



The effect of the commercial fish gelatin protein hydrolysate on rainbow trout (*Oncorhynchus mykiss*) fillet quality

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Abstract

Willing to increase the shelf life of the food matrix, studies regarding new preservations methods are developed to preserve fish deterioration. The application of natural additives as preservatives is increasingly common, with a current emphasis on the growing use of protein hydrolysates, in substitution to artificial ones. These compounds have been the subject of recent studies, focusing on determining their functional properties and the best form of applying peptide chains. The present study aimed to evaluate the effects of the protein hydrolysate obtained by the enzymatic hydrolysis of commercial fish gelatin added to rainbow trout fillets (*Oncorhynchus mykiss*) packed under vacuum and maintained under refrigeration. The fillets were distributed into three sample groups (controls and hydrolysate addition at 1:10 and 1:1 ratios). Microbiological and physico-chemical assessments were carried out. The results were compared and correlations observed regarding fish preservation assessments. A relationship between pH, redox potential and N-TVB was verified when compared to microbial development, especially with regard to psychrotrophic aerobic heterotrophic bacteria count. It was concluded that the application of protein hydrolysate as an additive is promising and can be effective in the control of food quality and maintenance of freshness for a longer period.

Keywords: bioactive peptides; food additive; microbiological analysis; shelf life; food quality.

Practical Application: Employment of protein hydrolysate obtained from the enzymatic hydrolysis of gelatin in the preservation of the rainbow trout.

1 Introduction

Fish is a highly perishable foodstuff. Because of this, significant interest in preservation methods is noted concerning the control of certain facilitators of undesirable physical-chemical reactions and microbial development, with the aim of reducing biological collective health threats and costs (Barcellos et al., 2016). Intrinsic [Hydrogenionic potential (pH), water activity, oxidation potential composition] and extrinsic (relative humidity, temperature, presence or absence of oxygen) food product factors may facilitate, hinder or inactivate food disease agent development (Huss et al., 2003).

One of the strategies applied in this regard by the industry is the use of additives as food ingredients, which serve several purposes according to specific technological properties, such as the preservation of physico-chemical, microbiological or sensorial qualities, and also includes maintenance of consumption conditions for longer periods of time. Studies on the functionalities of protein hydrolysates (PH), recognized and listed as "Generally Recognized as Safe (GRAS)" have become routine (Food and Drug Administration, 2010) among several technologically-applied additives.

Protein hydrolysates may be obtained through the hydrolysis of peptides from animal gelatin products. Gelatin from mechanically separated fish meat, mainly carcasses and muscle chips, contains

considerable amounts of protein (93%) and displays high storage stability. Furthermore, it is a higher value option for the use of waste than a production of flours and feed. These components present high nutritional value and digestibility, as well as a balanced amino acid composition (Venugopal et al., 1996). Gelatin hydrolysis can be carried out through the addition of different enzymes, such as Alcalase™ 2.4, in order to accelerate the process (Benjakul & Morrissey, 1997).

Decreases in pathogenic food microbiota amounts can be achieved by direct PH addition, either superficially, directly into the food composition or on to the food packaging, aiming for antimicrobial contact and action on product surfaces (Soares et al., 2009).

Food product pH, redox potential and total volatile nitrogen bases (TVB-N) values can be altered by the presence of microorganisms. pH can be influenced by the simple and immediate interaction of matrix molecules with the oxygen present in the environment, whereas TVB-N can be influenced by factors indirectly related to lipid oxidation, such as enzymatic processes and microbial metabolism. Increased biogenic amines during the *post-mortem* period is due to the high amount of proteolytic enzymes present in the intestinal tract, in association to the rapid autolytic process of animal products. More effective

Received 24 Sept., 2019

Accepted 19 Nov., 2019

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control measures to prevent contamination by these substances have focused on inhibiting microbial growth and reducing decarboxylase activity (Cardozo et al., 2013).

Rainbow trout is a commercially important fish species, highly consumed, with high market value, presenting a rich composition, mainly due to the presence of polyunsaturated fatty acids (PUFAS) of the omega-3 type (Instituto Galego de Formação em Aquicultura, 2015).

Studies have been increasing the commercial value of species such as salmonids. Therefore there has been a significant enhance in the financial share of world trade, which has led them to occupy the position of greater merchandise in terms of individual value in 2013 (Food and Agriculture Organization of the United Nations, 2016).

According to the Brazilian Association of Truticultors, statistically the annual production of trout revolves around 2,000 t, that is, 23% of the national consumption of salmonids (Porto-Foresti et al., 2002). The climate and temperature of the waters of Nova Friburgo in Rio de Janeiro, Brazil, for example, are favorable to the optimization of the process (Serviço Brasileiro de Apoio às Micro e Pequenas Empresas, 2015).

The aim of the present study was to evaluate the effects of a bioactive PH obtained by the enzymatic hydrolysis of commercial fish gelatin on the bioconservation and safety of vacuum-packed rainbow trout fillets (*Oncorhynchus mykiss*) maintained under refrigeration.

