply in Brazil (MAPA) corroborates with the world inspection departments in the controls and risk evaluations of these diseases (RIISPOA, 2017). In terms of innovation in technological processes for the addition of barriers to microbiological control, Brazil, however, for regulatory reasons, is behind developed countries such as Canada, USA, Japan and China and their regulations, that foresee the use of antimicrobial interventions, such as the use of peracetic acid, which has proven scientific basis as to its effectiveness for pathogen control. In the specific case of Brazil, the use of peracetic acid as a coadjuvant technology in the function of antimicrobial agent in carcasses and/or parts of butcher's animals is approved by the Health Surveillance Agency - ANVISA, according to Resolution RDC No. 2 of January 8, 2004, in enough quantity to obtain the desired effect, without leaving residues in the final product(ANVISA, 2004). However, this approval does not provide for residues in the final product, and using coadjuvant technologies may result in the unintended but unavoidable presence of residues or derivatives in the final product(ANVISA, 2021). For this reason, the

tolerance of the residual limits of PAA and its stabilizer (HEDP) concerning safety under the toxicological aspect, are requirements of the regulatory body of animal protein, enlightening the regulatory barrier in Brazil.

To progress on regulatory issues and achieve the food industry's goals for food safety, continued scientific investigations into progress on regulatory issues and achieve the food industries goals for food safety, and continued scientific investigations into microbial interventions in poultry processes are required. Research on final products that may be contaminated and that will be present on the consumer's table is a critical factor in improving the safety of poultry products and complying with legislation to achieve microbiological standards for Salmonella (DEUMIER, 2006; SCOTT et al., 2015; USDA- FSIS, 2015).

In the face of the mentioned, this paper demonstrates by reviewing literature studies, the efficacy and safety of the use of peracetic acid as a technological barrier in poultry meat processes, and also evaluates the scientific impact regarding the research of this antimicrobial on sensory aspects and the residual of HEDP in raw poultry meat, as an incentive for the use of PAA in the pre-requirement programs implemented in slaughterhouses,

1.2 Methodology

Published and current national and international legislation and reference books regarding microbiological and technological aspects were consulted and served as a basis for the investigation to retrieve studies on the effect of the application of peracetic acid.

Then, a web search containing scientific sources was conducted using the Science Direct, Web of Science, Pubmed and Springer platforms. The keywords used to retrieve the relevant information were: food safety, microbial intervention, poultry process, peracetic acid and HEDP.

The survey covered the period from 2008 to 2021 and geographical restrictions were not imposed.

The search screening was based on the title, abstract and conclusion of the searched scientific articles. Articles classified as relevant met the criteria of i) providing relevant data and counts of bacteria for the study (Enterobacteriaceae, total mesophil count, Salmonella *and Campylobacter*) ii) types of treatment, iii) comparison with other relevant antimicrobials, and iv) impact on the sensory and technological aspect of chicken meat by the action of antimicrobials.

After evaluating the selected documents, two standardized forms were designed to extract data from the selected articles. The first form aimed to collect general information from the studies and included information about the type of reference, the purpose of the study, where and when the study was conducted, and the kind of article.

The second form aimed to collect analytically relevant data for the scope of the review. It included information on the investigated bacteria, the analytical method used, the enumeration unit, data on the type of sample, application parameters of the antimicrobial solutions, kind of treatment, log reduction with the use of the antimicrobials, and the sensory aspects when cited in the articles. Only scientific publications that reported qualitative or quantitative microbiological reductions directly attributed to treatments using PAA, or its comparison with another intervention were used for data extraction.

The forms enabled the structuring of the bibliographic information and the detailing of this information as to the type of bacteria analyzed, the analysis methodology applied for the detection of the microorganism, the type of sample used (chicken cut or carcass), whether the microbiota was native or inoculated on a laboratory scale, the treatment applied with its controlled process parameters (immersion, spraying, chemical concentration and application

time), the log reduction after antimicrobial intervention and whether the citations addressed studies on the technological aspects of meat.

Microbiota of chicken meat and its impact

Batz, (2011) estimated in his study 14 food-borne pathogens that cause 14.1 billion (2009 dollars) in disease costs annually and among these, five pathogens cause more than ninety percent of this health burden driven: *Salmonella* enterica, *Campylobacter* spp, *Listeria monocytogenes*, *Toxoplasma gondii*, and Norovirus. Among these 14 pathogens found in food overall, a ranking of societal impact and risk was performed, using as criteria disease costs, number of patients, hospitalizations, and deaths. Salmonella is the first pathogen of relevance and is the biggest cause of food-borne diseases than any other pathogen, causing high numbers of hospitalizations and deaths. In a second place is *Toxoplasma gondii* and in third place is the pathogen *Campylobacter*.

Since Salmonella is the primary pathogen of relevance, foods involved in salmonellosis outbreaks are associated with a wide variety of foods regulated by FSIS and FDA, with significant risks associated with poultry, poultry products, and eggs (WHO, 2022). This suggests that the reduction of the Salmonella load requires an enormous effort from the regulatory agencies as well as from the slaughterhouses in order to guarantee process control and consequently reduce the risk of cross-contamination in the poultry process(USDA- FSIS, 2015).

Contaminated poultry has the greatest public health impact among foods. The most significant disease relevance is due to contamination with Salmonella *and Campylobacter* which are among the top three in the ranking of the top 10 food-borne pathogens of public health concern(BATZ, 2011; FORSYTHE, 2013; WHO, 2022).

Understanding the bacterial community profile through poultry processing can help the industry to know itself and consequently produce better poultry products. A study by Chen et al. (2020) evaluated the most abundant phyla found in chicken carcasses in the

during the production process. In general, 98.2% of the organisms belonged to the phyla Firmicutes, Proteobacteria, Bacteroidetes and Actinobacteria as schematically shown in figure 1.

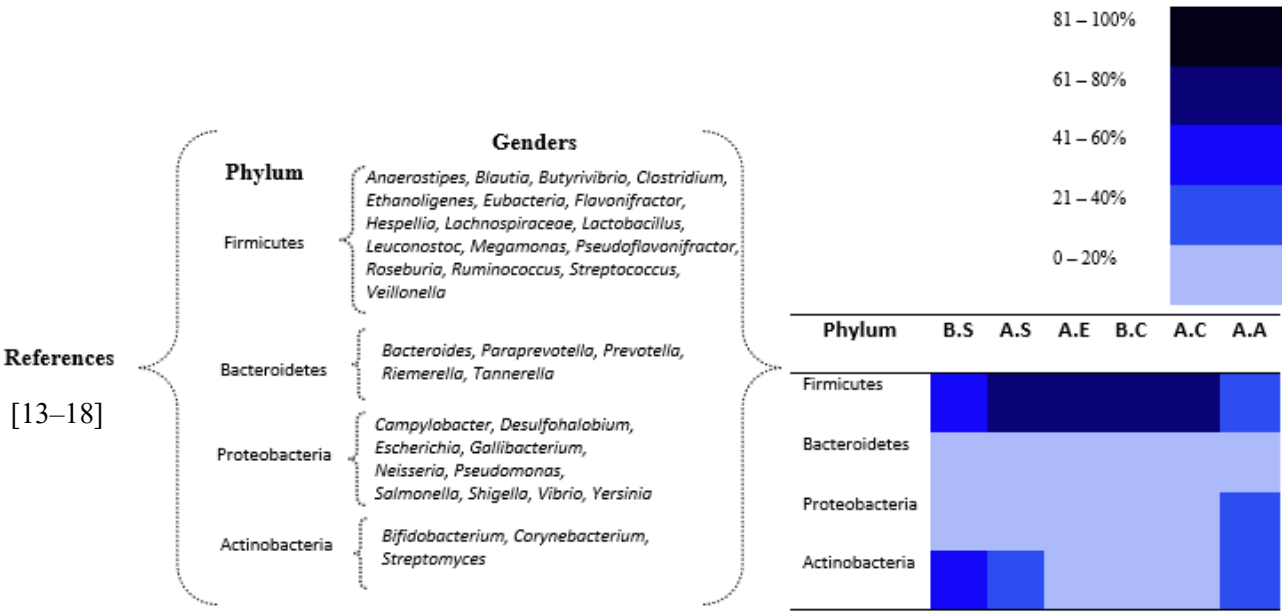

Fig. 1: Relative taxonomic unit abundance at phylum level in six sample groups across the poultry processing line

Where: BS group: samples collected before scalding; AS group: samples collected after scalding; A.E group: samples collected before evisceration; BC group: samples collected before immersion cooling (chiller); AC group: samples collected after immersion cooling (chiller); AA group: post-air cooling samples

When comparing the relative abundance of phylum between the beginning of processing (the BS sample group) and the end of processing (the AA sample group), it can be seen that the only phylum that became more predominant was the Proteobacteria phylum, as it increased its relative abundance over the course of the process. This phylum increased 32-fold in relative abundance from 0.9% in the sample group before BS evisceration to 28.8% in the sample group after AA air cooling (last step of the process). This can be explained by the prevalence of two

pathogens within the phylum, that is Salmonella and *Campylobacter*. The study in question also evaluated the prevalence of these pathogens and their presence was observed in all the stages of the production process evaluated with a prevalence of 89% for *Campylobacter* and 20% for Salmonella in the analyzed chicken carcass samples (CHEN et al., 2020).

1.3 Chicken slaughter and contamination points

From the second half of the twentieth century to the present day, agribusiness in Brazil has achieved significant advances in order to compete globally with the major world powers, enabling technological advancement of poultry production which is impressive for its dynamism and competence achieved in recent decades (ABPA, 2021).

Plants processing large quantities of chicken increasingly need hygienic practices during processing. It is essential to ensure food safety during slaughter, to control the spread of stool and cecal content, as these are the main hosts of enteric pathogens such as Salmonella, *E. coli*, and *Campylobacter* (DEMIROK et al., 2013; PROJAHN et al., 2019).

Based on the routine of a slaughterhouse in the mining region of Brazil, the poultry slaughter processing is detailed below.

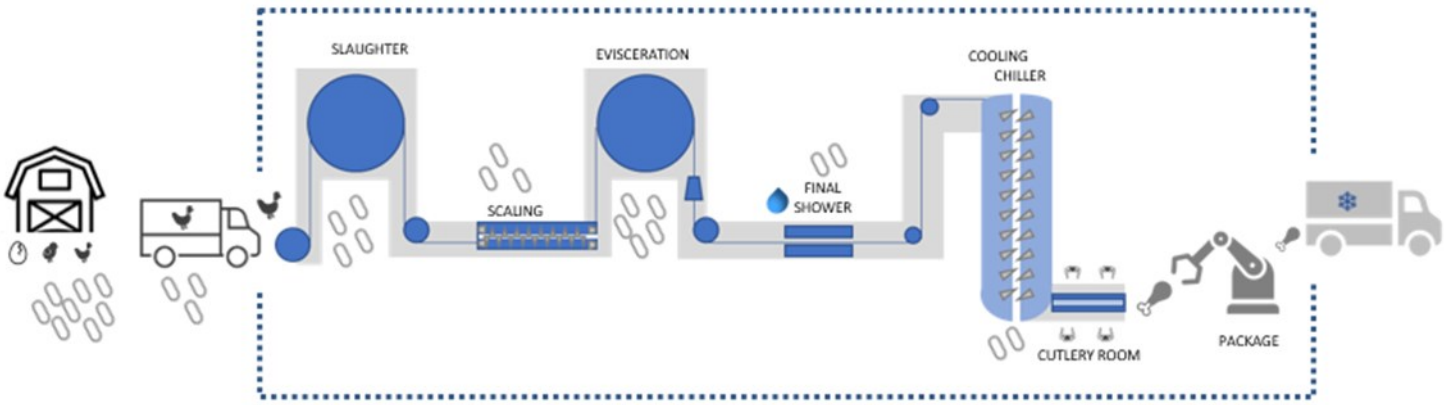

Fig. 2: An overview of the poultry meat production chain, (Farm-to-Fridge), indicating the main processing steps and the prevalence of bacteria present in the poultry microbiota at each step

1.4 Reception and Hanging

Approximately 24 hours before slaughter, the feed is removed and the chicken will be fasted. Properly performed pre-slaughter fasting aims to reduce the amount of fecal material in the animal's gastrointestinal contents. This prevents defecation during transport and the associated microbial spread among the chickens in the transport cases on the way to slaughter. This step is crucial to ensure the process of efficient evisceration, a prolonged fasting time causes changes in the animal's metabolism, friable viscera and consequent intestinal rupture in the eviscerator, causing cross-contamination(FOLEY et al., 2011; GARCÍA-SÁNCHEZ et al., 2017; MARMION et al., 2021).

The transportation of the birds from the poultry farm to the slaughterhouse is done by special trucks, equipped with specific cages to transport them. In these cages, the quantity of birds housed depends on the average weight of the bird, but always guarantees the animal's well-being.

On arrival at the company, the Federal Inspection visually inspect the birds in the lot to be slaughtered. After this procedure, the birds, still in the truck, are housed in an area with fans and water sprinklers, to ensure rest and reduce stress during transport, with controlled temperature and humidity (RIISPOA, 2017).

1.5 Slaughter

The stunning and bleeding room, where the bird arrives after being hung, should have low light and be away from loud noises to reduce stress to the animal. In this area stunning by electronarcosis is performed. The stunning tanks contain water, with a flow rate dependent on

the volume of birds, and the frequency, amperage, and voltage are standardized. The stunning time is a maximum of 12 seconds(MAPA, 1998).

The birds are bled manually with a knife, which aims to cut the carotid arteries and jugular veins. The knives are replaced every 30 minutes and sterilized at a minimum temperature of 85ºC. After this process, the bird spends at least 3 minutes in a bleeding tunnel, so most of the blood can drain away (MAPA, 1998).

A study has evaluated the prevalence of enteric Salmonella in slaughter processing equipment after complete sanitization procedures, and found that Salmonella persisted significantly in the first equipment of the slaughter line (OBE et al., 2020).

The study in focus, corroborates the fact that slaughter and bleeding are documented points at which about 60% of carcasses can carry the highest Salmonella cell burden, about 6.1 log CFU/ g, found during the processing chain(BOUBENDIR et al., 2021; RIVERA-PÉREZ; BARQUERO-CALVO; ZAMORA-SANABRIA, 2014). This can be explained by the high contamination of feces, dirt, and feathers that are inherent to the birds, as well as possible stress factors that lead to changes in the bird's metabolism, from fasting until the moment of slaughter.

1.6 Scalding and plucking

Scalding is done by immersing the bird in tanks containing water at a controlled flow rate and temperature, and the water is heated by steam. The water temperature in the scalding tank is between 54 and 63 °C. The birds go through this process to physically remove dirt from the skin and feathers and mainly to open and soften the feather follicles, facilitating their removal in the plucking machines(RIISPOA, 2017).

The scalding process is the first critical control point in poultry processing, since the step can result in the initial attachment of Salmonella to the skin. These Salmonella

contaminated carcasses can result in cross-contamination with the other carcasses via the scalding water and transporting the contamination throughout the subsequent stages of processing(MCBRIDE et al., 1980). Maintaining a constant renewal of the water in the scalding tank reduces the possibility of cross-contamination in the step, as this is the first viable point for the application of antimicrobial agents by the renewal water, helping to reduce microbial counts (CHEN et al., 2020).

Then the carcasses go through the plucking machines to remove the feathers using rubber fingers and water heated by steam at a temperature between 60 and 70 °C. These machines are regulated for each size and average batch weight, slope, height, and finger hardness. Maintenance must occur frequently to avoid carcass fractures, scratches, torn skin, and bruises that compromise the quality of the final product. After plucking, the carcasses go through the initial shower with controlled flow to remove possible dirt and feathers.

Washing can occur at various stages of the production cycle, and depending on the regulations of each country, chemical antimicrobials can be used as a technological barrier in microbiological control (BOUBENDIR et al., 2021; BUESS et al., 2019; GOMES DA SILVA et al., 2021).

1.7 Evisceration

The evisceration process begins with the pre-inspection conducted by the Federal Inspection (FI). As carcaças passam pelo processo de extração de cloaca, abertura de abdômen e exposição das vísceras. Then, at the exit of the eviscerator, the carcasses have their packages of offals aligned with the respective carcass so that the FI can also inspect the package of offals. After this process, the carcasses are released and proceed to the crop and trachea extractor and neck extractor (RIISPOA, 2017).

The carcasses then proceed to the monitoring stage, where the carcasses that still have visible gastrointestinal contamination are removed. Then, they go through the final shower with controlled flow, to remove possible residues that still exist, and proceed to the water immersion cooling system (*chiller*) (MAPA, 1998).