2 Materials and methods

2.1 Commercial fish gelatin hydrolysate preparation

The PH was obtained by applying the methodology proposed by Nikoo et al. (2014) and modified by Lima et al. (2018), at the Biochemistry Laboratory of the Center of Technology and Food Agribusiness-Agricultural Research Company (EMBRAPA), Brazil. The process comprises the hydrolysis of commercial fish gelatin (Brazilian Ministry of Health registration: 6.6660.0006), composed of proteins derived from different fish species by-products. The gelatin was dissolved in water at 55 °C, pH 6 and hydrolysis was performed using Alcalase™ 2.4 (donated by Novozymes Latin America Ltda.) in a 2.0 L jacketed reactor (working volume of 1.5 L) under controlled agitation, pH and temperature. The reaction was carried out for 90 min, at constant pH through the continuous addition of NaOH. The reaction was stopped by heating the reaction medium at 85 °C for 10 min, followed by cooling.

2.2 Fish sample preparation

The samples were a total of 16 kg of packaged commercial trout fillets weighing about 350 g purchased in packages containing two fillets each one, from the same lote, of aquaculture from Friburgo, in Rio de Janeiro, Brazil. They were transported frozen in an isothermal container to the Animal Origin Product Microbiological Control Laboratory, at the Veterinary Faculty - UFF for bacteriological analyses, and to the State Center for Research on Food Quality (CEPQA) belonging to the Rio de

Janeiro State Agricultural Research Company (PESAGRO-RJ) for physicochemical analyses. The fillets were thawed under overnight refrigeration, discarding the tail and the liquid lost in the thawing process, homogeneously divided in two samples of 150 g. Randomized and distributed into three groups: control and containing PH solution at 1:10 and 1:1 ratios. The PH was added with a pipette and vacuum-packaged maintained at 2 to 4 °C throughout the experiment.

2.3 Microbiological analyses

PH microbiological parameter assessments followed Brazilian regulations (Brasil, 2001) for “vitamin and mineral supplements and similars, presenting as powders, capsules, dragees and similars”, and were chosen due to similarities to the assessed product. Item “C” concerning regulations on “other powdered products, capsules, dragees and similars, such as gelatin, guarana, catuaba, marapuama, lecithin and others, isolated or in mixture”, establish coliform counts at 45 °C, coagulase positive *Staphylococcus* and the detection of *Salmonella* spp. In addition to the recommended analyses, Mesophilic Aerobic Heterotrophic Bacteria (MAHBC), Psychrotrophic Aerobic Heterotrophic Bacteria (PAHBC) and *Escherichia coli* counts were also performed in order to assess hygienic sanitary processing conditions.

The fillet analyses were carried out according to the established Brazilian regulation (Brasil, 2001) for “fish, fish roe, crustaceans and cephalopod molluscs *in natura*, cooled or frozen and not consumed raw”, while *Salmonella* spp. and *Staphylococcus* coagulase positive analyses were performed in accordance to 3M™ instruction manuals (3M Petrifilm™, 2013a, d). Coliform counts was carried out at 45 °C for total coliforms (3M Petrifilm™, 2013b). These assessments began on day zero (1st analysis point – T₀), when the hydrolyzed product was applied to the samples, followed by an analysis every 24-hour interval, totaling six sampling points (T₀, T₂₄, T₄₈, T₇₂, T₉₆ and T₁₂₀) in 120 hours. According to Brazilian legislation (Brasil, 2017), the refrigerate temperature for fishery products should be maintained up to 5 °C for no more than 7 days. The packages of the fillets recommended 2 or 3 days of validity, because they are frozen fillets. All analyses were performed in duplicate and counts were expressed as log CFU/g (logarithm colony forming unit per gramme).

The MAHBC and PAHBC determination methodologies differ only with regard to the plate incubation time and temperature, as described in the “Compendium of Methods for the Microbiological Examination of Foods” (American Public Health Association, 2015). For PAHBC, Petrifilm™ plates were used for aerobic counting (3M Petrifilm™, 2013c), following the established incubation instructions of 4 °C for ten days.

2.4 Physico-chemical analyses

The physico-chemical analyses were carried out in accordance to the “Official analytical methods of physico-chemical control of hake and its derivatives” manual (Association of Official Analytical

Chemists, 1990, 2005). Assessments were carried out on day zero (1st analysis point – T₀), when the hydrolyzed product was applied to the samples, followed by an analysis every 24-hour interval, totaling six sampling points (T₀, T₂₄, T₄₈, T₇₂, T₉₆ and T₁₂₀) in 120 hours. All analyses were performed in duplicate.

pH and redox potential determinations were performed using a potentiometer (Tecnal™, SP, Brazil) and expressed as meq/kg. Amounts of thiobarbituric acid-reactive substances (TBARS) were determined by the methodology described by Benjakul & Bauer (2001). The optical densities of the sample supernatants were determined at 532 nm using a UV-160 spectrophotometer (Thermo-Waltham™, MA, USA) and the results were expressed as mg of malonaldehyde (MA)/kg sample. The TVB-N, analysis, often used as spoilage indicator for fresh seafood maintained on ice (Haaland & Njaa, 1988), was performed using a Kjeldahl apparatus. TVB-N and the results were expressed as 100 g/sample (Lyman et al., 1953).

2.5 Statistical analyses

Descriptive statistics were applied in order to perform an exploratory analysis.

The bacteriological and physico-chemical results were assessed by the Kruskal-Wallis non-parametric test, at a significance level of 5% (p ≤ 0.05) in order to compare the three experimental groups. The Mann Whitney test at a significance level of 2% (p ≤ 0.02) was applied for comparisons between pairs. Pearson’s linear correlation coefficient was used to assess potential associations among the assessed variables and Spearman’s correlation coefficient was applied for comparisons between means physico-chemical and microbiological values, at a significance level of 5% (p ≤ 0.05).

All data were processed using the IBM Statistical Package for the Social Sciences (SPSS), version 17.0.

3 Results and discussion

3.1 The effect of the protein hydrolysate on rainbow trout fillet microbiota

In the PH microbiological evaluation, no contamination was detected.

All samples (controls and hydrolysate addition at 1:10 and 1:1 ratios) tested negative for *Salmonella* spp. in accordance to Brazilian RDC n° 12 ANVISA determinations (Brasil, 2001). No contamination was observed for 25 g of the samples confirmed rainbow trout fillet safety conditions.

Regarding MAHBC, the lowest observed value was of 4.28 log CFU/g in the control group on the T₇₂ of the experiment, while the highest was 7.63 log CFU/g in the 1:10 PH ratio group on the T₉₆. The other growth apex was noted in the 1:10 ratio group on the T₄₈, at 7.10 log CFU/g. The means of the evaluations were of 6.57 log CFU/g with a standard deviation of 5.54 log CFU/g (Figure 1).

The MAHBC value at the T₀ was of 5.37 log CFU/g in the control group, while PAHBC in the same point displayed a lower value, ranging from below the limit of detection in the control group to 2.0 log CFU/g in the 1:10 PH ratio group.

With the exception of the 1:10 ratio group regarding MAHBC, the remaining samples were all within the established limits (International Commission on Microbiological Specifications for Foods, 1986) and considered safe for consumption until the end of the experiment. Thus, PH addition did not stimulate microbiota growth. Initial bacterial loads and bacterial development throughout the food production chain are obstacles to product commercialization, and usually lead to economic losses (Franco, 2012).

No MAHBC and PAHBC limits are established in Brazilian legislation regarding microbiological food standards (Brasil, 2001). As

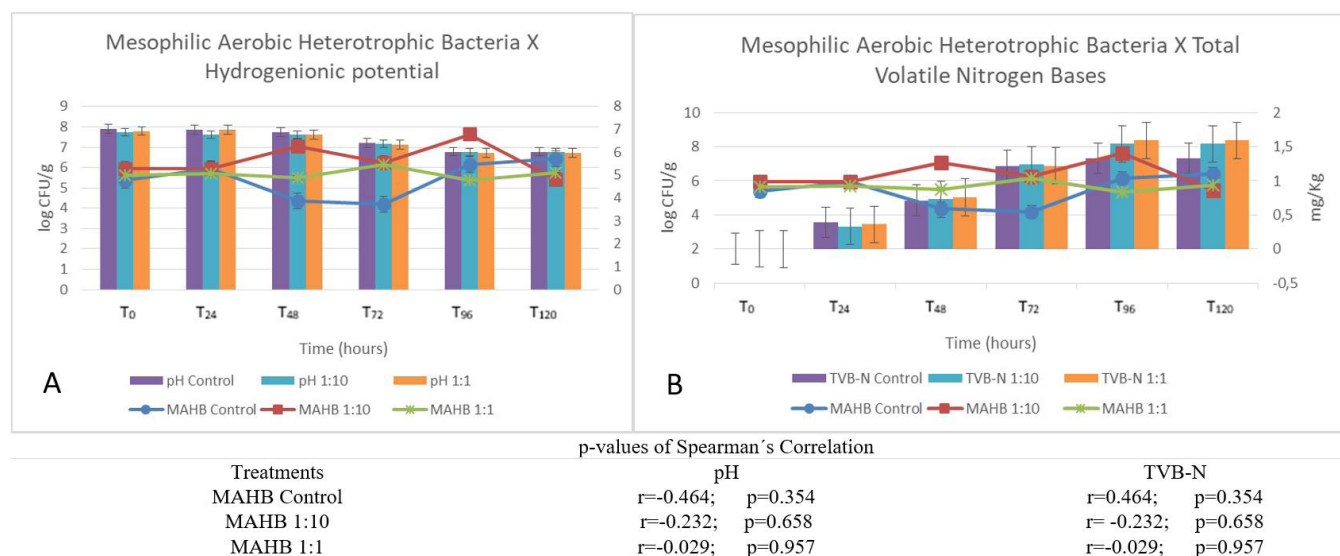


Figure 1. Association between mean MAHB counts (log CFU/g) in relation to the mean physico-chemical parameter values (p > 0.05) of rainbow trout fillets in the three sample groups: control, PH addition at 1:10 and 1:1 ratios during 120 hours, assessed at six analysis points: (A) MAHB X pH; (B) MAHB X TVB-N (mg/kg).

a result the standards established by the International Commission on Microbiological Specifications for Foods (ICMSF) were applied, which specify a limit of 7.0 log CFU/g (International Commission on Microbiological Specifications for Foods, 1986) for these microorganisms. This value was surpassed in the 1:10 group at the apex of the T₄₈ and T₉₆. However, although some samples extrapolated this standard, 95% of the samples presented values below 7.04 log CFU/g. The importance of mesophilic bacteria assessments is related to the evaluation of the sanitary conditions of the studied matrix and eminent risks to collective health, since all pathogenic bacteria present in food products belong to this group (Franco & Landgraf, 2008).