The evisceration stage is the most sensitive stage in controlling the spread of contamination. This is because it is the time when all the bird's intestinal contents are exposed. This process, either manually or mechanically, can cause ruptures in the caecal coating. In addition, the high load (about 11 log CFU/g) of bacteria per gram of caecal contents provides a strong potential of contamination if exposed(WAITE; TAYLOR, 2014). For this reason, the operational sanitary controls evaluating the efficiency of the equipment alongside the fulfillment of the pre-slaughter fasting procedure, help in the control of the discrimination of the contamination in the evisceration stage (RIVERA-PÉREZ; BARQUERO-CALVO; ZAMORA-SANABRIA, 2014).

In addition, equipment and surfaces are particularly vulnerable to cross-contamination of carcasses in this part of the plant. A study conducted in France recovered *Campylobacter jejuni* from the eviscerator before cleaning and disinfection, and this pathogen persisted in the equipment and was recovered again after the disinfection procedure, which corroborates the relevance of pre-slaughter procedures to reduce contamination within slaughterhouses (OBE et al., 2020; PEYRAT et al., 2008).

A strategy adopted to reduce the microbial count throughout the process is the application of antimicrobials at this stage, through the initial washing showers (before evisceration) and the final shower, at the end of the evisceration process (EFSA, 2008).

1.8 Carcass cooling (chiller)

Pre-cooling of carcasses is done using chillers, which transport the carcasses through three cooling stages, making the bird reach a maximum temperature of 4°C. The chilled water enters countercurrent at a flow rate according to the carcass weight. In the first stage, called pre-chiller, the residence time should not be longer than 30 minutes and the cooling and washing of the carcasses begin, the water temperature inside this chiller has a maximum limit of 16°C(MAPA, 1998).

The second and third stages, like the first, will lower the carcass temp, and the water temperature should be a maximum of 4°C. The retention time of the carcasses in these two chillers is approximately 120 minutes. After the cooling period, which takes about two hours, the carcasses leave the chiller with a maximum of 4°C and are sent for rehanging. At this stage the carcasses are re-hung and directed to the dismembering and deboning area. The period from re-hanging to the next area should ensure a satisfactory drip.

Effective cooling can reduce bacterial levels that may have been elevated during the previous stages, especially plucking and evisceration, paying special attention to the pathogens of relevance in the poultry process, Salmonella, *E. coli* and *Campylobacter*, as they exhibit high thermosensitivity at cold temperatures (BOUBENDIR et al., 2021; HARDIE et al., 2019). Its effect, however, depends on factors such as initial microbial load, chemical additions to the cooling system, water flow rate, and cooling capacity (AE KIM et al., 2017; DEMIROK et al., 2013; MARMION et al., 2021).

1.9 Cutting area

Leaving the chiller, the carcasses go through the conveyor to the cutting sector. In the cutting area, where the ambient temperature is a maximum of 12ºC, the cuts are completely removed, where the bone is removed and the skin may or may not be removed and proceeds to the packaging process (MAPA, 1998).

Afterwards, the cuts are weighed and packed in printed plastic packaging (separately by type of product), then they go to the freezing tunnel, which operates at a temperature between -25°C or colder, until the product reaches a minimum temperature. -12°C for the internal market and -18°C for the external market, being ready to be shipped (MAPA, 1998).

In this stage of the process, the immersion of cuts after the cooling system, was presented as the most used in the published literature, this corroborates ensuring the safety of the final product (BOURASSA et al., 2019; CANO; MENESES; CHAVES, 2021).

1.10 Organic acids used in pathogen control

When selecting an antimicrobial intervention, establishments should ensure that the interventions and levels of antimicrobials used are safe and appropriate.

To this end, Directive 7120.1 (USDA-FSIS, 2021) regulates the use of antimicrobial agents from safe and suitable ingredients in the production of meat, poultry, and eggs.

Table 1: Summary of Updates to list of substances used as an antimicrobial intervention

Substance	Amount
Watery solution of citric and hydrochloric acids;	sufficient for purpose
Lactic acid;	Solutions of 2% -5% lactic acid and a minimum 2:1 ratio of lactic acid to sodium lactate
Watery mixture of sodium diacetate, lactic acid, nisin preparation, and pectin;	Not to exceed a 20% solution of the aqueous mixture, and not to exceed a 0.2% nisin concentration
Aqueous mixture of peracetic acid (PAA), hydrogen peroxide (HP), acetic acid (AA) and (HEDP), optionally sulfuric acid (SA);	2000 ppm PAA, 1474 ppm HP, and 136 ppm HEDP
Aqueous mixture of peracetic acid and xanthan gum;	Concentration not to exceed 1500 ppm PAA, 800 ppm HP, 133 ppm HEDP, and 0.5% xanthan gum
Bacteriophage preparation of up to 6 phages targeting Salmonella;	up to a level of 1 x 10^8 PFU/g of food
Aqueous solution of citric and hydrochloric acids adjusted to a pH of 1.0 to 2.0;	sufficient for purpose
Aqueous solution of citric and hydrochloric acids adjusted to a pH of 0.5 to 2.0;	sufficient for purpose
Aqueous solution of sulfuric acid and sodium sulfate.	Concentration sufficient to achieve a targeted pH range of 1-2.2; delivered at a minimum system pressure of 0.5 psi;

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However, just the Directive 7120.1 is not enough scientific support for the use of interventions by establishments, because it does not contain effectiveness data or all the critical operational parameters. Thus, the scientific opinions issued by the health authorities of each country are extremely relevant for determining operational criteria, since the opinions issued by the health authorities are based on several studies and evidence.

Antimicrobial agents can be used at three stages in poultry processing: (i) on hot eviscerated carcasses or parts (pre-cooling) by short-term spray or immersion; (ii) on carcasses in the chiller system; (iii) on chilled carcasses or parts (post-cooling) by short-term immersion. Usually the antimicrobial is added to the water in equipment already present in the processing line(EFSA, 2014a).

Ebel et al. (2019) have researched 167 poultry slaughterhouses in the U.S. that produce chicken carcasses and cuts and identified that most establishments use peracetic acid (PAA) as the main antimicrobial intervention for both carcass application (immersion and spray) and immersion cuts.

Sukumaran (2015) have studied the combination of organic acids (peracetic acid, chlorine, cetylpyridinium chloride, and lauric arginate) with phages. In their study, the best combination for Salmonella reduction was the use of 400 ppm PAA per 20 seconds of chicken skin immersion, enabling a reduction of up to 2.5 log. In the same study, the efficacy of using only PAA was observed, achieving a reduction of up to 1.7 log of the pathogen, a result greater than the combination of the use of phages with the other organic acids.

Furthermore, the results of several studies over the past seven years suggest the use of PAA as an effective antimicrobial strategy for application in slaughterhouses to reduce Salmonella and *Campylobacter* in carcasses, corroborating the popularity of PAA (AE KIM et al., 2017; BARCO et al., 2015; CANO; MENESES; CHAVES, 2021; NAGEL et al., 2013; SCOTT et al., 2015; SUKUMARAN et al., 2015; WALSH et al., 2018; ZHANG et al., 2019).

Peracetic acid (PAA), also known as peracetic acid or peroxyacid, is a mixture of peracetic acid, acetic acid, hydrogen peroxide, and 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP), optionally octanoic acid and sulfuric acid. Its use in raw poultry products has been approved by the Food and Drug Administration (FDA) at a maximum concentration of 2,000 ppm peroxyacids (USDA-FSIS, 2021).

1.11 Regulatory framework in the evaluation of the safety and efficacy of peracetic acid solutions

PAA solutions are produced from water, acetic acid, hydrogen peroxide (HP), and HEDP. Alternatively, octanoic acid and sulfuric acid can be used. PAA formation is the result

of an equilibrium reaction between HP and acetic acid. Although acetic acid and hydrogen peroxide are known to have antimicrobial properties, their effects within these solutions are minimal. Acetic acid reacts with hydrogen peroxide to generate peracetic acid with which it is in equilibrium (BERTRAM et al., 2019; EFSA, 2014a) .

Hence, the amount and presence of acetic acid and hydrogen peroxide are critical to the concentration of peracetic acid and thus the antimicrobial effect. HEDP has no antimicrobial effect, it functions as a stabilizer in the solution, preventing metal ions from catalyzing the degradation of peracetic acid and hydrogen peroxide (JECFA, 2006).

Acetic acid is oxidized by hydrogen peroxide, resulting in peracetic acid and water, this reaction tends to equilibrium. The percentage of conversion of acetic acid into peracetic acid depends on the relative molar ratio between the reactants, their concentrations and the presence of the acid catalyst. Generally based on formulated peracetic acid, it uses the substance of HEDP as a catalyst and stabilizer of the formulation. It is known that peracetic acid solutions are considered stable, as well as the concentrated product. The most common market concentrations range from 10% to 15%, mainly due to reactivity and instability at higher concentrations. Studies on the kinetics of synthesis and decomposition of peracetic acid show that the use of substances with sequestering characteristics, such as HEDP, increases the shelf life of concentrated products by approximately one year after manufacture (JECFA, 2006). Zhang, 2018 in his study on thermodynamic properties of an emerging chemical disinfectant, peracetic acid reports that Oxidizing acetic acid with hydrogen peroxide in the presence of a catalyst such as sulfuric acid is a common approach for PAA synthesis or perhydrolysis (2018).

The maximum use concentration of PAA, hydrogen peroxide, and HEDP for application in poultry products are described in Table 2:

Table 2: Allowed concentration of PAA, H_2O_2 and HEDP solutions in industrial poultry product operations

Intended Use	PAA (ppm)	H_2O_2 (ppm)	HEDP (ppm)
Water used in processing, ice or brine, spraying, immersion, rinsing, cooling system, low temperature immersion baths (below 4.4°C), or scalding water for whole or cut poultry, including carcasses, parts and organs.	2000	1474	136

Source:(FDA, 2020)

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) estimated the intake of each component of the peracetic acid solution based on the residual amounts expected to be present in the treated food (meat and vegetables) at the time of consumption (JECFA, 2006).

The ingestion of acetic acid is not a concern, since its use as vinegar on food would result in a much higher exposure than using peracetic antimicrobial solutions (EFSA, 2014b).

JECFA indicates in its conclusion that because the peroxide compounds reactivity, only octanoic acid, acetic acid and HEDP remained in foods that are treated with the antimicrobial solution and that are not subsequently washed, processed or cooked (JECFA, 2006).

The EFSA assessment of 2005 cites experiments performed to establish residues of peracetic acid, peroxyoctanoic acid and HEDP (EFSA, 2005). In these experiments, the residues of peroxyacids and hydrogen peroxide in chicken carcasses after 2, 5 and 10 min of peroxyacid spraying (200 mg/L) and immersion for 60 min at below 4 °C were below the detection limit of 1 mg/L. Because of the low levels of peroxide compounds observed and the chemical instability/reactivity, it is unlikely that these substances will remain on the poultry

carcasses and therefore there is no need to perform a safety assessment for these substances. Regarding HEDP, six chicken carcasses were treated with two different solutions. Solution 1 contained 200 mg / L peroxyacids (as peracetic acid) and 10 mg / L HEDP, and solution 2 contained 30 mg / L peroxyacids and 1.5 mg / L HEDP. All chicken carcasses were sprayed for 15s with solution 1 at room temperature. Three of the chicken carcasses were then immersed for 60 min in a 3ºC bath with solution 1, and the other three chicken carcasses were immersed for 60 min in a 2ºC bath with solution 2. Chicken carcasses treated with solution 1 in the bath gave a residual amount of 120-170 μg HEDP per kg carcass. In the case of solution 2 in the bath, the residual amount was 40-50 μg HEDP per kg carcass, close to detection limit (LOD).

Based on the previous information, it can be seen that HEDP can remain in the carcasses after treatment, which are not subjected to further washing or processing.

According to the JECFA risk assessment, several studies (human, rat, rabbit, dog, and monkey) have been conducted on the elimination of HEDP after oral administration. Collectively, the data indicated that the absorption of HEDP from the gastrointestinal tract is quite limited and that its metabolism is insignificant. Some accumulation in bone has been observed, with a half-life in rats of about 12 days (JECFA, 2006).

HEDP did not induce mutations in a bacterial gene mutation assay (Ames test) nor in an in vitro mammalian cell gene mutation test in L5178Y TK +/- mouse lymphoma cells (JECFA, 2006).

It is worth noting that studies related to HEDP absorption in humans and animals corroborate the safety of the stabilizer with respect to immersion bath at high concentrations. This conclusion, though, is only applicable for PAA working solutions containing up to 130 mg HEDP / L in combination with 3-minute immersion times. When longer contact times are applied, EFSA recommends that HEDP concentrations should be correspondingly reduced.

Furthermore, as already mentioned, for long duration baths, HEDP residues remain on carcasses after microbial treatment (EFSA, 2014a).

Various amino acids and amino acid derivative compounds, such as peptides and proteins, can be oxidized by the peroxyacids present in the PAA solution (EFSA, 2005). Although there are several possibilities for amino acid oxidation, it was concluded that "no significant level of amino acid by-products will be produced after peroxyacid treatment, since the levels of free amino acids in chicken meat just before aging are very low" (EFSA, 2008). The application of peroxyacids solution can also cause oxidation of fatty acid lipids with one or more double bonds(EFSA, 2008). In this regard, in 2005, EFSA concluded that no significant differences were observed in TBARS (thiobarbituric acid reactive substances) values or fatty acid profiles when comparing treated samples with raw or cooked samples (EFSA, 2005).

Both the risk assessments conducted by JECFA and EFSA conclude that the use of the evaluated solutions is not of health concern.

The scientific opinion on the evaluation of the safety and efficacy of peracetic acid solutions for pathogen reduction in poultry carcasses and meat (EFSA, 2014a), concludes that no toxicity concerns have been identified regarding peroxyacid residues due to the high instability described. No concerns are indicated, either, regarding residual acetic acid and octanoic acid.

Moreover, concerning the question of the safety of possible reaction products of hydrogen peroxide and peroxyacids with lipids and proteins/amino acids from poultry carcasses, due to the low content of amino acids on the carcass surface it was concluded that no risk was expected, including short-term treatment with higher peroxide concentrations (EFSA, 2014a).

In regards to lipid peroxidation, no by-products were identified in the experiments present in the EFSA, 2005 risk assessment (EFSA, 2005, 2014a).

Given the aforementioned, regarding the safety and efficacy of peracetic acid solutions, EFSA (2014a) corroborates with the safety of PAA, however, as far as the stabilizer HEDP is concerned, with regard to long term immersion baths, it is recommended to control HEDP residues in poultry carcasses and the monitoring of HEDP concentration in the PAA working solution should be considered in HACCP plans. The recommendations provided in the scientific opinion of the European Food Safety Authority support the risk assessment of the HEDP stabilizer based on the mentioned studies.

1.12 1-Hydroxyethylidene 1,1-disphosphonic acid (HEDP) Stabilizer

1-Hydroxyethane-l, l-diphosphonic acid (HEDP) belongs to a group of synthetic chelating agents, which are widely used for various applications, for example as corrosion inhibitors, antioxidants, antifouling agents, dispersants in cooling water circulations, and as building blocks in industrial cleaning products and detergents. Due to its ability to slow or prevent the precipitation of minerals, especially Ca salts from aqueous solutions at substoichiometric concentrations (threshold effect), HEDP has found preferential application in the area of water conditioning and water softening, partially in combination with Zeolite (FISCHER, 1993).

As recommended by EFSA (2014a) a method for the determination of HEDP residues in poultry carcasses, poultry meat and poultry meat products should be developed and validated.

Figure 3, demonstrates the minimum molecular structure of the HEDP molecule defined as $C_2H_8O_7P_2$. By basic stoichiometry, each molecule of HEDP has two moles of phosphorus, that is, phosphorus works as an indicator of the presence of HEDP, because in the other raw materials used in the composition of PAA, there is no phosphorus present.

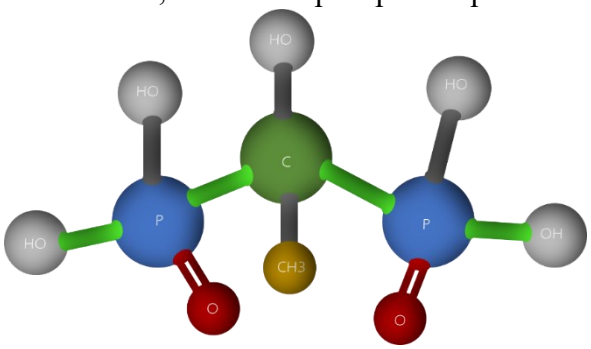


Fig. 3: Molecular Structure HEDP- C2H8O7P2

Source: Own

To understand the mechanism of HEDP as a stabilizer for PAA, it is important to note that peracetic acid dissociates rapidly into water and acetic acid, especially upon contact with proteins (GAMBLE et al., 2016; WALSH et al., 2018) . Peracetic acid provides only a large initial reduction in bacteria due to the organic material containing nitrogen in the muscle. After one minute of contact time, there is no measurable PAA residue remaining and therefore HEDP is an additive present in the peracetic composition to prevent precipitations in chemical reactions and allow it to happen (JECFA, 2006; MESROBIAN, 2009) .