Concerning PAHBC, values between zero and 3.0 log CFU/g in the 1:10 ratio group in the T₇₂ were observed, with means of 1.91 log CFU/g and standard deviation of 1.18 log CFU/g. These low values are due to the fact that the samples were initially frozen and maintained under refrigeration during the experiment. High PAHBC counts lead to the acceleration of the deterioration process, mainly due to protease, lipase and phospholipase syntheses (International Commission on Microbiological Specifications for Foods, 1986) (Figure 2).

A fast development of psychrotrophic microorganisms was observed in all rainbow trout fillet groups, including in the T₁₂₀, when other microorganism counts had already decreased. This may be associated to bacterial ecology, due to the capacity of bacterial microbiota alterations during storage, when these organisms may adapt to the imposed environmental conditions (Gram & Dalgaard, 2002). The decomposition generated by the presence of psychrotrophic bacteria in food is especially justified by their ability to produce different enzymes (International Commission on Microbiological Specifications for Foods, 1986).

The 3M Petrifilm™ method (2013a) is used to determined *S. aureus* counts. The Brazilian regulation (Brasil, 2001) indicates a limit of 3.0 log CFU/g only for coagulase positive *Staphylococcus*,

an enterotoxin-producing microorganism commonly associated with food poisoning. However, as *S. aureus* is also a coagulase positive bacterium, the limit established in Brazilian regulation for coagulase positive *Staphylococcus* was applied.

The development of *S. aureus* is noteworthy, due to the fact that this microorganism is able to grow in different pH ranges (Franco & Landgraf, 2008). Values ranged from zero in the control group in the T₀ and T₁₂₀ of the 1:1 group. One sample presented 3.0 log CFU/g in the control group at the T₇₂. The means were of 2.30 log CFU/g and standard deviation of 2.04 log CFU/g. Count decreases were observed in all groups between the T₉₆ and T₁₂₀, indicating a declining phase, justified by drops in nutrient levels and probable competition with other microorganisms (Figure 3).

In relation to *S. aureus*, total coliforms and *E. coli*, lower counts were identified and maintained throughout the test. In the T₉₆ shows a reduction, suggestive of the beginning of bacterial growth decline stage. The presence of these microorganisms indicate the hygienic-sanitary condition of the assessed fish, and aid in identifying the contamination stage.

In the T₀ the initial isolation, it was obtained significant counts of coliforms group. But the variation log CFU/g counts have a tendency in the control and 1:1 groups, down to below limit of detection. The highest value was determined as 3.34 log CFU/g in the 1:10 ratio group in the T₄₈, with means of 2.28 log CFU/g and standard deviation of 1.48 log CFU/g (Figure 4).

This is similar to what was observed for *E. coli*, with a minimum of zero in some evaluations, including in the T₁₂₀ for all groups. The development peak was observed at 2.78 log CFU/g in the 1:10 ratio group in the T₄₈. While in the 1:1 group, counting was only observed in the T₀ samples, with a highest value of 1.85 log CFU/g, and no positive values detected at any other analysis points. The means and standard deviation were of 1.94 log CFU/g and 1.57 log CFU/g, respectively (Figure 5).