Since HEDP is essential to stabilize the reaction and according to EFSA's study that ensures that HEDP residues remain on carcasses after microbial treatment for long duration immersions, the search for HEDP residues in meat matrix is relevant in cold storage processes, thus ensuring the LOD (EFSA, 2014a) .

1.13 Impact of using organic acids on meat technological aspects

It is known that one of the significant factors influencing consumer preference for chicken meat is the meat color at the point of purchase, an important attribute in the appearance evaluation. Color preferences are a subjective characteristic of meat, as perceived by the consumer. Consumers tend to prefer chicken meat that is very similar in color to what they are used to consume (MANJANKATTIL et al., 2021).

To measure, classify and reproduce color readings, it was necessary to develop objective instrumental parameters. The food color is most often measured in terms of the CIE scale, where the L *, a *, b * values plus the shade angle and chroma are quantified. The CIE Lab color space defines the L *, a * and b * values in relation to an international color standard measurement, adopted by the Commission International Eclairage (CIE) in 1976. In this method, the L * value is the brightness component, ranging from 0 to 100 (black to white); a * and b * range from -120 to +120 with a * ranging from green if it is negative to red if it is positive and b * ranging from blue if it is negative to yellow if it is positive(AN et al., 2010; YAM; PAPADAKIS, 2004). The poultry meat color is instrumentally measured by means of a colorimeter that measures the CIE values L *, a * and b *; the colorimeters only measure a meat area between 2-5 cm^2 (MANJANKATTIL et al., 2021).

Studies evaluating the color change in chicken legs treated with lactic acid were not satisfactory when compared to the control sample. Experiments conducted between 2003 to 2017 on red meat cuts treated with lactic acid by immersion with concentration of 2%, 4% and 6%, found that meat treated with lactic acid had higher L* values compared to untreated meat (MANZOOR et al., 2020; WIDEMAN; O'BRYAN; CRANDALL, 2016).

Studies that addressed color analysis in chicken meat treated with malic acid, benzoic acid both with concentration ranged from 0.1 mg/mL to 5 mg/mL and citric acid (2%), found that these acids did not cause significant changes when compared to control samples (WIDEMAN; O'BRYAN; CRANDALL, 2016).

The texture, mainly of chicken breast, is a subjective meat characteristic that is relevant in the definition of product quality by the consumer. Antimicrobial treatments can affect these characteristics (WIDEMAN; O'BRYAN; CRANDALL, 2016) because they generally have a low pH, which can cause a sharp decrease in pH after slaughter. If the pH value decreases rapidly right after slaughter, it can cause alterations in the muscle structure, resulting in pale, flaccid meat with low water retention capacity, which is then called PSE (pale, soft, exudative). For instance, PAA has a very low pH (2.6 - 3.4) and it is essential to study its reaction on the sensory aspect of meat (KATARIA et al., 2020).

For this reason, studies that corroborate the identification or not of sensory alterations in meats submitted to antimicrobial treatment are necessary.

Among the most widely used techniques to evaluate the meat resistance to mechanical and crumbling effects are those that apply shear force, which measures the force required to cut food, using universal texturometers. To help instrumental evaluation of the tenderness in meats, texturometers use specific blades for each type of material, the Warner Bratzlerblade model has been one of the most used for chicken meat analysis. Lyon and Lyon (AN et al., 2010; 1998) emphasize the importance of uniformity and sample size as well as orientation of the muscle fibers in relation to the cutting blades used in instrumental testing to ensure uniform results. In shear tests, that use Warner Bratzler type blades, the samples to be analyzed must be uniform and the cut through the blade must be perpendicular to the direction of the muscle fibers (AN et al., 2010; LYON; LYON, 1998).

Another study to evaluate the identity and quality of products with great applicability is the sensory analysis by trained judges, through the discriminative control difference test. It determines the probability of difference or similarity between two products, that is, if there is a significant difference between the test and control (untreated) samples, and estimates their degree of difference. The judges perform the recognition of basic tastes and aromas, and present discrimination of samples by triangular tests, in order to ensure recognition of the difference among the evaluated products (LAWLESS; HEYMANN, 1984).

Regarding the technological effects, Nagel et al. (2013) evaluated the sensory attributes in chicken (breast) meat treated with antimicrobial solutions containing peracetic acid, including appearance, flavor, texture, juiciness and general acceptance after treatment and after 24 hours of storage at +4°C. There were no significant differences ($P \leq 0.05$) between chicken breast treated with 400 ppm and 1,000 ppm peracetic acid solution in immersion in post chiller system when compared to the control sample considering the appearance attribute. The chicken meat appearance after all treatments was rated as "slightly equal". Similarly, the panelists were unable to determine differences between the various treatments for the flavor attribute.

The treatment with 1,000 ppm peracetic acid was rated as "moderately tender," while the treatment with 400 ppm was identified as "slightly tender" for the texture sensory evaluation. When the participants evaluated the juiciness of the breast fillets, none of the treatments showed significant differences ($P \leq 0.05$) from the control sample and were perceived as "slightly moist". Bauermeister et al. (2008) , also evaluated sensory aspects and in their study, the application of PAA in different concentrations did not differ significantly from the control ($P < 0.05$).

Manjankattil (2021)evaluated the subjective characteristic of meat regarding color and in his study, the color evaluation parameters L* a*b* did not differ significantly between the control sample and the sample treated with PAA ($P < 0.05$).

It is relevant to highlight that among the articles selected for the review, only the aforementioned authors approached the study regarding the sensory aspects of meat; however, two of them approached consumer perception through sensory analysis and only one brought the subjective analysis of color.

No articles were found that addressed the meat subjective characteristic of tenderness. It is known that the reduction in pH can directly influence the meat quality, particularly tenderness, thus studies that address the subjective analysis of tenderness are relevant. Furthermore, in general, there is a need for further studies to investigate the changes that can influence the meat's subjective characteristics and consumer perception.

1.14 Use of peracetic acid as an antimicrobial in chicken

Figure 4 represents the steps of the systematic review process summarized in sixty-three references with pertinent data related to the poultry process and the use of the antimicrobial peracetic acid.

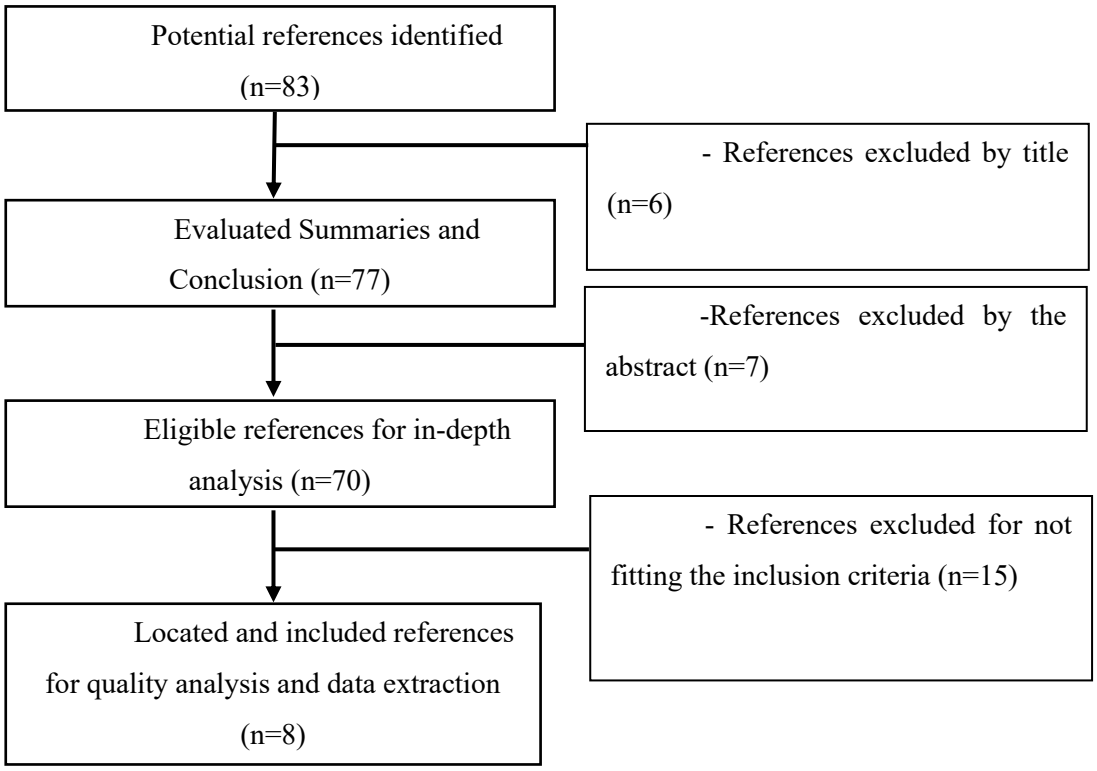


Fig. 4: Flow diagram representing the systematic review process results

The studies found to support this review were sufficient for structuring the bibliographic information and detailing this information, for example, type of bacteria analyzed, the treatment applied with its controlled process parameters (immersion, spraying, chemical concentration and application time), among others. In this way, the diagram contributes to the selection of key articles to be prioritized.

1.15 Experimental summary on the effectiveness of PAA and other antimicrobials

Alongside PAA, a range of antimicrobials are being researched for decontamination in poultry meat.

Table 3 presents research studies that address the effectiveness of these antimicrobials and their comparison with PAA. The addressed antimicrobial were caprylic acid, chlorine, cetylpyridinium chloride, combination of sulfuric acid and sodium sulfate (Amplon), use of specific phages, lauric arginate, and lysozyme.

Table 3: Comparison of PAA treatment effectiveness with other antimicrobials for decontamination in chicken meat

Reference	Bacteria	Sample	Environment	Application	Treatment	Reduction (log10 UFC/g)
(AE KIM et al., 2017)	Total mesophil count	Chicken carcass	After chiller	Immersion	750 ppm PAA/ 15 sec	4,08
(ZHANG et al., 2019)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	0,60% CPC/10 sec	≅ 3,7
(SUKUMARAN et al., 2015)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	0,60% CPC/20 sec	≅ 3,7
(SUKUMARAN et al., 2015)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	0,60% CPC/30 sec	≅ 3,7
(SUKUMARAN et al., 2015)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	700 ppm PAA /10 sec	3,50
(SUKUMARAN et al., 2015)	Salmonella Thyphymurium	Chicken Thigh	Laboratory	Immersion	0,60% CPC/30 sec	≅ 3,5
(SUKUMARAN et al., 2015)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	0,35% CPC/10 sec	≅ 3,5
(SUKUMARAN et al., 2015)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	0,35% CPC/ 20 sec	≅ 3,5

(SUKUMARAN et al., 2015)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	0,35% CPC/30 sec	≅ 3,5
(AE KIM et al., 2017)	*Campylobacter*	Chicken carcass	Before evisceration	Aspersion	Amplon (ph 1,3)	3,25
(KATARIA et al., 2020)	*Campylobacter coli*	Chicken Wing	Laboratory	Immersion	500 ppm PAA/10 sec	≅ 2,5
(KATARIA et al., 2020)	*Campylobacter coli*	Chicken Wing	Laboratory	Immersion	500 ppm PAA/60min	≅ 2,5
(SUKUMARAN et al., 2015)	*Salmonella Thyphymurium*	Chicken Thigh	Laboratory	Immersion	0,60% CPC/20 sec	≅ 2,5
(SUKUMARAN et al., 2015)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	1000 ppm PAA /10 sec	≅ 2,5
(SUKUMARAN et al., 2015)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	1000 ppm PAA /20 sec	≅ 2,5
(SUKUMARAN et al., 2015)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	1000 ppm PAA /30 sec	≅ 2,5
(MANJANKATTIL et al., 2021)	Salmonella Heidelberg	Chicken Thigh	Laboratory	Immersion	1% CA + 500 ppm de PAA	2,34
(SUKUMARAN et al., 2015)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	700 ppm PAA /20 sec	2,30
(AE KIM et al., 2017)	*Campylobacter*	Chicken carcass	After chiller	Immersion	750 ppm PAA/ 15 sec	2,23
(SUKUMARAN et al., 2015)	Salmonella	Chicken Skin	After chiller	Immersion	400 ppm PAA+ Phage/20 sec	2,2 - 2,5
(SUKUMARAN et al., 2015)	Salmonella	Chicken Skin	After chiller	Immersion	50 ppm PAA + Fago/ 20 sec	2,2
(NAGEL et al., 2013)	Salmonella Thyphymurium	Chicken carcass	After chiller	Immersion	1000 ppm PAA/ 20 sec	2,14
(NAGEL et al., 2013)	*Campylobacter jejuni*	Chicken carcass	After chiller	Immersion	1000 ppm PAA/ 20 sec	2,03
(NAGEL et al., 2013)	Salmonella Thyphymurium	Chicken carcass	After chiller	Immersion	400 ppm PAA/ 20 sec	2,02
(KATARIA et al., 2020)	*Campylobacter coli*	Chicken Wing	Laboratory	Immersion	50 ppm PAA/10 sec	≅ 2
(KATARIA et al., 2020)	*Campylobacter coli*	Chicken Wing	Laboratory	Immersion	50 ppm PAA/60 min	≅ 2
(SUKUMARAN et al., 2015)	Salmonella Thyphymurium	Chicken Thigh	Laboratory	Immersion	700 ppm PAA /10 sec	≅ 2
(SUKUMARAN et al., 2015)	Salmonella Thyphymurium	Chicken Thigh	Laboratory	Immersion	700 ppm PAA /20 sec	≅ 2

(SUKUMARAN et al., 2015)	Salmonella Thyphymurium	Chicken Thigh	Laboratory	Immersion	700 ppm PAA /30 sec	≅ 2
(SUKUMARAN et al., 2015)	Salmonella Thyphymurium	Chicken Thigh	Laboratory	Immersion	1000 ppm PAA /10 sec	≅ 2
(SUKUMARAN et al., 2015)	Salmonella Thyphymurium	Chicken Thigh	Laboratory	Immersion	1000 ppm PAA /20 sec	≅ 2
(SUKUMARAN et al., 2015)	Salmonella Thyphymurium	Chicken Thigh	Laboratory	Immersion	1000 ppm PAA /30 sec	≅ 2
(SUKUMARAN et al., 2015)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	700 ppm PAA /30 sec	2,00
(NAGEL et al., 2013)	*Campylobacter jejuni*	Chicken carcass	After chiller	Immersion	400 ppm PAA/ 20 sec	1,93
(KUMAR et al., 2020)	Salmonella Typhimurium	Chicken Breast	Laboratory	Immersion	1000 ppm PAA /30 sec	1,92
(KUMAR et al., 2020)	*Campylobacter coli*	Chicken Breast	Laboratory	Immersion	1000 ppm PAA /30 sec	1,87
(KATARIA et al., 2020)	Salmonella Typhimurium	Chicken Wing	Laboratory	Immersion	500 ppm PAA /60min	≅ 1,8
(KUMAR et al., 2020)	Salmonella Typhimurium	Chicken Breast	Laboratory	Immersion	500 ppm PAA /30 sec	1,77
(SUKUMARAN et al., 2015)	Salmonella	Chicken Skin	After chiller	Immersion	400 ppm PAA /20 sec	1,7
(SUKUMARAN et al., 2015)	Salmonella	Chicken Skin	After chiller	Immersion	Chlorine 30 ppm + Phage/ 20 sec	1,7
(KUMAR et al., 2020)	*Campylobacter coli*	Chicken Breast	Laboratory	Immersion	500 ppm PAA /30 sec	1,68
(SUKUMARAN et al., 2015)	Salmonella Thyphymurium	Chicken Thigh	Laboratory	Immersion	0,35% CPC/10 sec	≅ 1,6
(SUKUMARAN et al., 2015)	Salmonella Thyphymurium	Chicken Thigh	Laboratory	Immersion	0,35% CPC/ 20 sec	≅ 1,6
(SUKUMARAN et al., 2015)	Salmonella Thyphymurium	Chicken Thigh	Laboratory	Immersion	0,35% CPC/30 sec	≅ 1,6
(SUKUMARAN et al., 2015)	Salmonella Thyphymurium	Chicken Thigh	Laboratory	Immersion	0,60% CPC/10 sec	≅ 1,6
(MANJANKATTI L et al., 2021)	Salmonella Heidelberg	Chicken Breast	Laboratory	Immersion	1% CA + 1200 ppm de PAA	1,57
(AE KIM et al., 2017)	*Campylobacter*	Chicken carcass	After chiller	Immersion	Amplon (Ph 1,4/ 15 sec)	1,53
(MANJANKATTI L et al., 2021)	Salmonella Heidelberg	Chicken Thigh	Laboratory	Immersion	0,5% CA + 500 ppm de PAA	1,52