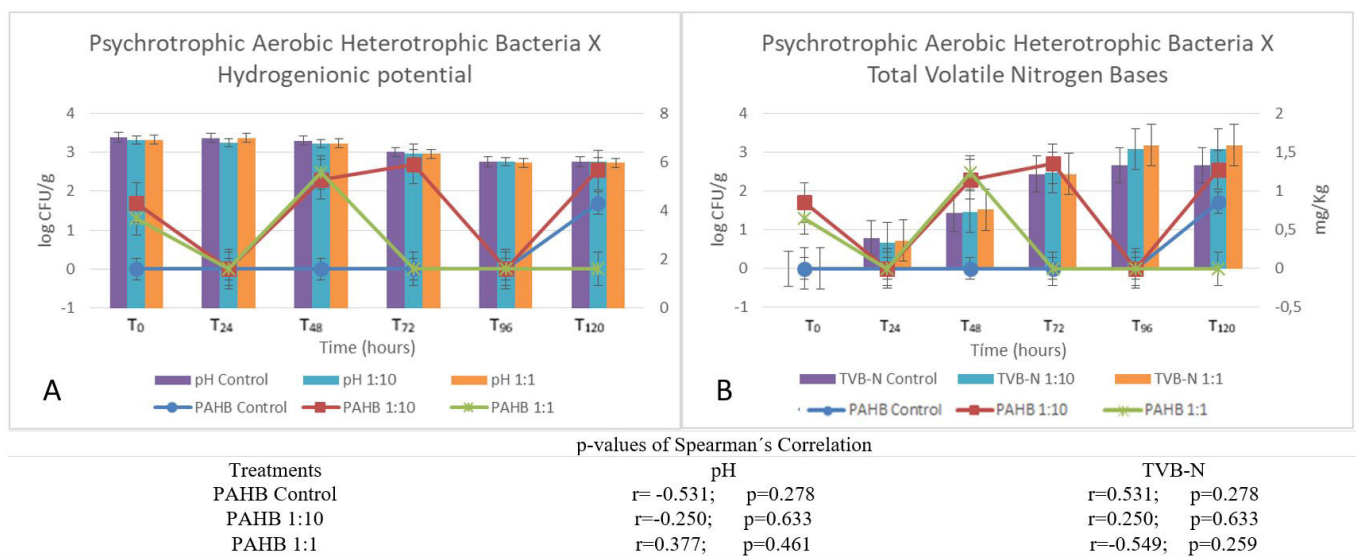


Figure 2. Association between mean PAHBC counts (log CFU/g) in relation to the mean physico-chemical parameter values ($p > 0.05$) of rainbow trout fillets in the three sample groups: control, PH addition at 1:10 and 1:1 ratios during 120 hours, assessed at six analysis points: (A) PAHBC X pH; (B) PAHBC X TVB-N (mg/kg).

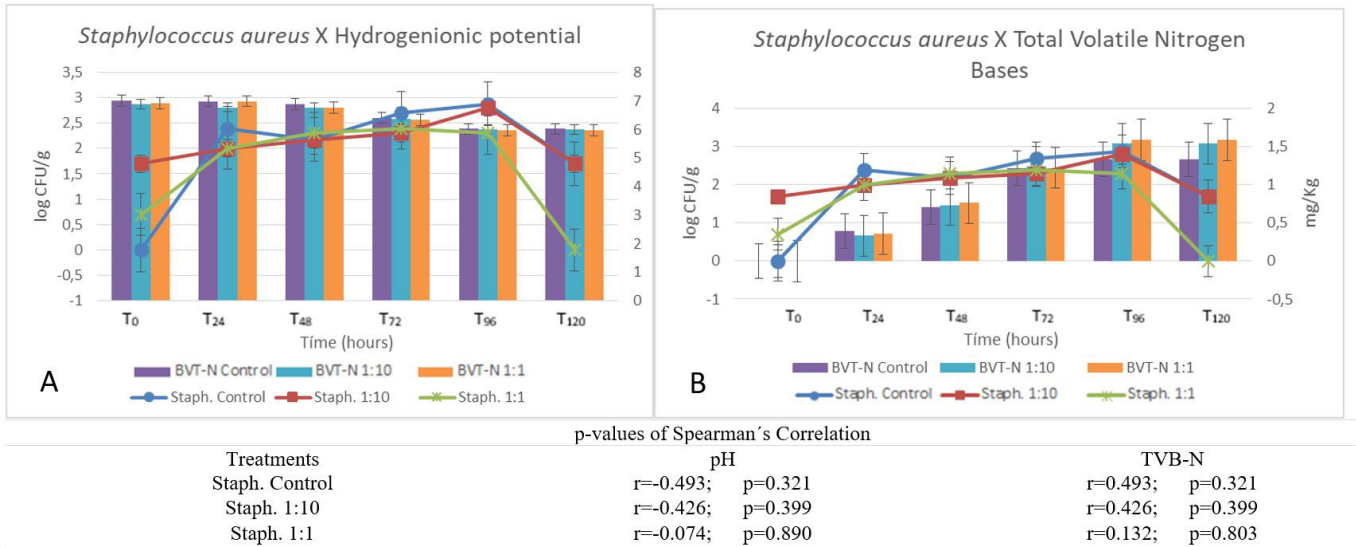


Figure 3. Association between mean values *Staphylococcus aureus* counts (log CFU/g) in relation to the mean physico-chemical parameter values ($p > 0.05$) of rainbow trout fillets in the three sample groups: control, PH addition at 1:10 and 1:1 ratios during 120 hours, assessed at six analysis points: (A) *Staphylococcus aureus* X pH; (B) *Staphylococcus aureus* X TVB-N (mg/kg).