(KUMAR et al., 2020)	Salmonella Typhimurium	Chicken Breast	Laboratory	Aspersion	1000 ppm PAA /10 sec	1,45
(MANJANKATTI L et al., 2021)	*Salmonella Heidelberg*	Chicken Breast	Laboratory	Immersion	2% CA	1,41
(KUMAR et al., 2020)	Salmonella Typhimurium	Chicken Breast	Laboratory	Immersion	250 ppm PAA /30 sec	1,33
(KATARIA et al., 2020)	Salmonella Typhimurium	Chicken Wing	Laboratory	Immersion	50 ppm PAA /60 min	≅ 1,3
(BAUERMEISTER et al., 2008)	Salmonella Thyphymurium	Chicken carcass	Chiller	Immersion	200 ppm PAA/10 min	1,30
(MANJANKATTI L et al., 2021)	Salmonella Heidelberg	Chicken Breast	Laboratory	Immersion	1200 ppm PAA/2min	1,25
(MANJANKATTI L et al., 2021)	Salmonella Heidelberg	Chicken Breast	Laboratory	Immersion	2% CA + 1200 ppm de PAA	1,25
(KUMAR et al., 2020)	*Campylobacter coli*	Chicken Breast	Laboratory	Aspersion	1000 ppm PAA /10 sec	1,24
(KUMAR et al., 2020)	Salmonella Typhimurium	Chicken Breast	Laboratory	Aspersion	500 ppm PAA /10 sec	1,23
(KUMAR et al., 2020)	*Campylobacter coli*	Chicken Breast	Laboratory	Immersion	250 ppm PAA /30 sec	1,23
(SUKUMARAN et al., 2015)	Salmonella	Chicken Skin	After chiller	Immersion	CPC 0,2% +Fago/ 20 sec	1,2-1,3
(BAUERMEISTER et al., 2008)	Salmonella Thyphymurium	Chicken carcass	Chiller	Immersion	100 ppm PAA/10 min	1,20
(MANJANKATTI L et al., 2021)	Salmonella Heidelberg	Chicken Breast	Laboratory	Immersion	1% CA	1,19
(AE KIM et al., 2017)	*Campylobacter*	Chicken carcass	Chiller Simulator	Immersion	Amplon (Ph 1,4/ 15 sec)	1,15
(KUMAR et al., 2020)	Salmonella Typhimurium	Chicken Breast	Laboratory	Immersion	100 ppm PAA /30 sec	1,01
(KUMAR et al., 2020)	Salmonella Typhimurium	Chicken Breast	Laboratory	Aspersion	250 ppm PAA /10 sec	1,01
(KATARIA et al., 2020)	Salmonella Typhimurium	Chicken Wing	Laboratory	Immersion	500 ppm PAA/10 sec	≅ 1
(SUKUMARAN et al., 2015)	Salmonella	Chicken Skin	After chiller	Immersion	LAE 200 PPM +fago/ 20 sec	0,8 - 1
(MANJANKATTI L et al., 2021)	Salmonella Heidelberg	Chicken Thigh	Laboratory	Immersion	1% CA	0,98
(BAUERMEISTER et al., 2008)	Salmonella Thyphymurium	Chicken carcass	Chiller	Immersion	25 ppm PAA/10 min	0,90

(KUMAR et al., 2020)	*Campylobacter coli*	Chicken Breast	Laboratory	Aspersion	500 ppm PAA /10 sec	0,85
(NAGEL et al., 2013)	Salmonella Thyphymurium	Chicken carcass	After chiller	Immersion	40 ppm chlorine / 20 sec	≅0,8
(NAGEL et al., 2013)	Salmonella Thyphymurium	Chicken carcass	After chiller	Immersion	5000 ppm Lisozima/ 20 sec	≅0,8
(BAUERMEISTER et al., 2008)	*Campylobacter jejuni*	Chicken carcass	Chiller	Immersion	200 ppm PAA/10 min	0,80
(KUMAR et al., 2020)	*Campylobacter coli*	Chicken Breast	Laboratory	Immersion	100 ppm PAA /30 sec	0,78
(KUMAR et al., 2020)	*Campylobacter coli*	Chicken Breast	Laboratory	Aspersion	250 ppm PAA /10 sec	0,74
(MANJANKATTIL et al., 2021)	Salmonella Heidelberg	Chicken Thigh	Laboratory	Immersion	0,5% CA	0,70
(NAGEL et al., 2013)	Salmonella Thyphymurium	Chicken carcass	After chiller	Immersion	40 ppm chlorine/ 20 sec	≅0,7
(NAGEL et al., 2013)	*Campylobacter jejuni*	Chicken carcass	After chiller	Immersion	40 ppm chlorine / 20 sec	≅0,7
(NAGEL et al., 2013)	*Campylobacter jejuni*	Chicken carcass	After chiller	Immersion	5000 ppm Lisozima/ 20 sec	≅0,7
(KUMAR et al., 2020)	Salmonella Typhimurium	Chicken Breast	Laboratory	Aspersion	100 ppm PAA /10 sec	0,65
(SUKUMARAN et al., 2015)	Salmonella	Chicken Skin	After chiller	Immersion	CPC 0,2%/ 20 sec	0,6 - 0,7
(NAGEL et al., 2013)	Salmonella Thyphymurium	Chicken carcass	After chiller	Immersion	Water	≅0,6
(NAGEL et al., 2013)	*Campylobacter jejuni*	Chicken carcass	After chiller	Immersion	40 ppm chlorine / 20 sec	≅0,6
(KATARIA et al., 2020)	Salmonella Typhimurium	Chicken Wing	Laboratory	Immersion	50 ppm PAA/10 sec	≅0,57
(SUKUMARAN et al., 2015)	Salmonella	Chicken Skin	After chiller	Immersion	Chlorine 30 ppm/ 20 sec	0,5 -0,6
(SUKUMARAN et al., 2015)	Salmonella	Chicken Skin	After chiller	Immersion	LAE 200 PPM/ 20 sec	0,5 -0,6
(SUKUMARAN et al., 2015)	Salmonella Thyphymurium	Chicken Thigh	Laboratory	Immersion	0,003% Chlorine/ 10 sec	≅0,5
(SUKUMARAN et al., 2015)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	0,003% Chlorine/ 10 sec	≅0,5
(SUKUMARAN et al., 2015)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	0,003% Chlorine/ 20 sec	≅0,5

(SUKUMARAN et al., 2015)	*Campylobacter jejuni*	Chicken Thigh	Laboratory	Immersion	0,003% Chlorine/ 30 sec	≅0,5
(AE KIM et al., 2017)	Total mesophil count	Chicken carcass	Chiller Simulator	Immersion	Amplon (Ph 1,4/ 15 sec)	≅0,5
(NAGEL et al., 2013)	*Campylobacter jejuni*	Chicken carcass	After chiller	Immersion	Water	≅0,5
(SUKUMARAN et al., 2015)	Salmonella	Chicken Skin	After chiller	Immersion	50 ppm PAA	0,4 - 0,6
(AE KIM et al., 2017)	Total mesophil count	Chicken carcass	Before evisceration	Aspersion	Amplon (ph 1,3)	≅ 0,4
(BAUERMEISTER et al., 2008)	*Campylobacter jejuni*	Chicken carcass	Chiller	Immersion	25 ppm PAA/10 min	0,40
(SUKUMARAN et al., 2015)	Salmonella Thyphymurium	Chicken Thigh	Laboratory	Immersion	0,003% Chlorine/ 20 sec	≅ 0,3
(BAUERMEISTER et al., 2008)	*Campylobacter jejuni*	Chicken carcass	Chiller	Immersion	100 ppm PAA/10 min	0,30
(KUMAR et al., 2020)	*Campylobacter coli*	Chicken Breast	Laboratory	Aspersion	100 ppm PAA /10 sec	0,28
(SUKUMARAN et al., 2015)	Salmonella Thyphymurium	Chicken Thigh	Laboratory	Immersion	0,003% Chlorine/ 30 sec	≅ 0,2
(MANJANKATTIL et al., 2021)	Salmonella Heidelberg	Chicken Thigh	Laboratory	Immersion	500 ppm PAA/2min	NG
(AE KIM et al., 2017)	Total mesophil count	Chicken carcass	After chiller	Immersion	Amplon (Ph 1,4/ 15 sec)	NG
(AE KIM et al., 2017)	Salmonella	Chicken carcass	After chiller	Immersion	750 ppm PAA/ 15 sec	Qualitative Analysis
(AE KIM et al., 2017)	Salmonella	Chicken carcass	Before evisceration	Aspersion	Amplon (ph 1,3)	Qualitative Analysis
(AE KIM et al., 2017)	Salmonella	Chicken carcass	Chiller Simulator	Immersion	Amplon (Ph 1,4/ 15 sec)	Qualitative Analysis
(AE KIM et al., 2017)	Salmonella	Chicken carcass	After chiller	Immersion	Amplon (Ph 1,4/ 15 sec)	Qualitative Analysis

Key1:Ca: Caprilic Acid; PC: Positico Control; NG: No grow detected; CPC: Chloride of Cetilpiridinio; LAE: arginate lauric

Most of the studies focused on relevant bacteria in the poultry process, such as Salmonella Heidelberg, Salmonella Thyphymurium, *Campteriaylobacter jejuni, Campylobacter coli*, as well as indicator bacteria and total mesophil count.

Different cuts were evaluated in the studies, among them chicken breast and cuts with skin, thigh, wing, skin, and chicken carcass. The range of cuts allowed a detailed analysis of the antimicrobials effectiveness in the worst case scenario, because the chicken wing and thigh cuts are completely covered with the skin and can provide protection to microorganisms and prevent antimicrobial exposure (KATARIA et al., 2020).

Most of the studies (80% of them) covered in the research were conducted in laboratory settings, with the meat matrix inoculated with a cocktail of resistant strains, treated with the antimicrobial under study, and then evaluated for log reduction of the bacteria. It is worth noting that high inoculations as in the case of laboratory scale studies, in general, do not reflect the reality of a production process, however the reduction found by studies using PAA alone or in combination with another antimicrobial, ranged from 0.65 to 4.08 log where 85% of the results, showed reduction above 1 log, which is prominently expressive.

The way of applying antimicrobials that has been most addressed in the studies is the immersion of chicken cuts or carcasses for a controlled time and concentration, the longer the immersion time, the lower the concentration used.

Kumar et al. (KUMAR et al., 2020) evaluated both immersion and spray methods with chicken breast fillets treated with PAA. The study highlighted that all levels of PAA reduced log of Salmonella reaching a 1.45 log reduction in the sprinkling process when the highest concentration (1000 ppm) was used and 1.92 log reduction in the cut immersion process, also at the highest concentration. It can be observed that for both addressed methods, the increase in logarithmic reduction occurred as the concentration of the antimicrobial solution increased. The study also showed satisfactory results for the reduction of *Campylobacter Coli*, where at the

highest concentration a 1.87 log and 1.24 log reduction were obtained for the immersion and aspersion processes respectively. Thus, the application of the PAA treatment by immersion was more effective in reducing Salmonella and *Campylobacter* populations in chicken breast fillets when compared to the spray method; however, the results found with the spray method of application were also significant.

An interesting approach to antimicrobial application methods was the evaluation of chicken carcasses after the scalding step, before entering the evisceration process. The study in focus evaluated the effectiveness of the Amplon antimicrobial by the carcass sprinkling method and assessed the total mesophil count (APCs) and *Campylobacter* counts. The reduction of APCs on chicken carcasses did not show significant differences in the bacteria recovered ($P< 0.05$) between the control and treated groups, indicating that spraying Amplon did not affect the APC populations on chicken carcasses; but, regarding *Campylobacter*, spraying with the antimicrobial resulted in a log reduction of 3.25 (AE KIM et al., 2017). This application strategy after the scalding stage contributes to the reduction of the microbial load throughout the production process and not only at the end of the process, since the stage before the treatment (scalding) may result in the initial fixation of bacteria on the skin, carrying the contamination along the subsequent stages of processing (MCBRIDE et al., 1980).

Manjankattil et al. (MANJANKATTIL et al., 2021) , present two compounds, caprylic acid (CA) and PAA, that were tested, alone or in combination, both compounds resulted in a significant reduction in the survival of Salmonella Heidelberg in breasts and thighs with PAA applied alone or in combination has been the most effective of all treatments in the study, achieving a 2.34 log reduction in the combination of 1%CA + 500 ppm PAA.

Kataria et al. (KATARIA et al., 2020) evaluated the effectiveness of PPA on chicken wing cuts treated with 50 and 500 ppm PPA for 10 seconds and 60 minutes of immersion. The survey showed better results for *Campylobacter* reduction: at the highest concentration for both

10 seconds and 60 minutes the study found an approximately 2.5 log reduction in *Campylobacter* count.

Also using immersion treatment, the effectiveness of 6 different treatments of antimicrobial solutions, 0.003% chlorine, 700 ppm peracetic acid (PAA), 1000 ppm PAA, 0.35% cetylpyridinium chloride (CPC) and 0.6% CPC for 10, 20 and 30 seconds contact time on S reduction, was evaluated. Typhimurium e *Campylobacter jejuni* The S. Typhimurium study using PAA treatment reduced by approximately 2 log for both concentrations (700 and 1000 ppm) and times tested, and CPC treatment also showed a satisfactory reduction of approximately 1.6 log for all times. The results with chlorine treatment did not show significant reductions when compared to the control ($P > 0.05$). For *C. jejuni* the results treated with PAA and CPC were more effective when compared to the Salmonella results, showing at their best, reductions of approximately 3.5 log when subjected to PAA treatment and approximately 3.7 log when treated with CPC. Regarding chlorine, no significant ($P > 0.05$) reductions in microbial counts were observed (ZHANG et al., 2019).

Bauermeister et al. (BAUERMEISTER et al., 2008) conducted a comparative evaluation of chicken carcasses treated with different levels of PAA to reduce Salmonella and *Campylobacter* compared to chicken carcasses treated with chlorine in the chiller system of a commercial processing facility. The study showed that all levels of PAA reduced CFU/sample of Salmonella and *Campylobacter* significantly ($P<0.05$) more than chlorine.

Concerning post-chiller applications, another paper discussed the effectiveness of post-chiller water treatments consisting of 40 ppm total chlorine, 400 ppm or 1000 ppm peracetic acid (PAA), and 1000 ppm or 5000 ppm lysozyme against Salmonella and *Campylobacter spp.* Comparing the antimicrobial efficacy of the various antimicrobials, PAA showed the best reduction ($P \leq 0.05$) in S. Typhimurium and *Campylobacter jejuni* in chicken carcasses. The best logarithmic reductions were 2.14 and 2.03 log cfu / mL for the treatments using the highest

concentration (1000 ppm), which corroborates with the studies presented in Table 3, demonstrating that raising the concentration of PAA for the immersion system corroborates with the logarithmic reduction (NAGEL et al., 2013).

An interesting trend is the combination of PAA and a second antimicrobial agent to improve the effectiveness of the chemical antimicrobial. Sukumaran et al. (SUKUMARAN et al., 2015) evaluated chicken skin samples inoculated with a cocktail of Salmonella. Typhimurium, S. Heidelberg and S. Enteritidis immersed in an antimicrobial solution of different chemical antimicrobials (LAE lauric arginate, CPC, PAA or chlorine) for 20 seconds and then were surface treated with a Salmonella-specific bacteriophage (phage) solution applied via spray. When the phage was applied sequentially with chemical antimicrobials, all treatments resulted in significant reductions of Salmonella. The application of PAA (400 ppm) followed by the spray application of the phage, resulted in the greatest Salmonella reduction of 2.2-2.5 log CFU/cm2. In conclusion, the greatest reductions in Salmonella counts were achieved in chicken skin by sequential application of chemical antimicrobials followed by phage spray, this sequential application (chemical antimicrobial + phage) may provide additional obstacles for pathogen reduction in carcasses or poultry parts.

Peroxyacetic acid (PAA) is the most popular antimicrobial in poultry farming, replacing chlorine and other compounds. Cano et al. (2021) compared in their review research, the effectiveness of PAA versus other antimicrobials for the decontamination of raw poultry carcasses and parts. For this purpose, the author reviewed 26 articles that compared PAA with more than 20 different antimicrobials, using different applications (spray or immersion), concentrations and exposure times. The compounds often found as a comparator were chlorine, lactic acid, and cetylpyridinium chloride. The pathogens involved in the studies were mostly Salmonella and *Campylobacter*. The review also corroborates the efficacy of the antimicrobial action of PAA for pathogen control.

1.16 Final considerations

PAA used as an antimicrobial intervention for poultry, either alone or when combined with other antimicrobial compounds, is consistently effective in reducing pathogens (Salmonella and *Campylobacter*) and microbial loads in the course of the process, and is a unique strategy as technological barriers in poultry processes.

The method of immersing the cuts after the cooling system has been the most widely used in the published literature, which ensures the safety of the final product. The treatment at the end of the production process avoids possible recontamination of the product through cross contamination. Nonetheless, application at critical steps of the poultry slaughter process, such as after scalding and during evisceration, is a unique strategy to contain microbial spread, reducing the initial load of incoming bacteria and stabilizing control throughout the process.

More research is needed and should be conducted addressing studies with the strain found in the plant alongside its actual initial count. Most studies approach research by inoculating the meat matrix with a cocktail of resistant strains, applying treatment with the antimicrobial under investigation, and then evaluating the logarithmic reduction of the bacteria. It is important to emphasize that the reality of the slaughter process may bring different variables: strains that may be more resistant than the inoculation strains, initial microbial counts, and variations in the application of antimicrobials in the slaughter line. In general, the scenario inside a laboratory does not reflect the reality of a production process

In regards to the technological effects of the PAA antimicrobial on poultry meat, further studies are needed to further investigate the modifications that may influence the subjective characteristics of the meat as well as consumer perception.

Finally, to ensure the risk assessment of the HEDP stabilizer corroborating with the safety of the EAP, studies that present HEDP residuals for long-term immersion bath applications in poultry carcasses, poultry meat, and poultry meat products are of paramount importance to enable the validation of an effective method that can determine and quantify this residual. Due to the basic stoichiometry, phosphorus functions as an indicator of the presence of HEDP and perhaps, could be a pathway for the development of a method to be validated. Thus, future studies that present this approach and that corroborate the safety of the stabilizer in the formulation of PAA will contribute to the approval of meat sanitary regulations in several countries.

2 CAPÍTULO 2 - AVALIAÇÃO MICROBIOLÓGICA E FÍSICO-QUÍMICA DE CORTES DE FRANGO SUBMETIDOS À APLICAÇÃO DE ÁCIDO PERACÉTICO

RESUMO

O abate de aves em larga escala é um processo rápido e altamente automatizado o que possibilita a contaminação cruzada no decorrer do processo, caso os parâmetros de controle não sejam rigorosamente controlados. Para tal, o uso de ácidos orgânicos para a descontaminação em carnes de aves é uma estratégia importante para a redução de doenças transmitidas por alimentos. No Brasil, ainda não há a aprovação para o uso de coadjuvante de tecnologia que possam auxiliar no controle e disseminação da carga de doenças transmitidas por alimentos (DTA's) pelo MAPA. Diante do exposto, este trabalho investigou a aplicação do ácido peracético (PAA) nos cortes de peito e coxa de frango, para: (a) avaliar a eficácia do PAA como antimicrobiano em *Salmonella enterica*, por meio da redução de bactérias indicadoras de relevância no processo avícola e (b) avaliar o impacto do PAA sobre a cor, textura e perda de peso por cocção das amostras de peito de frango sem pele e coxas de frango com pele. Através do Delineamento Composto Central Rotacional (DCCR) as melhores condições das variáveis concentração e tempo de aplicação do ácido peracético nos cortes de peito e coxa de frango foram determinadas. Avaliando as características microbiológicas dos cortes tratadas com solução de ácido peracético foi possível a redução de até 2,90 para *Enterobacteriaceae* no peito de frango com as condições na região do ponto central (1500 ppm) e 3,65 também para *Enterobacteriaceae* em coxa de frango quando aplicado concentrações acima de 1800 ppm. O ácido peracético não influenciou as características tecnológicas da carne de frango uma vez que não alterou as características de aparência da carne in natura avaliadas por análises objetivas (cor, textura e perda de cozimento), o que poderia impactar na preferência e aceitabilidade do consumidor.

Palavras-chave: Microbiota de aves; segurança dos alimentos; intervenção microbiana; processo avícola; Delineamento composto central rotacional.

ABSTRACT

The poultry slaughter on a large scale is a fast and highly automated process, which makes cross-contamination possible during the process, if the control parameters are not strictly controlled. To this end, the use of organic acids for poultry meat decontamination is an important strategy for reducing foodborne illnesses. In Brazil, there is still no approval for the use of supporting technology that can help control and spread the burden of foodborne diseases (DTA's) by MAPA. Given the above, this work investigated the application of peracetic acid (PAA) in chicken breast and thigh cuts, to: (a) evaluate the effectiveness of PAA as an antimicrobial in *Salmonella enterica*, through the reduction of bacteria that indicate relevance in the poultry process and (b) evaluate the impact of PAA on the color, texture and weight loss by cooking of samples of skinless chicken breast and chicken thighs with skin. Through the Rotational Central Composite Design (DCCR) the best conditions of the variables concentration and time of peracetic acid application in chicken breast and thigh cuts were determined. Evaluating the microbiological characteristics of the cuts treated with peracetic acid solution, it was possible to reduce up to 2.90 for *Enterobacteriaceae* in chicken breast with the conditions in the central point region (1500 ppm) and 3.65 also for *Enterobacteriaceae* in chicken thigh when applied concentrations above 1800 ppm. Peracetic acid did not influence the technological characteristics of chicken meat since it did not change the appearance characteristics of fresh meat evaluated by objective analyzes (color, texture and loss of cooking), which could impact consumer preference and acceptability.

Keywords: Poultry microbiota; food safety; microbial intervention; poultry process; rotational central composite design.

2.1 MATERIAL E MÉTODOS

Os experimentos foram realizados em um abatedouro de aves localizado em Uberlândia – MG e as análises microbiológicas para Enterobactérias e Contagem de Mesófilos Aeróbios realizadas no Laboratório de Saúde Animal – Uberlândia, acreditado pela ISO/IEC 17025. As análises físico-químicas (cor, propriedades mecânicas e perda de peso por cocção) foram realizadas nos Laboratórios de Engenharia de Alimentos da Universidade Federal de Uberlândia no campus de Patos de Minas.

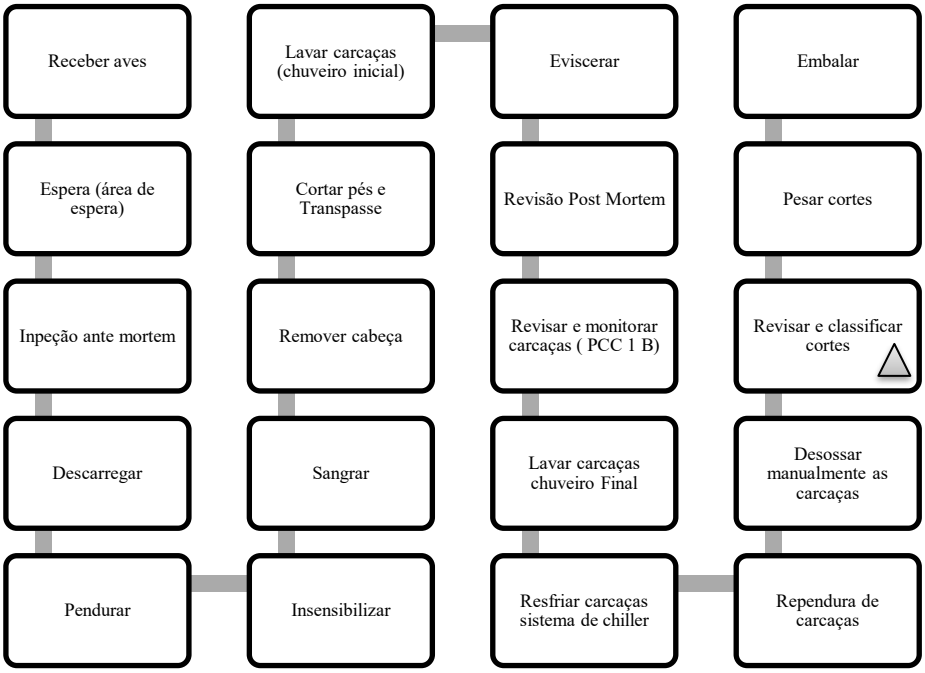
2.2 Cortes de frango

Para o teste foram selecionados dois cortes representativos em volume de produção no abatedouro, os quais representam desafios microbiológicos diferentes (um corte sem pele e outro com pele), sendo eles o peito de frango e a coxa de frango. O peito de frango representa o corte de maior valor agregado para a empresa produtora e a escolha da coxa de frango está pautada no desafio microbiológico do corte, por fornecer proteção ao microrganismo e prevenir a exposição das bactérias ao antimicrobiano.

2.3 Aplicação de ácido peracético nos cortes de frango

O ácido peracético foi aplicado aos cortes por imersão em solução antimicrobiana pós etapa de resfriamento (chiller). O fluxograma abaixo contempla o processo produtivo de abate de frango e discrimina o ponto de aplicação da solução de PAA conforme delineamento experimental que se segue.

Figura 1 - Fluxograma do processo de abate e locais na linha de aplicação da solução antimicrobiana.



△ Ponto de coleta das amostras para a aplicação do ácido peracético por imersão dos cortes em solução antimicrobiana, em uma simulação do sistema de imersão de cortes (DIP).

2.4 Equipamentos de dosagem e aplicação da solução antimicrobiana

A Figura 2 mostra o equipamento desenvolvido para realizar a imersão de cortes, que possui um sistema fechado em Inox, da marca Morris.

Figura 2 – Equipamento para imersão de cortes em solução antimicrobiana

Fonte: Empresa Morris Equipment

O estudo foi conduzido em ambiente relevante, dentro do abatedouro, porém simulando o funcionamento do equipamento de imersão “DIP”. As amostras de peito e coxa de frango foram submersas em solução antimicrobiana de ácido peracético por tempo e concentração determinados, conforme delineamento experimental. A imersão dos cortes se deu por meio de containers de polietileno com sistema manual para realizar a retirada dos cortes.

2.5 Solução Inspexx (ácido peracético)

O ácido peracético é responsável pelo efeito técnico (antimicrobiano) pretendido do produto Inspexx™ 150, nome comercial para uma mistura aquosa de ácido peracético (PAA, CAS n ° 79-21-0), peróxido de hidrogênio (HP, CAS n ° 7722-84-1), ácido acético (CAS Reg. No. 64-19-7), ácido editrônico-1-hidroxietilideno-1,1-ácido difosfônico (HEDP), Reg. CAS 2809-21-4) (EFSA, 2005; JECFA, 2006).

A composição do produto utilizado na experimentação, o Inspexx™ 150, possui concentração de ácido peracético variando de 14,4% a 16,0% (Inspexx 150®).

2.6 Avaliação microbiológica dos cortes de frango

Foram conduzidos estudos em bancada para verificar nos cortes o uso de solução antimicrobiana de ácido peracético em diferentes concentrações, tempos e geometrias, sendo avaliado a redução microbiana na superfície de carnes de frango. As amostras foram coletadas durante a linha de processamento de abate, após a etapa de revisão e classificação dos cortes. O ponto da amostra tratada representa exatamente onde o equipamento de imersão de cortes seria alocado.

Cada amostra foi enumerada para bactérias mesófilas aeróbias (CTM), utilizando água peptonada como diluente e placas de Petrifilme para Contagem de Mesófilos Aeróbios (AOAC 990,12; 3M) e para *Enterobacteriaceae* sp. com placas de Petrifilme *Enterobacteriaceae* (AOAC 2003.1- Validação: Enumeração de *Enterobacteriaceae* em Alimentos Selecionados; 3M).

A rinsagem foi realizada por meio da agitação dos cortes no diluente (água peptonada), por aproximadamente 1 minuto. As partes foram retiradas da embalagem e o líquido remanescente, homogeneizado. Desse homogeneizado foram realizadas as diluições

necessárias e destas, foram inoculados 1 mL nas placas de Petrifilm 3M, específicas para cada um dos microrganismos analisados.

Na sequência as placas foram incubadas, respeitando o binômio tempo e temperatura definidos pela metodologia, após a incubação as placas foram contadas de acordo com as orientações do fabricante e os resultados calculados e expressos em UFC/g, conforme cálculo abaixo:

$$\boldsymbol{UFC/g = ((UFC\ x\ V\ x\ D))/P}$$

Onde:
UFC = Unidades Formadoras de Colônias obtidas na placa
V = Volume de diluente usado para rinsagem (mL)
D = Diluição usada na placa
P = Peso das amostras (g)

A resposta avaliada foi a redução logarítmica das bactérias indicadoras analisadas (*Enterobacteriaceae* e Contagem de Mesófilos Aeróbios) quando comparadas com a amostra controle.

2.7 Esquematização das amostras

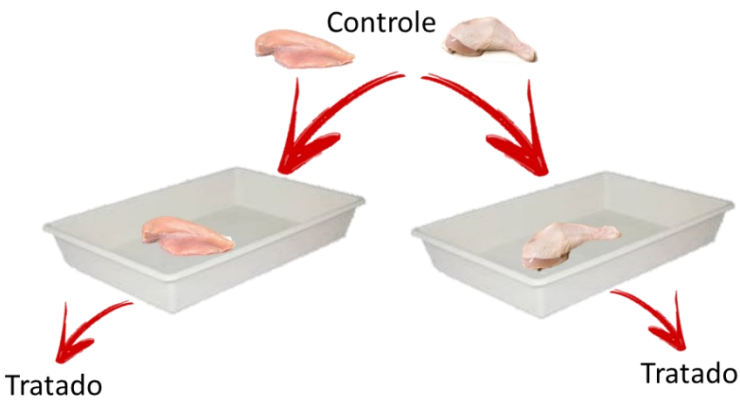
Para a imersão dos cortes, foi marcado o horário de entrada e a saída do corte no simulador do DIP, sendo as coletas das amostras realizadas conforme etapa abaixo, para cada experimento definido no DCCR.

Etapa: Foram marcado o horário de entrada e a saída do corte no simulador do DIP e dentre esses foram coletadas as amostras para o grupo “Controle “e “Tratado” da seguinte maneira:

(i) 3 amostras de peito e 3 amostras de coxas coletadas antes de entrar no simulador do DIP sem o uso do ácido peracético, identificadas como “Controle”;

(ii) 3 amostras de peito e 3 amostras de coxa coletadas após a imersão no simulador do DIP com ácido peracético, identificadas como “Tratado”.

Figura 3 - Esquema ilustrativo de amostras



2.8 Plano amostral para determinação da amostragem

A definição do tamanho amostral para a realização do experimento se baseou nas recomendações de Morettin (2006). Por meio dos resultados de análises microbiológicas realizados nos testes preliminares com o ácido peracético no frigorífico de Uberlândia, a definição do número de amostras se deu utilizando a média e desvio padrão dos dados para o ponto de aplicação pós resfriamento.

O dimensionamento foi realizado considerando o intervalo de confiança de 90% e erro aceitável de 5% sobre a média, conforme equação abaixo.

$$\boldsymbol{n = (z\alpha/2.\sigma)\text{^}2)/E}$$

Onde:

n – número de amostras

Zα/2 – para o nível de confiança desejado de 90% o valor de Z = 1,645

σ – Desvio padrão

E – Erro de 5%, calculado pela média

2.9 Delineamento Composto Central Rotacional para o sistema de imersão dos cortes em solução de ácido peracético

Para a determinação da amostragem no processo de imersão dos cortes (simulação do sistema DIP), utilizou-se o histórico dos resultados de análise microbiológica, realizados no teste *offline* com o ácido peracético no abatedouro em questão. Considerando o pior cenário no cálculo da amostragem que garantirá 90% de confiança com 5% de erro sobre a média, será necessário utilizar no mínimo 84 amostras para os testes em cortes.

Com o objetivo de determinar as melhores condições operacionais no processo de controle microbiológico de cortes de frango foi realizado previamente um Delineamento Composto Central Rotacional (DCCR) com duas variáveis (tempo e concentração do ácido acético), totalizando 11 ensaios, com 3 réplicas no ponto central, apresentados na Tabela 1.

Os experimentos foram divididos em três dias de produção, contemplando a cada dia experimental, um ponto central. Foi fixado os mesmos horários de coleta das amostras para evitar que possíveis variáveis aleatórias possam interferir no resultado.

Tabela 1 - Matriz do Delineamento Composto Central Rotacional no estudo da imersão de cortes (coxa e peito)

Variável independente	Ponto axial inferior **-α (-1.41)**	Nível inferior **(-1)**	Ponto Central **(0)**	Nível superior **(+1)**	Ponto axial superior **+ α (+1.41)**
Tempo (s) (X_1)	17,95	20	25	30	32,05
[ácido (ppm)] (X_2)	1006.5	1150	1500	1850	1993.5

Como respostas a este planejamento, as variáveis dependentes foram as contagens de bactérias mesófilas aeróbias (Y1) e *Enterobacteriaceae* (Y2).

Após obtidos os resultados dos experimentos, estes foram analisados por superfície de resposta e por análise canônica pelo software Statistica® 10.0.