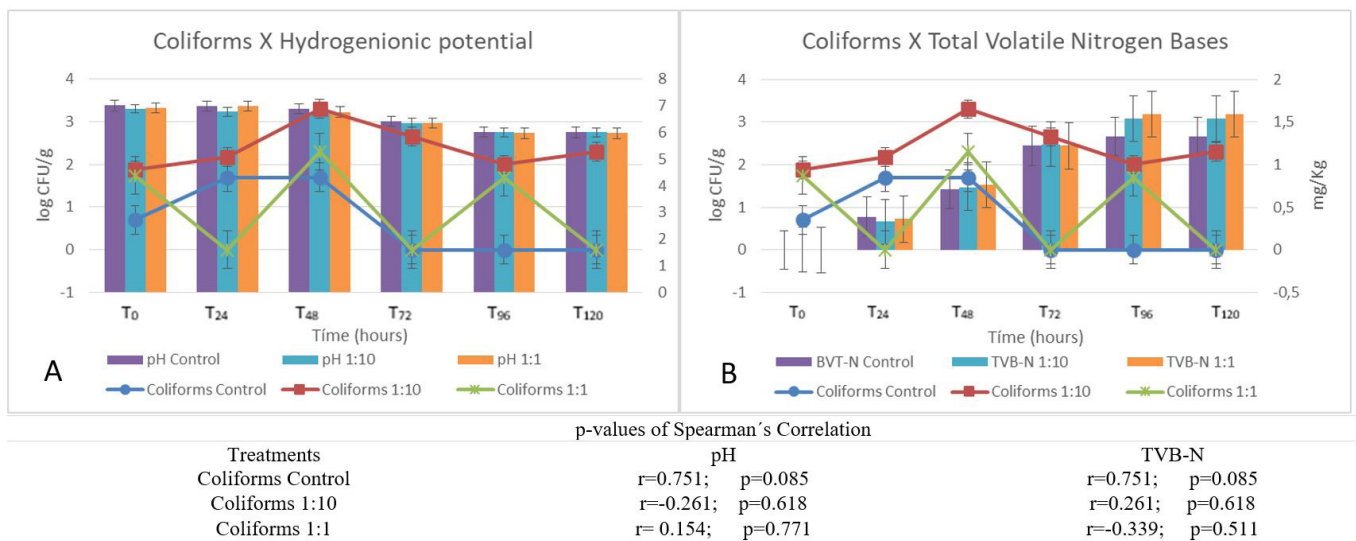


Figure 4. Association between mean values coliforms group counts (log CFU/g) in relation to the mean physico-chemical parameter values ($p > 0.05$) of rainbow trout fillets in the three sample groups: control, PH addition at 1:10 and 1:1 ratios during 120 hours, assessed at six analysis points: (A) coliforms X pH; (B) coliforms X TVB-N (mg/kg).

No limits are established in Brazilian (Brasil, 2001) or international standards for total and fecal coliforms in *in natura* fish. The presence of this microbiota in the food matrix can be indicative of fecal and/or environmental contamination. Fontes et al. (2007), in an experiment carried out on fish samples, adopted the standards described by Ribeiro (1974) for raw fish, i.e. the absence of total coliforms and *E. coli*. A total of 30% of the samples analysed by this author were in non-conformity regarding total coliforms, and *E. coli* was not detected in any of the samples.

No standard for total coliforms is available (Brasil, 2001; Huss, 1997; International Commission on Microbiological

Specifications for Foods, 1986), so a limit of 3.0 log CFU/g was considered, as the final product is considered unsatisfactory for consumption when total coliforms exceed this hygienic indicator value (Brasil, 2001). For *E. coli*, the established limit for thermotolerant coliforms was applied, from 11 to 500 NMP/g (International Commission on Microbiological Specifications for Foods, 1986). Only samples from the 1:10 ratio group in the T₄₈ for both microorganisms exceeded this standard.

No statistically significant difference ($p > 0.05$) between sample groups was noted for the bacteriological analyses.

Peptides with antimicrobial activity have been isolated from several species. For example, American lobster hemolymph