2.10 Caracterização física dos cortes tratadas com solução antimicrobiana de ácido peracético

a) Análise dos cortes de frango

Para a avaliação objetiva da carne de peito de frango e coxa de frango, foram realizadas análises de cor e textura (força de cisalhamento). Para a realização dessas análises, além da análise de perda de peso por cocção, foram retiradas mais três amostras de cada ensaio do DCCR, sendo uma amostra destinada para a análise de cor, outra para a análise de textura na matriz cárnea crua e a outra para a análise de perda de cocção. Os filés de peito e coxa de frango foram armazenados a - 20º C até o momento da avaliação das análises e foram descongelados previamente ao momento de cada análise.

Os resultados foram analisados por meio de análise estatística - software Statistica® 10.0 para realizar uma Análise de Variância (ANOVA) usando o teste de comparação de Tukey para determinar a diferença estatisticamente significativa das médias em um intervalo de confiança de 95%.

b) Análise de cor instrumental

A avaliação da cor foi realizada nas amostras descongeladas de filé de peito de frango cru sem a pele e nas amostras de coxa cru com a pele, por meio de um colorímetro CR-400 (Minolta, Konica, São Paulo, São Paulo, Brasil). As medidas foram tomadas de acordo com Bilgili et al. (1998) por meio do sistema CIELab com os parâmetros L* (luminosidade), a* (intensidade do verde até vermelho) e b* (intensidade do amarelo ao azul) (Ramos e Gomide, 2007). As características da medição foram: área de medição de 11 mm de diâmetro, ângulo de observação de 2°, iluminante C com componente especular incluído.

c) Análise de textura instrumental

As amostras utilizadas para avaliação da força de cisalhamento foram mantidas sob refrigeração (4 ºC ± 2 ºC) por 24 horas antes do início das análises. Imediatamente após a retirada das amostras do refrigerador, foi removido pele e ossos dos cortes e após, os cortes

foram cortados em paralelogramos de 1 cm de altura x 1 cm de largura x 4 cm de comprimento seguindo a orientação paralela das fibras musculares (AN et al., 2010; LYON; LYON, 1998). Cada amostra de filé de peito de frango deu origem a quatro paralelogramos destinados à avaliação da força de cisalhamento, conforme a Figura 5. Para as amostras de coxa, cada amostra deu origem a três paralelogramos destinado a avaliação de força de cisalhamento. A análise da textura instrumental foi realizada para as amostras de peito e coxa cruas, bem como para as amostras assadas, as quais foram submetidas a cocção em forno elétrico com temperatura estabilizada a 170 °C até que atingissem 71 °C no seu centro geométrico, sendo o tempo total de cozimento em torno de 35 minutos para o peito e 25 minutos para a coxa.

Figura 4 – Padrão de retirada dos paralelogramos para avaliação da força de cisalhamento.

Fonte: própria

Para a realização dessa análise foi utilizado texturômetro universal TAXT Plus (Stable Micro System, Surrey, UK) acoplada a uma lâmina *Warner Bratzler Shear Force (WBSP),* conectado a um microcomputador para interpretação dos dados pelo software Texture Expert^{R}. Durante os testes foi adotado uma velocidade de pré-teste de 10 mm/s e de 5mm/s, tendo como carga de 25 Kg (AN et al., 2010; MANZOOR et al., 2020; TADEU, 2016). O resultado final de cada amostra de filé foi calculado pela média das repetições (paralelogramos) de um mesmo corte e o valor da força de cisalhamento expresso em Newton.

d) Análise de perda de peso na cocção nos cortes de frango

Antes da avaliação da perda de peso à cocção, as amostras de filés de peito de frango e coxas de frango foram descongeladas sob refrigeração (4 ºC ± 2) por 24 h e, após esse período, foi avaliada a perda de peso à cocção as amostras de cortes. Para tal, foi utilizado grelhas de 30 cm de comprimento x 25 cm de largura, depositadas sobre bandejas (assadeiras) de alumínio com aproximadamente 25 cm de comprimento x 20 cm de largura x 4,5 cm de profundidade. Foi realizada a pesagem dos cortes e a temperatura foi monitorada a cada 10 minutos por meio de termômetros a laser e termômetro espeto até atingirem 71 °C no seu centro geométrico, sendo o tempo total de cozimento em torno de 35 minutos para o peito e 25 minutos para a coxa. As amostras foram submetidas à cocção em forno elétrico com temperatura estabilizada a 170°C.

Após o tratamento térmico, os conjuntos contendo as amostras foram retirados do forno e deixados esfriar por meia hora à temperatura ambiente e em seguida, pesados. A perda de peso à cocção foi obtida pela diferença entre o peso da amostra crua e cozida. As perdas de peso foram expressas em porcentagem do peso da amostra original.

Após a etapa de pesagem para avaliação da perda de cocção, as amostras assadas foram submetidas a avaliação de textura instrumental, conforme descrito no item “c”.

2.11 RESULTADOS E DISCUSSÃO

O Delineamento Composto Central Rotacional (DCCR) possibilitou a obtenção do modelo matemático reduzido obtido por uma regressão múltipla e de superfícies de resposta, que indicam a influência do tempo de exposição e da concentração do ácido sobre a ação descontaminante do ácido peracético nos cortes de peito e coxa de frango. As equações obtidas descrevem o comportamento da redução logarítmica das bactérias indicadoras de processo, Contagem de Mesófilos Aeróbias (CTM) e *Enterobacteriaceae* (EB), quando aplicado o ácido peracético. Avaliou-se pela tabela da anova as variáveis, tempo e concentração, que influenciaram significativamente no resultado ($p < 0,05$).

2.11.1 Análise dos resultados do Delineamento Composto Central Rotacional nos cortes de Peito de Frango

Os resultados da avaliação estatística para a variável Contagem de Mesófilos Aeróbias (Y_1) e *Enterobacteriaceae* (Y_2) em peito de frango estão apresentados na Tabela Anova (Tabela 2).

Tabela 2: Tabela anova para indicação das variáveis significativas nas respostas de a) Contagem de Mesófilas Aeróbicas (CTM) e b) Enterobactérias (EB).

Ys	Respostas	Unidade	Modelo	R^2	Fcal	Ftab
Y1	Contagem de Mesófilos Aeróbios	UFC/ g	Y_1= 1,74+0,24 (X_2)	79,60%	15,40	5,12
Y2	*Enterobacteriaceae*	UFC/ g	$Y_2 = 2,41-0,50\ (X_1)^2\ -0,58\ (X_2)^2$	82,90%	12,58	4,46

Os modelos de regressão múltipla obtidos para a redução microbiana de mesófilos aeróbicos, Equação 1 ($R^2 = 0,7957$) (CTM) e para a redução de enterobactérias Equação 2 ($R^2 = 0,8286$) (EB) são descritos com os termos significativos, resultante do DCCR em relação a ação descontaminante do ácido peracético em peito de frango, por meio da análise da redução logarítmica das bactérias indicadoras, mediante a tempo e concentração, respectivamente.

Os modelos apresentam valores de R^2 elevados, indicando a adequação do modelo empírico aos dados observados, com capacidade de predição de 80% para a variável dependente CTM e 83% para EB. É possível afirmar que o efeito é estatisticamente significativo haja vista F calculado maior que F tabelado o que leva em consideração os graus de liberdade da amostra. Observa-se que para a redução logarítmica de Contagem de Mesófilos Aeróbios há a influência significativa da concentração do ácido (X_2) linear, o tempo não foi significativo. Para *Enterobacteriaceae* há a influência significativa da concentração do ácido $(X_2)^2$ quadrático e tempo quadrático $(X_1)^2$.

As Figuras 6 e 7 apresentam as superfícies de resposta e curvas de nível resultantes do DCCR, para o corte peito de frango, elas representam a influência das variáveis independentes na redução da concentração logarítmica das bactérias mesófilas e das enterobactérias, respectivamente. As regiões apresentadas em vermelho escuro são as que mais reduziram a concentração logarítmica dos microrganismos.

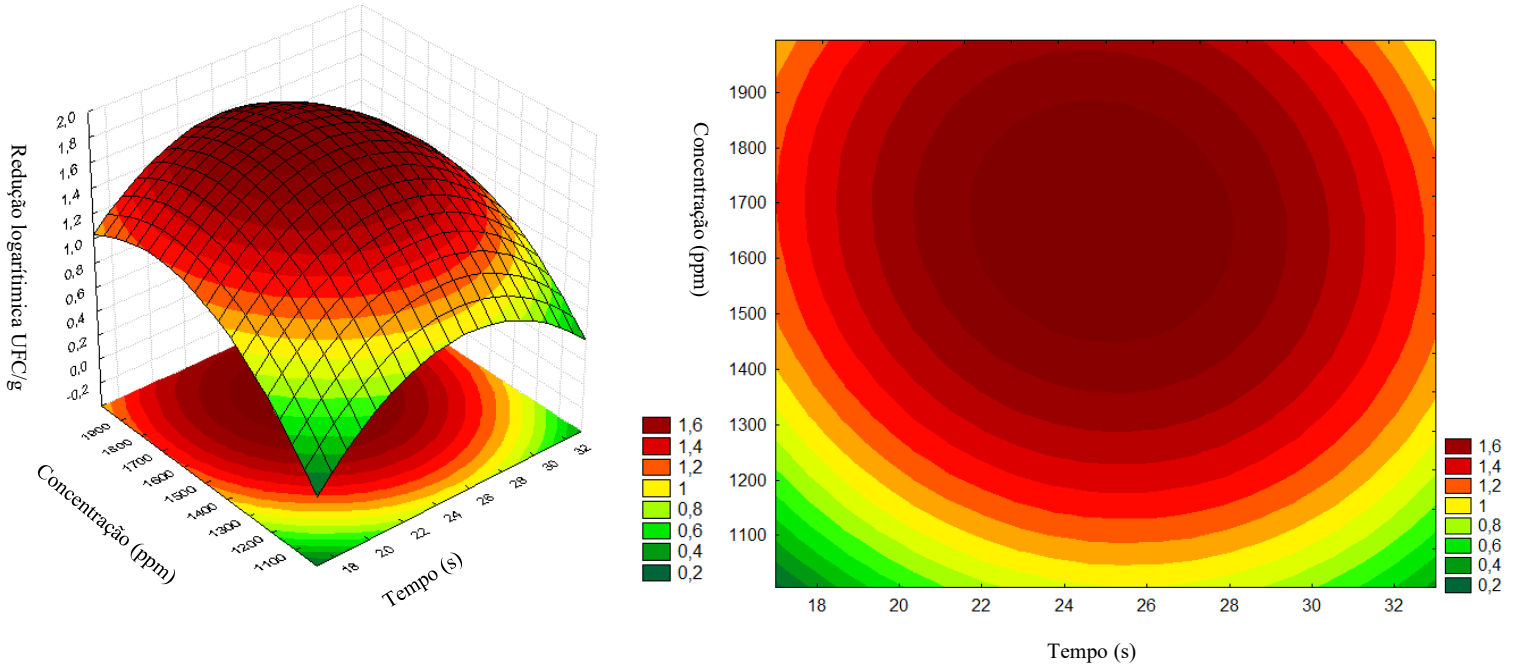


Figura 6: Superfícies de resposta e curvas de nível para a redução logarítmica de bactérias mesófilas (Y_1) em peito de frango em função do tempo (X_1) e da concentração de ácido peracético (X_2).

Analisando as superfícies resposta e as curvas de nível expressas nas Figuras 6 e 7 é possível inferir as regiões ótimas das variáveis independentes tempo e concentração do ácido peracético para a melhor efetividade na redução logarítmica.

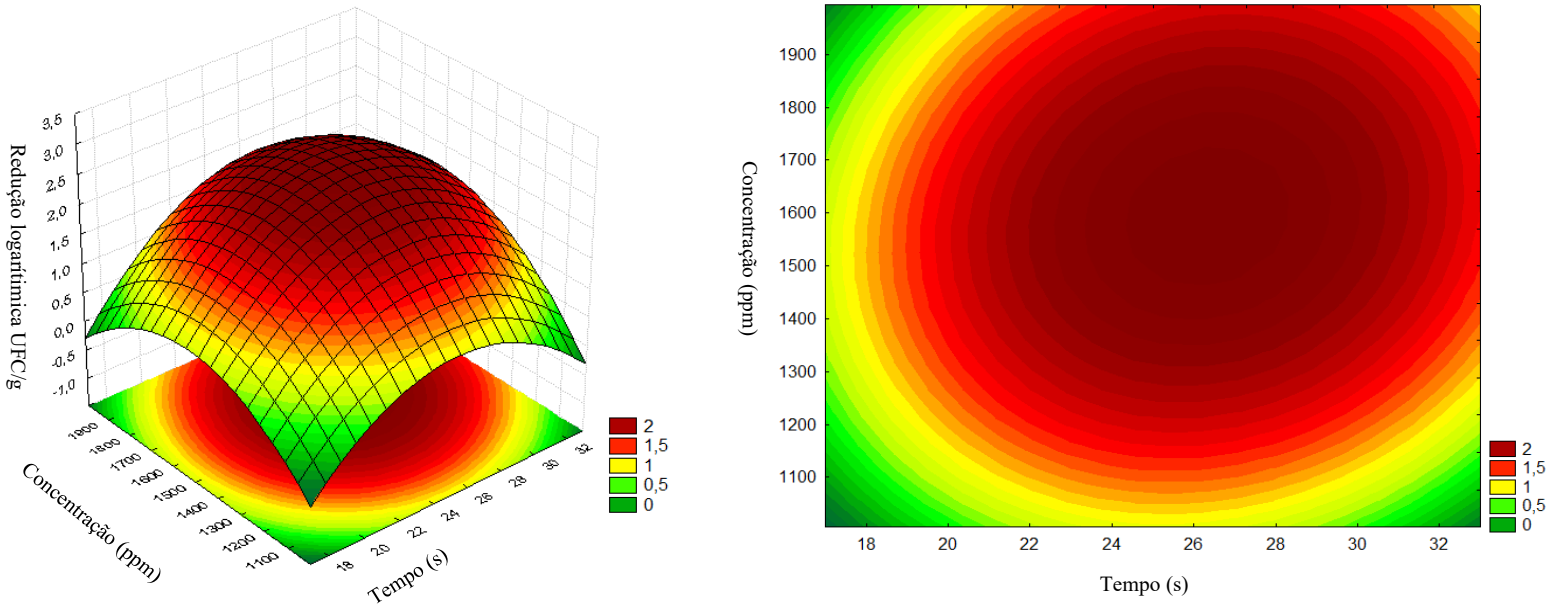


Figura 7: Superfícies de resposta e curvas de nível para a redução logarítmica *Enterobacteriaceae* (Y_2) em peito de frango em função do tempo (X_1) e da concentração de ácido peracético (X_2).

Comparando as superfícies de resposta obtidas na Figura 6 e na Figura 7 do DCCR, é possível observar que ambas as bactérias indicadoras se comportaram de forma similar e apresentam os melhores resultados no que diz respeito à redução das bactérias indicadoras do processo, quando aplicado as condições na região de Ponto Central.

Por meio das curvas de superfície resposta observou-se que quando aumentamos o tempo do produto exposto ao antimicrobiano, não foram obtidos os melhores resultados em redução logarítmica, o que não era esperado, uma vez que teoricamente quanto maior a concentração utilizada e o tempo do produto em contato com o antimicrobiano, maior seriam as reduções logarítmicas. Isso pode ser explicado baseado no trabalho de Mesrobian (2009), que relata que o ácido peracético fornece apenas uma grande redução inicial nas bactérias devido ao material orgânico que contém nitrogênio no músculo. Após um minuto de tempo de contato, não há resíduo de PAA mensurável restante, atestando que às condições ótimas de operação para redução de bactérias, não necessariamente se encontram nos maiores tempos, o que também fica evidenciado com o coeficiente negativo que multiplica o termo de tempo quadrático $(X_1)^2$ para EB e o tempo não ter sido significativo para CTM.

Vale ressaltar a eficácia do antimicrobiano como descontaminante em carne de aves, mesmo na menor concentração (1006,5 ppm) obteve-se uma redução de 0,90 e 1,29 log para CTM e EB, respectivamente. A região ótima para Y_1 e Y_2 estão na região do ponto central (1500 ppm a 25 s) com reduções de 1,90 e 2,90 respectivamente. Também foi observado uma maior redução para EB quando comparado a redução para CTM, embora, em ambos os casos as reduções foram veementemente expressivas. Assim, para garantir uma melhor redução tanto de bactérias mesófilas quanto de enterobactérias no peito de frango o interessante é utilizar 1500 ppm.

Manjankattil (2021), avaliou em seu estudo a redução de *Salmonella Heidelberg* em peito de frango imersos em solução de PAA a 1200 ppm por 2 minutos, encontrando uma redução de 1,25 log e Kumar (2020), que também avaliou a redução de Salmonella *e Campilobacter* em filés de peito de frango em imersão de solução de PAA com concentração de (250 a 1000 ppm) e tempo de 10 e 30 segundos, evidenciou uma redução de 1,92 log na maior concentração a 30 segundos.

Os estudos corroboram com a eficácia do antimicrobiano e com as reduções encontradas nesse estudo, através do DCCR. Importante ressaltar o estudo de Manjankattil, onde percebe-se que o aumento do tempo não necessariamente aumenta a redução logarítmica.

2.11.2 Análise dos resultados do Delineamento Composto Central Rotacional nos cortes de coxa de frango

Os resultados da avaliação estatística para a variável Contagem de Mesófilas Aeróbias (Y_1) e *Enterobacteriaceae* (Y_2) em coxa de frango estão apresentados na Tabela Anova (Tabela 3).

Tabela 3: Tabela Anova para indicação das variáveis significativas nas respostas de a) Contagem de Mesófilas Aeróbicas (CTM) e b) Enterobactérias (EB).

Ys	Respostas	Unidade	Modelo	R^2	Fcal	F Tab
Y1	Contagem de Mesófilos Aeróbios	UFC/ g	Y_1= 1,68+0,47 (X_2)	91,10%	17,12	5,12
Y2	*Enterobacteriaceae*	UFC/ g	Y_2 = 2,38-0,87 (X_1) ² + 0,74 (X_2)	94,20%	15,36	4,46

As Equações 3 (R^2 = 0,91149) e 4 (R^2 = 0,94154) descrevem o modelo de regressão múltipla, com os termos significativos, resultante do DCCR em relação a ação descontaminante do ácido peracético em coxa de frango, por meio da análise da redução logarítmica das bactérias indicadoras, mediante a tempo e concentração respectivamente.

Os modelos apresentam valores de R^2 elevados, indicando a adequação do modelo empírico aos dados observados, com capacidade de predição de 91% para a variável dependente bactérias mesófilas aeróbias e 94% para *Enterobacteriaceae*. É possível afirmar que o efeito é estatisticamente significativo haja vista F calculado maior que F tabelado o que leva em consideração os graus de liberdade da amostra. Observa-se que para a redução logarítmica de Contagem de Mesófilos Aeróbios há a influência significativa da concentração do ácido (X_2) linear, e o tempo não foi significativo. Para *Enterobacteriaceae* há a influência significativa da concentração do ácido (X_2) linear e tempo quadrático $(X_1)^2$, todavia o coeficiente que multiplica o termo de tempo quadrático $(X_1)^2$ é negativo.

Além disso, de acordo com o coeficiente positivo que multiplica o termo de concentração linear (X_2) nos modelos para as duas respostas, com o aumento da concentração de ácido peracético reduz a concentração de microrganismos analisados.

As Figuras 9 e 10 apresentam as superfícies de resposta e curvas de nível resultantes do DCCR, para o corte coxa de frango. As regiões apresentadas de vermelho escuro são as que mais reduziram a concentração logarítmica dos microrganismos.

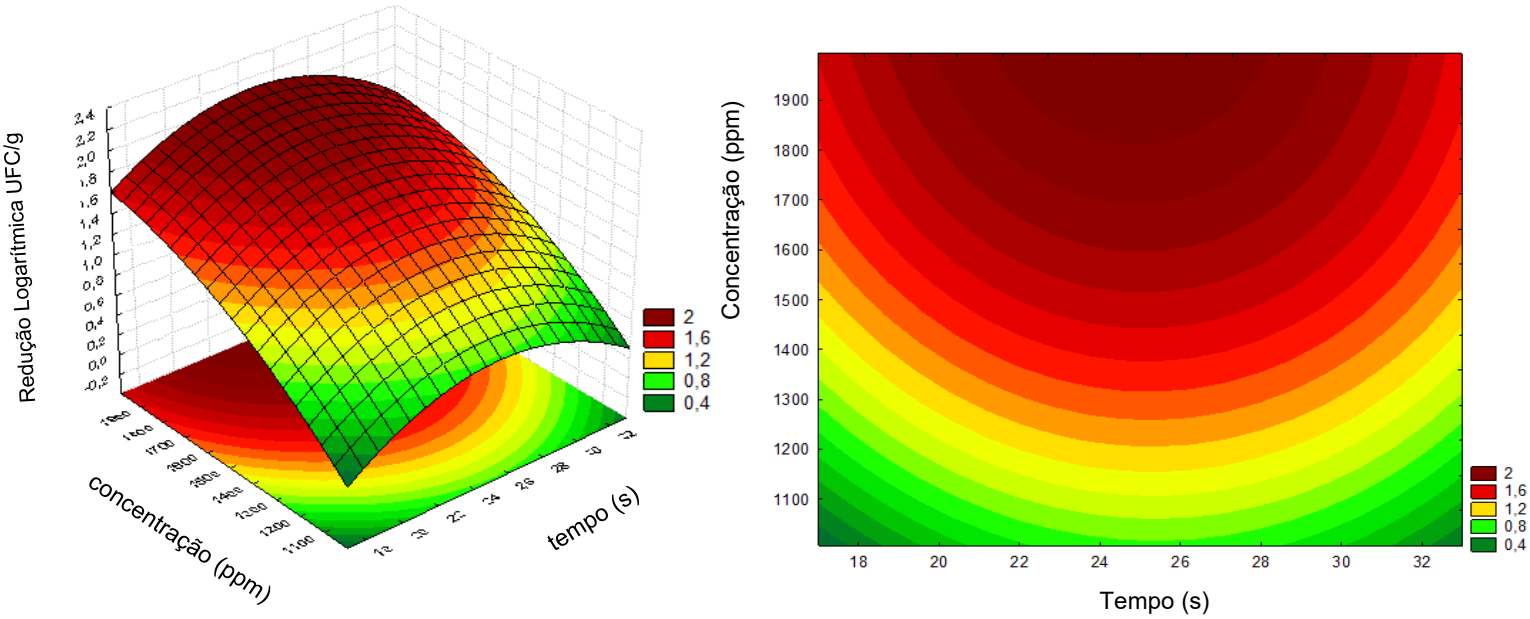


Figura 9: Superfícies de resposta e curvas de nível para a redução logarítmica de bactérias mesófilas (Y1) em coxa de frango

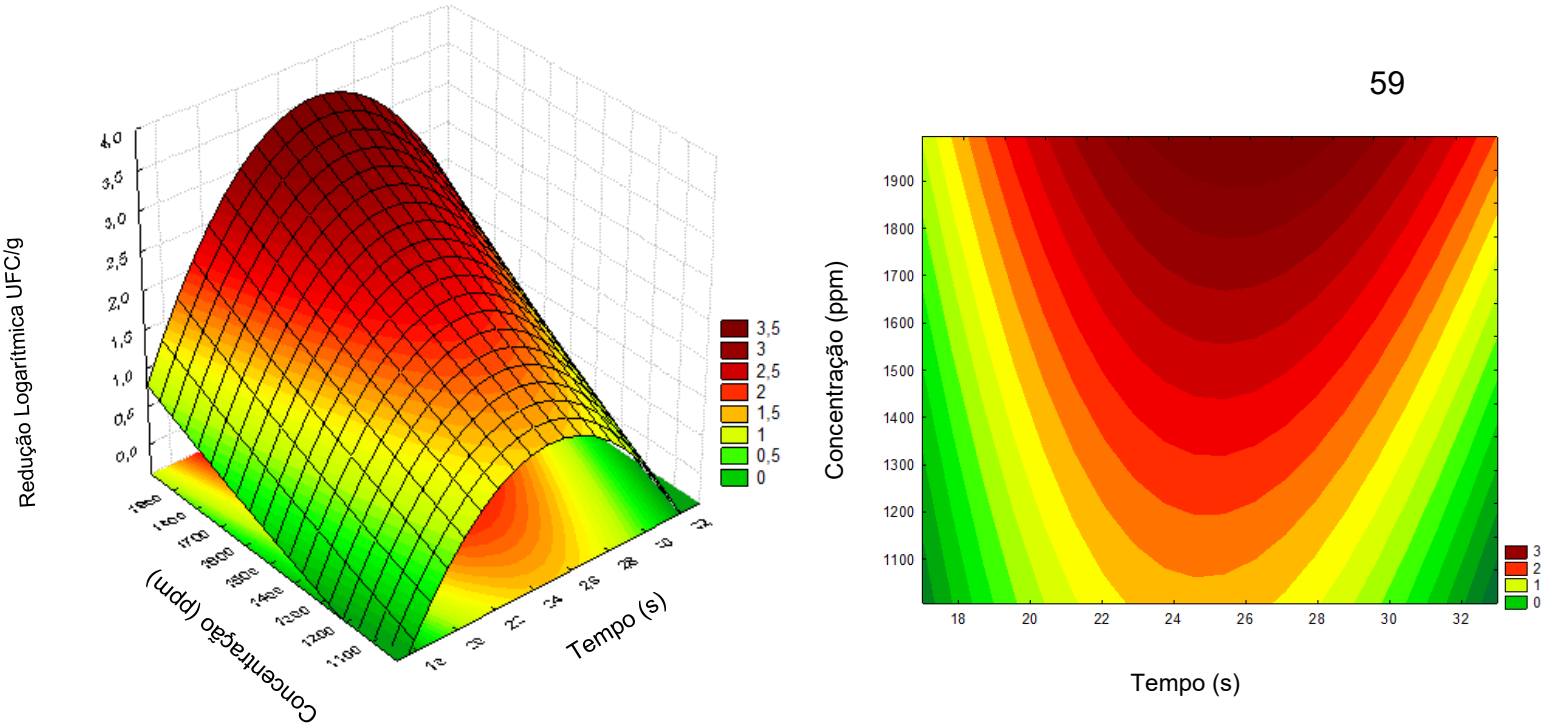


Figura 10: Superfícies de resposta e curvas de nível para a redução logarítmica *Enterobacteriaceae* (Y2) em peito de frango

Através das superfícies de resposta observa-se que para CTM e para EB as melhores reduções logarítmicas em ambos os casos se concentram quando aplicado concentrações acima 1800 ppm apresentando reduções logarítmicas de 2,14 e 3,65 log para CTM e EB respectivamente.

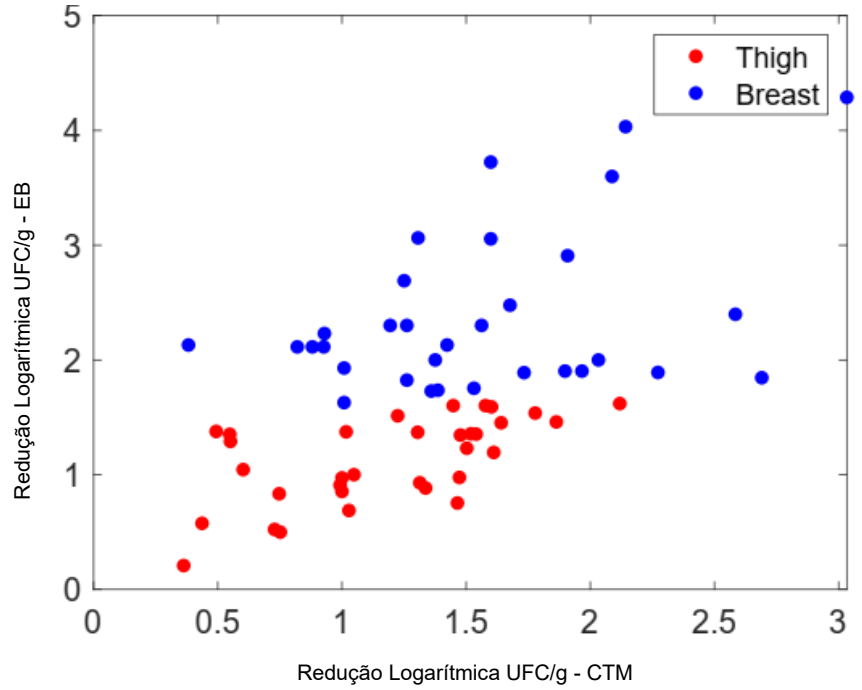


Figura 11: Dispersão dos pontos discretos dos experimentos realizados no DCCR para peito (●) e coxa (●) de frango utilizando o PAA.

Observa-se na Figura 11 que a aplicação do ácido peracético em coxa de frango, assim como para o corte de peito de frango corrobora com a eficácia do antimicrobiano. Vale ressaltar que em todas as aplicações os resultados foram expressivos, mas que a redução logarítima da concentração de microorganismos depende da parte do frango analisada. Observa-se que na Figura 11 a redução da concentração de EB foi mais significativa em todos os tratamentos da coxa em relação ao peito, já a redução de CTM tiveram resultados mais expressivos no peito, mas a maioria dos tratamentos da coxa e do peito tiveram reduções de CTM próximas.

Quando aplicado a menor concentração 1006,5 ppm, obteve-se resultados de redução de 1,05 e 1,38 log para CTM e EB respectivamente. Corroborando com a eficácia do antimicrobiano, os pontos centrais (1500 ppm por 25 segundos) apresentaram resultados para as respostas Y_1 e Y_2 de 1,88 e 2,17 log, respectivamente. Também se observou que quando o tempo de contato com o antimicrobiano era aumentado, a redução logarítmica não aumentava concomitantemente ao aumento da concentração o que fica evidenciado com o coeficiente negativo que multiplica o termo de tempo quadrático $(X_1)^2$ para EB e o tempo não ter sido significativo para CTM.

De encontro ao corte peito de frango, no qual as melhores reduções foram encontradas na região de ponto central, para o corte de coxa de frango, os melhores resultados se deram com o aumento da concentração. Os resultados comprovam a hipótese testada de que há a influência da superfície de contato da ave na ação do antimicrobiano.

Os cortes de coxa de frango estão completamente cobertas com a pele e podem fornecer proteção aos microrganismos e prevenir a exposição a antimicrobiano (KATARIA et al., 2020). Dessa forma, podemos correlacionar o desafio microbiano do corte de coxa com os resultados encontrados no estudo por meio do DCCR, haja visto a necessidade de concentrações acima de 1800 ppm para obter as maiores reduções logarítmicas, o que não ocorre no corte de peito de frango.

Zhang (2019), também estudou o corte coxa de frango por imersão avaliando os patógenos S. Heidelberg e *Campylobacter jejuni,* em imersão de solução de PAA com concentração de 700 e 1000 ppm e tempo (10 a 30 segundos). O estudo encontrou reduções para S. Typhimurium de aproximadamente 2 log para ambas as concentrações (700 e 1000 ppm) e reduções de aproximadamente 2,5 log para *Campylobacter jejuni* a 1000 ppm, os tempos

não influenciaram na redução. O estudo de Zhang encontrou reduções similares ao desse estudo, embora tenha utilizado menores concentrações de PAA.

A efetividade do ácido peracético encontrada nesse estudo bem como nas referências supracitadas, são explicadas pelo mecanismo de ação do ácido peracético o qual é baseado na oxidação dos constituintes celulares, ou seja, na liberação de oxigênio ativo que interage com ligações de enxofre nas proteínas, enzimas e outros metabólitos dos microrganismos. Também interrompe a função osmótica, o transporte por lipoproteínas da membrana citoplasmática e causa deslocamento ou ruptura da parede celular, desta forma, facilitando sua ação contra microrganismos Gram-negativos. A sua ação na desnaturação de proteínas ajuda a explicar suas características esporicida. Age também sobre as bases da molécula de DNA, além de inativar a catalase, uma enzima que neutraliza a ação dos radicais livres de hidroxila (ACOSTA et al., 2022).

As principais vantagens do ácido peracético são a biodegrabilidade, atoxicidade e a ação efetiva em matéria orgânica. Além disto, após o uso há uma rápida decomposição transformando- se em ácido acético, água e oxigênio (EFSA, 2014a; THOMAS et al., 2020).

2.11.3 Análise dos resultados da caracterização física dos cortes tratadas com solução antimicrobiana de ácido peracético

Conforme descrito na metodologia, os experimentos foram divididos em três dias de produção, contemplando a cada dia experimental, um ponto central. Foi fixado os mesmos horários de coleta das amostras para evitar que possíveis variáveis aleatórias pudessem interferir no resultado. Dessa maneira, a análise comparativa dos resultados da caracterização física, se deu entre amostras tratadas e amostra controle de cada dia de coleta.

2.11.4 Análise dos resultados de cor instrumental

Os resultados da avaliação objetiva da cor de filés de peito de frango sem pele e a pele da coxa de frango após o tratamento com ácido peracético, nas concentrações e tempo conforme DCCR estão apresentados nas Tabelas 3 e 4 respectivamente. Não foi observada diferença

estatística significativa ($p < 0,05$) entre as amostras tratadas entre si, e quando comparadas ao controle para os parâmetros L* a* b*, independente da concentração utilizada.