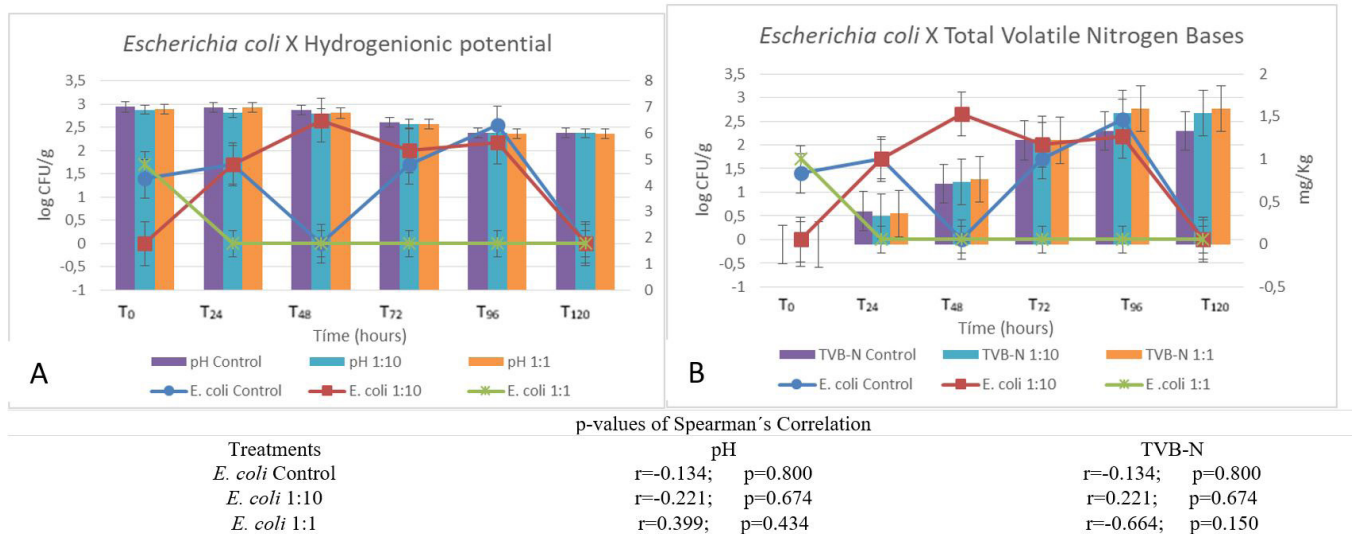


Figure 5. Association between mean values *Escherichia coli* counts (log CFU/g) in relation to the mean physico-chemical parameter values ($p > 0.05$) of rainbow trout fillets in the three sample groups: control, PH addition at 1:10 and 1:1 ratios during 120 hours, assessed at six analysis points: (A) *Escherichia coli* X pH; (B) *Escherichia coli* X TVB-N (mg/kg).

(*Homarus americanus*) has been reported to exhibit bacteriostatic activity against certain Gram-negative bacteria (Battison et al., 2008), while a cysteine-rich peptide has been isolated from oyster (*C. gigas*), capable of inhibiting the growth of both Gram-positive and Gram-negative bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*, as well as fungi (*Botrytis cinerea* and *Penicillium expansum*) (Liu et al., 2008).

3.2 Physico-chemical parameters versus microbial development

PH safety was evaluated, and physico-chemical characterizations carried out. Total protein values were determined at 17.4 mg/100g, water activity and freshness at 0.99, pH at 7.03 and redox potential at 21.3 mg/kg, while non-protein nitrogen was “undetected” (LOD: 0.001 mg/100g; LOQ: 0.008 mg/100g), ratifying the microbiological results, with the purpose of assuring the safety and quality of rainbow trout sample PH application.

In relation to physico-chemical rainbow trout fillet parameters, a decrease in mean pH values during storage was noted in the control group, from 7.01 in the T_0 to 6.02 in the T_{120} . Redox potential increased from 12.5 mg/kg to 34.5 mg/kg in the same group, even when packed under vacuum, while TVB-N increased from ≤ 0.1 to 1.33 mg/kg and TBARS from ≤ 0.01 to 2.47 mg/kg. No statistically significant difference ($p > 0.05$) was observed between the initial and final values for each group or during the different analysis points.

According to Brazilian regulations (Brasil, 2017), adequate fresh fish quality and identity standards are observed at $pH < 7.0$ and TVB-N values of up to 3.0 mg/kg, while the limit set by the USDA (2005) for TBARS ranges from 1 to 2 mg/kg. No time differences were detected between the sample groups ($p > 0.05$), the control and treatment groups or different PH dilutions. No PH influence in relation to pH, TVB-N, TBARS and redox

potential was observed ($p > 0.05$) when comparing the three sample groups.

An increased redox potential may be associated to enzymatic activity, which is, in turn, related to microbial growth (Figure 6).

According to Azeredo (2012), aerobic bacteria, mainly deteriorating bacteria and some pathogens, require the presence of oxygen and a high redox potential, usually between 35 mg/kg and 50 mg/kg, to multiply. Anaerobes, on the other hand, especially pathogens and some spoilage organisms, require low redox potential levels, usually below -15 mg/kg. Thus, the initial redox potential of the samples may have influenced bacteria development, including *Salmonella* spp., *Staphylococcus aureus*, total coliforms and *Escherichia coli*, which are able to grow in anaerobic environments (Franco, 2012).

Considering Pearson's correlation, was observed significant relationship between TBARS and TVB-N ($p < 0.001$), redox potential and TBARS ($p < 0.001$) and redox potential and TVB-N ($p < 0.001$). According to Brazilian regulations (Brasil, 2001) the TVB-N is the method frequently used to determine hydrolysis level in food, although the enzymatic action (TBARS) and microbial metabolism (redox) can be used as well with good correlation.

Advances in the deterioration of muscle proteins, either due to autolytic enzyme action or the presence of microorganisms, lead to the formation of volatile nitrogenous compounds, such as trimethylamine, dimethylamine and ammonia, which are identified in the TVB-N analysis. In addition other substances are also formed, with amounts varying with different storage periods (Moura et al., 2003), as verified by Shamshad et al. (1990), who detected an approximate two-fold increase in TVB-N values in prawns stored at room temperature, associating this result with increased microbial counts and enzymatic activity.

Moura et al. (2003) detected higher microbial counts (ranging from 1.0×10^4 to 3.0×10^7 CFU/g) with increasing pH values

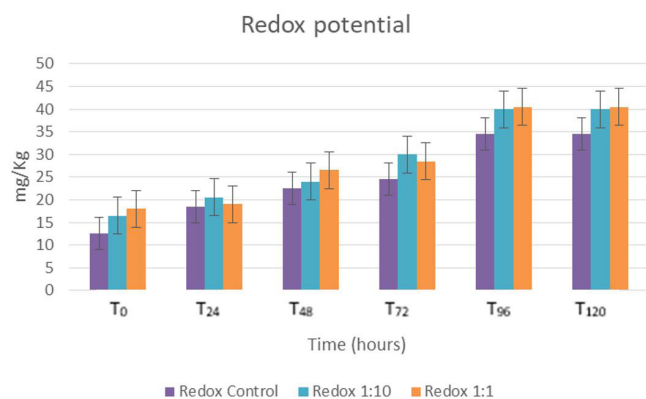


Figure 6. Graphical representation of mean values of redox potential (mg/kg) of rainbow trout fillets in the three sample groups: control, PH addition at 1:10 and 1:1 ratios over 120 hours, assessed at six analysis points ($p > 0.05$).