Tabela 4: Resultados da avaliação objetiva da cor (parâmetros de L* a* b*) em filés de peito de frango cru e sem pele

Data 27.09	**Cor L***	**Cor a***	**Cor b***
	Média ± desv. Padrão	Média ± desv. Padrão	Média ± desv. Padrão
C	39,16ª ± 2,20	1,34ª ± 0,51	6,76ª ± 1,26
T1	39,47ª ± 0,94	1,45ª ± 0,82	5,33ª ± 2,00
T3	36,65ª ± 1,27	1,27ª ± 0,42	6,38ª ± 2,38
T5	38,88ª ± 2,53	1,13ª ± 0,56	6,59ª ± 2,11
T9	38,41ª ± 5,53	1,14ª ± 0,85	6,58ª ± 1,35
Data 28.09	**Cor L***	**Cor a***	**Cor b***
	Média ± desv. Padrão	Média ± desv. Padrão	Média ± desv. Padrão
C	41,54ª ± 3,02	2,23ª ± 0,93	8,74ª ± 0,32
T10	41,44ª ± 2,74	2,11ª ± 0,79	6,42ª ± 2,85
T2	38,25ª ± 1,18	2,54ª ± 1,27	5,85ª ± 2,19
T4	40,78ª ± 2,11	2,45ª ± 1,40	7,00ª ± 1,01
T6	41,89ª ± 1,90	1,69ª ± 0,75	6,99ª ± 1,07
Data 29.09	**Cor L***	**Cor a***	**Cor b***
	Média ± desv. Padrão	Média ± desv. Padrão	Média ± desv. Padrão
C	46,27ª ± 4,23	2,11ª ± 1,11	5,67ª ± 1,42
T11	39,08ª ± 2,28	2,28ª ± 0,62	5,22ª ± 0,37
T7	41,97ª ± 2,97	1,90ª ± 0,49	6,82ª ± 0,58
T8	39,66ª ± 3,34	1,42ª ± 0,32	5,30ª ± 1,06

Médias seguidas de letras iguais na mesma coluna não diferem entre si pelo teste de Tukey ($P<0,05$). C = controle; T1 = 1150 ppm|20s; T2 = 1150 ppm|30s; T3 = 1850 ppm|20s; T4 = 1850 ppm|30s; T5 = 1500 ppm|17,95s; T6 = 1500 ppm|32,05s; T7 = 1006,5 ppm|25s; T8 = 1993,5 ppm|25s; T9 = 1500 ppm|25s; T10 = 1500 ppm|25s; T11 = 1500 ppm|25s.

Tabela 5: Resultados da avaliação objetiva da cor (parâmetros de L* a* b*) em pele de coxa de frango cru

04.10	**Cor L***	**Cor a***	**Cor b***
	Média ± desv. Padrão	Média ± desv. Padrão	Média ± desv. Padrão
C	22,90ª ± 0,63	-0,64ª ± 0,045	0,96ª ± 0,23
T1	23,26ª ± 2,38	-0,56ª ± 0,081	0,80ª ± 0,39
T3	22,97ª ± 1,44	-0,51ª ± 0,015	0,95ª ± 0,27
T5	22,74ª ± 1,74	-0,56ª ± 0,12	0,82ª ± 0,26

T9	25,06ª ± 0,44	-0,59ª ± 0,16	1,08ª ± 0,48
05.10	**Cor L***	**Cor a***	**Cor b***
	Média ± desv. Padrão	Média ± desv. Padrão	Média ± desv. Padrão
C	23,90ª ± 1,48	-0,64ª ± 0,045	0,73ª ± 0,10
T10	21,78ª ± 0,59	-0,60ª ± 0,15	0,87ª ± 0,20
T2	21,04ª ± 0,40	-0,45ª ± 0,062	1,03ª ± 0,36
T4	22,88ª ± 1,73	-0,57ª ± 0,038	0,71ª ± 0,062
T6	24,06ª ± 5,14	-0,63ª ± 0,050	1,00ª ± 0,40
06.10	**Cor L***	**Cor a***	**Cor b***
	Média ± desv. Padrão	Média ± desv. Padrão	Média ± desv. Padrão
C	25,34ª ± 1,32	-0,51ª ± 0,11	0,81ª ± 0,25
T11	25,65ª ± 0,24	-0,55ª ± 0,11	0,83ª ± 0,049
T7	20,77ª ± 3,19	-0,59ª ± 0,020	0,81ª ± 0,23
T8	22,64ª ± 2,53	-0,54ª ± 0,12	0,93ª ± 0,41

Médias seguidas de letras iguais na mesma coluna não diferem entre si pelo teste de Tukey (P<0,05). C = controle; T1 = 1150 ppm|20s; T2 = 1150 ppm|30s; T3 = 1850 ppm|20s; T4 = 1850 ppm|30s; T5 = 1500 ppm|17,95s; T6 = 1500 ppm|32,05s; T7 = 1006,5 ppm|25s; T8 = 1993,5 ppm|25s; T9 = 1500 ppm|25s; T10 = 1500 ppm|25s; T11 = 1500 ppm|25s.

Manjankattil et al (2021), avaliou os parametros de cor L* a* b, em coxa de frango com pele tratados e peito de frango tratados com 500 ppm e 1200 ppm de ácido peracético respectivamente. Os valores de cor entre os tratamentos e a amostra controle não diferiram significativamente (P < 0,05). Vale ressaltar que mesmo usando concentrações maiores de ácido peracético, também não foi observada variações estatisticamente significativas no presente estudo para os parâmetros de cor, o que é benéfico para a indústria, uma vez que não altera as características de aparência da carne in natura, o que poderia impactar na preferência e aceitabilidade do consumidor.

Mehall et al (2015), avaliou a influência do fosfato trissódico (10%), lactate de potássio (3%), metassilicato de sódio a 4% e cloreto de cetilpiridínio a 0,5% nas características instrumentais de cor em bifes bovinos. Nenhum efeito significativo (P < 0,05) de tratamento foi detectado para L*, a*, b*, demonstrado que o uso de antimicrobianos podem corroborar com a qualidade microbiológica do produto final, sem impactar no parâmetro de cor. Apesar dos resultados satisfatórios para diferentes antimicrobianos, o tratamento de carne bovina com ácido lático (2%, 3%, 4% e 5%), apresentou diferença estatística significativa para L*, a*, b* conforme estudo de Melcón et al (2017). Dessa forma, avaliar a cor durante os estudos com o uso de antimicrobianos é importante para evitar problemas de aceitabilidade pelos consumidores.

2.11.5 Análise dos resultados de textura instrumental

Os resultados da avaliação objetiva da força de cisalhamento de filés de peito de frango assado e coxa sem pele e sem osso de frango assado após o tratamento com ácido peracético, estão demonstrados na Tabela 5 e 6. Este parâmetro foi avaliado ambos os cortes crus e assados, no intuito de avaliar a possível influência do ácido peracético na textura da carne, caso as variáveis durante o processo de cozimento pudessem impactar no resultado.

Tabela 6: Resultados da avaliação da força de cisalhamento (N) em filés de peito de frango, cru e assado, após tratamento com ácido peracético

	Data 27.09	**Textura "Cru"**	**Textura "Assada"**
		Média ± desv. Padrão	Média ± desv. Padrão
C		20,71[a] ± 2,48	54,65[a] ± 4,37
T1		25,19[a] ± 4,06	56,50[a] ± 4,78
T3		21,22[a] ± 4,69	55,87[a] ± 4,29
T5		22,10[a] ± 5,40	54,77[a] ± 4,04
T9		24,77[a] ± 4,35	55,20[a] ± 4,05
	Data 28.09	**Textura "Cru"**	**Textura "Assada"**
		Média ± desv. Padrão	Média ± desv. Padrão
C		22,59[a] ± 3,94	58,41[a] ± 4,52
T10		22,24[a] ± 4,17	55,84[a] ± 4,33
T2		20,89[a] ± 4,61	56,64[a] ± 5,05
T4		23,25[a] ± 3,23	58,67[a] ± 4,65
T6		22,90[a] ± 4,28	59,02[a] ± 4,92
	Data 29.09	**Textura "Cru"**	**Textura "Assada"**
		Média ± desv. Padrão	Média ± desv. Padrão
C		21,00[a] ± 2,98	60,25[a] ± 4,44
T11		23,39[a] ± 5,27	58,77[a] ± 5,65
T7		20,30[a] ± 4,37	61,44[a] ± 5,00
T8		22,59[a] ± 3,52	62,55[a] ± 5,17

Médias seguidas de letras iguais na mesma coluna não diferem entre si pelo teste de Tukey (P<0,05). C = controle; T1 = 1150 ppm|20s; T2 = 1150 ppm|30s; T3 = 1850 ppm|20s; T4 = 1850 ppm|30s; T5 = 1500 ppm|17,95s; T6 = 1500 ppm|32,05s; T7 = 1006,5 ppm|25s; T8 = 1993,5 ppm|25s; T9 = 1500 ppm|25s; T10 = 1500 ppm|25s; T11 = 1500 ppm|25s.

Tabela 7: Resultados da avaliação da força de cisalhamento (N) em coxa de frango sem pele e sem osso, cru e assado, após tratamento com ácido Peracético

04.10	**Textura "Cru"**	**Textura "Assada"**
	Média	Média
C	51,53ª ± 4,15	16,21ª ± 3,24
T1	47,28ª ± 4,94	17,80ª ± 2,21
T3	51,51ª ± 3,50	18,97ª ± 4,95
T5	50,39 ª ± 3,40	16,58ª ± 3,84
T9	47,23ª ± 3,89	19,83ª ± 2,81
05.10	**Textura "Cru"**	**Textura "Assada"**
	Média	Média
C	49,21ª ± 4,03	17,77ª ± 3,55
T10	46,34ª ± 3,09	18,06ª ± 2,64
T2	47,35ª ± 4,29	18,12ª ± 4,59
T4	51,42ª ± 3,49	18,49ª ± 2,87
T6	47,94ª ± 4,77	17,78ª ± 4,35
06.10	**Textura "Cru"**	**Textura "Assada"**
	Média	Média
C	49,37ª ± 3,57	19,73ª ± 2,19
T11	48,70ª ± 3,50	18,56ª ± 3,39
T7	48,87ª ± 4,00	17,50ª ± 4,14
T8	50,02ª ± 3,71	21,74 ª ± 4,48

Médias seguidas de letras iguais não diferem entre si pelo teste de Tukey (P<0,05). C = controle; T1 = 1150 ppm|20s; T2 = 1150 ppm|30s; T3 = 1850 ppm|20s; T4 = 1850 ppm|30s; T5 = 1500 ppm|17,95s; T6 = 1500 ppm|32,05s; T7 = 1006,5 ppm|25s; T8 = 1993,5 ppm|25s; T9 = 1500 ppm|25s; T10 = 1500 ppm|25s; T11 = 1500 ppm|25s.

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Thomas et al (2019), avaliou a influência do ácido levunílico e do ácido lático em bifes bovinos cozidos na análise de a força de cisalhamento Warner-Bratzler. O estudo encontrou valores semenlhantes ($p < 0,05$) para os tratamentos avaliados quando comparados a carne sem tratamento antimicrobiano. A análise objetiva de força de cisalhamento Warner-Bratzler encontrada no presente estudo, corrobora com a análise subjetiva da literatura supracitada sugerindo que os antimicrobianos testados, não devem ter efeito adverso nas características tecnológicas da carne, todavia, não foram encontrados estudos que avaliaram a influência do ácido peracético na análise objetiva de força de cisalhamento.

Pela análise dos dados nota-se a presença de alguns desvios padrões elevados, os quais se justificam pelo fato das amostras serem destrutivas, ou seja, cortes de frangos diferentes

(influência de lotes, genética e peso médio por exemplo) e a própria posição da fibra no equipamento, direção perpendicular ou paralela a fibra também podem influenciar na medida.

2.11.6 Análise dos resultados de perda de peso nos cortes de frango

Os resultados da perda de peso de filés de peito de frango e coxa de frango assados após o tratamento com ácido peracético nas concentrações e tempo conforme DCCR estão apresentados na Tabela 7.

Tabela 8: Resultados da perda de peso por cocção em filés de peito de frango e coxa de frango após tratamento com ácido peracético.

	Peito de frango	**Coxa de frango**
Data 27.09	**Perda de peso por cocção**	**Perda de peso por cocção**
	Média ± desv. Padrão	Média ± desv. Padrão
C	55,88ª ± 1,82	13,44ª ± 1,25
T1	61,57ª ± 4,54	15,25ª ± 1,45
T3	56,99ª ± 2,22	14,75ª ± 1,07
T5	60,70ª ± 3,99	14,24ª ± 0,49
T9	59,79ª ± 4,94	14,60ª ± 2,03
Data 28.09	**Perda de peso por cocção**	**Perda de peso por cocção**
	Média ± desv. Padrão	Média ± desv. Padrão
C	57,98ª ± 4,23	13,98ª ± 1,69
T10	54,16ª ± 4,35	14,04ª ± 1,63
T2	60,63ª ± 3,09	14,18ª ± 1,53
T4	60,57ª ± 4,57	13,03ª ± 1,52
T6	58,66ª ± 1,88	14,81ª ± 0,11
Data 29.09	**Perda de peso por cocção**	**Perda de peso por cocção**
	Média ± desv. Padrão	Média ± desv. Padrão
C	59,93ª ± 2,18	16,20ª ± 0,57
T11	58,21ª ± 4,64	14,29ª ± 1,12
T7	56,71ª ± 4,12	14,96ª ± 1,55
T8	60,44ª ± 3,95	14,03ª ± 0,96

Médias seguidas de letras iguais na mesma coluna não diferem entre si pelo teste de Tukey (P<0,05). C = controle; T1 = 1150 ppm|20s; T2 = 1150 ppm|30s; T3 = 1850 ppm|20s; T4 = 1850 ppm|30s; T5 = 1500 ppm|17,95s; T6 = 1500 ppm|32,05s; T7 = 1006,5 ppm|25s; T8 = 1993,5 ppm|25s; T9 = 1500 ppm|25s; T10 = 1500 ppm|25s; T11 = 1500 ppm|25s.

Nos resultados apresentados na Tabela 9 observa-se que o uso do ácido peracético, não influenciou na perda de peso ao descongelamento para nenhum dos tratamentos avaliados pois não apresentou diferença estatística ($P < 0,05$) em relação ao controle. O ácido peracético que interagiu com a pele e com o corte durante a aplicação e no armazenamento dos cortes até a realização das análises não contribuiu para a diminuição da capacidade de retenção de água, e dessa forma não levou à ocorrência de diferenças entre os tratamentos.

Thomas et al (2019), também avaliou o a influência do uso de antimicrobianos em bifes bovinos (ácido lático e ácido levunílico) na avaliação de perda de cozimento concluindo que o uso desses antimicrobianos nos parâmetros avaliados, não influenciou na perda de cozimento, quando comparados a amostra controle (sem tratamento com antimicrobianos).

2.12 Conclusão Final

(i) O ácido peracético é consistentemente eficaz na redução de patógenos (Salmonella e Campylobacter) e cargas microbianas no decorrer do processo;

(ii) Os estudos presentes no artigo de revisão demonstraram que o PAA sozinho ou em combinação com outro antimicrobiano, variou de 0,65 a 4,08 log onde 85% dos resultados, mostraram redução acima de 1 log, que é expressivamente proeminente;

(iii) O método de imersão dos cortes após o sistema de resfriamento tem sido o mais utilizado na literatura publicada;

(iv) O fósforo funciona como um indicador da presença de HEDP e pode ser um caminho para o desenvolvimento de um método a ser validado

(v) Em todas as concentrações utilizadas no estudo do capítulo 2, a menor redução logarítmica encontrada foi de ≅ 1 log o que é expressivo, atingindo reduções de 2 log para EB no peito de frango e 3,65 também para EB em coxa de frango.

(vi) Há influência da superfície de contato da ave (peito sem pele e coxa com pele) para a redução logarítmica uma vez que, para o corte coxa de frango há a necessidade de concentrações acima do ponto central para obter as maiores reduções logarítmicas, o que não ocorre no corte de peito de frango.

(vii) Por meio dos gráficos de superfície resposta e curvas de níveis conclui-se que, para o corte peito de frango a região próxima ao ponto central (1500 ppm) é a melhor condição de aplicação do PAA e para a coxa de frango, as melhores condições se encontram em concentrações acima de 1800 ppm.

(viii) O PAA utilizado como intervenção antimicrobiana para aves, é consistentemente eficaz para a redução de bactérias indicadoras do processo (CTM e EB) e consequentemente a redução de patógenos. Sendo assim, o uso do PAA em pontos finais do processo e/ou combinados ao longo do processo produtivo, é uma estratégia singular criando barreiras tecnológicas nos processos de aves.

(ix) O ácido peracético não influenciou nas características tecnológicas da carne de frango uma vez que não alterou as características de aparência da carne *in natura* avaliadas por análises objetivas (cor, textura e perda de cozimento), o que poderia impactar na preferência e aceitabilidade do consumidor.

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