(ranging from 7.09 to 8.10), noting a correlation between PAHBC and pH. In addition, correlations were also identified between TVB-N (ranging from 27.6 to 73.0 mgN/100g) and pH, and between TVB-N and PAHBC. The pH parameter displays the best correlations between the variables determined herein, as opposed to TBARS (values between 0.17 and 2.12 mgMA/kg), which did not display any significant correlation with the assessed parameters.

Lactic acid bacteria are commonly used as natural food preservatives, due to the action of their metabolites and bacteriocin production, which displays antimicrobial action (Vignolo et al., 2012).

Behnam et al. (2015) evaluated the effect of nisin on the shelf life of chilled rainbow trout (*Oncorhynchus mykiss*), reporting a consonance between physico-chemical and microbiological analyses. These authors identified lower aerobic mesophilic, psychrotrophic and lactic acid heterotrophic bacteria counts in the treated groups compared to the control group. Thus, they concluded that, despite increased storage time, established limits were not exceeded due to vacuum packaging and a gradual increase of carbon dioxide levels in the packages, which may have an inhibitory effect on Gram-negative microorganisms. This was similar to what was observed in the present study, where, although increased microorganism counts were noted during the evaluation period, none of the samples exceeded regulatory limits. This is probably associated to the vacuum packaging, as, with increasing storage time, decreasing total coliform and *Escherichia coli* counts, both Gram-negative organisms, were verified. Despite no statistically significant difference ($p > 0.05$) between the three groups, possible effect was observed evidenced in the enhancement of the exponential phase of the treated groups in relation to the control.

Physico-chemical parameters can, thus, be associated to microbial development and product quality, in order to allow shelf life extension or reduction.

Regarding the identified associations between MAHB/PAHB/*Staphylococcus aureus* counts and pH, no

statistically significant difference was observed ($p > 0.05$) when comparing the mean values of the control group. The same was noted for mean MAHB/ PAHB/ *Staphylococcus aureus* counts and TVB-N. These results indicate no significant correlation ($p > 0.05$), although TVB-N assessments are recommended for storage time evaluation and graphically demonstrated a joint evolution.

Regarding MAHB results, the reduction of pH led to increase bacterial development of group 1:10 and consequently of the values of TVB-N (Figure 1). Similarly to that observed with the values of the *S. aureus* counts found in the three sample groups (Figure 3). Concerning the growth of PAHB, it was evidenced that the reduction of pH generated a reduction of the microbiota of the group treated with PH 1:10, facilitating the development of resistant microorganisms due to decreased competition and increasing levels of TVB-N (Figure 2).

Moura et al. (2003) also used these associations for data validation, detecting significant relationships between PAHB counts and pH ($r = 0.85$) and between PAHB and TVB-N ($r = 0.68$), thus reinforcing the effectiveness of the comparison methodology regarding microbiological and physico-chemical parameters to determine food product quality.

Hosseini et al. (2016) adding fish gelatin coating with incorporated oregano essential oil applied to rainbow trout (*O. mykiss*) and observed effectiveness in reducing TVB-N and redox potential products, besides the inhibiting growth of MAHB and PAHB. In the present study, similar results were found, confirming the potential of the product. However, as no statistically significant difference was verified, it is necessary to optimize its application to obtain better results.

In the same way Yıldız & Yangılar (2016) with trout coating with edible film with whey protein formulations, verified efficacy in controlling microbial development and prolonged product life. During the storage of the treatment samples, TVB-N and TBARS values determined the effect of edible film coating on the rate and extent of the product quantitatively in fish. The PH applied in our study shows the same effects preventing the oxidative products, but when compared control and treatments not observed significant difference, demanding diversify PH adding forms in other points of fillet processing.

4 Conclusions

Commercial PH obtained from fish gelatin does not stimulate microbial development in rainbow trout fillets. Although no statistically significant difference was verified between the values of the sample groups, a potential effect was observed, with an increase of the exponential phase and reduction of the stationary phase of the assessed bacteria. According to the microbiological and physico-chemical results, the PAHBC proliferation was associated to pH decrease, redox and TVB-N increase. Studies should be done to optimize the application and improve the efficacy of PH, since it has proven to have an effect on the preservation of fish freshness according to the standards suggested in the legislations.

Acknowledgements

This work was supported and financed by CAPES and UFF. The authors are grateful to Embrapa Agroindústria de Alimentos and to Centro Estadual de Qualidade de Alimentos - Empresa de Pesquisa Agropecuária do Estado do Rio de Janeiro (CEPQA-PESAGRO/RJ).

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