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ScienceDirect

Procedia Food Science 7 (2016) 13 – 16

Procedia Food Science

9th International Conference on Predictive Modelling in Food

Effect of UV-C radiation on shelf life of vacuum package *Colossoma macropomum* x *Piaractus mesopotamicus* fillets

Fernanda de Oliveira Bottino[a], Bruna Leal Rodrigues[a], Joanna Damazio de Nunes Ribeiro[a], César Aquiles Lázaro[a,b], Carlos Adam Conte-Junior[a]*

[a]*Departamento de Tecnologia de Alimentos, Faculdade de Veterinária, Universidade Federal Fluminense (UFF), Niteroi, Rio de Janeiro, Brazil*
[b]*Facultad de Medicina Veterinaria, Universidad Nacional Mayor de San Marcos (UNMSM), San Borja, Lima, Peru*

Abstract

Colossoma macropomum x Piaractus mesopotamicus (CP) is a freshwater fish with greatest commercial importance in Brazil. Fillets of CP are highly perishable food and preservation technology with UV-C could improve food safety and extend shelf life. Fillet samples were submitted at UV-C (55.83 mJ/cm^2) and examined for mesophilic and psychrotrophic count and biogenic amines over 6 days. UV-C reduced the bacterial growth and number of colonies in the stationary phase; also increase the levels of cadaverine, putrescine and histamine. The results suggest that UV-C enhanced the shelf-life of CP fillets by at least 50%.

Peer-review under responsibility of Department of Food Science, Faculty of Food Engineering, University of Campinas.

Keywords: UV-C; modelling growth; biogenic amines

1. Introduction

Colossoma macropomum x *Piaractus mesopotamicus* (CP) represents a greatest economic importance in Brazilian aquaculture[1], representing approximately 15.4% (60.463 metric tons) of the total annual production[2]. Healthy fish products are demanded by society. However, the chemical properties of this product during the chain production, promotes the increasing of bacterial load and the formation of metabolites from decarboxylation of amino acids by spoilage bacteria such as biogenic amines that are considered good parameter of food quality[3]. As an

* Corresponding author. Tel.: 55(21) 2629- 9545
E-mail address: carlosconte@id.uff.br

Peer-review under responsibility of Department of Food Science, Faculty of Food Engineering, University of Campinas.
doi:10.1016/j.profoo.2016.02.077

alternative to reduce these losses, studies about preservation technologies have been constantly performed aiming improve quality and durability to products of fish farming. UV-C radiation consists in a new non-thermal technology employed for superficial decontamination of several food matrices[4,5]. It is low cost and easy implementation, no production of reactivity, chemical waste or undesirable by-products which might alter sensory[6].This study aimed to assess the effects of UV-C radiation on microbiological and physicochemical parameters of vacuum-packed fillets with skin of the hybrid *Colossoma macropomum* x *Piaractus mesopotamicus* during storage at 4ºC.

2. Material and methods

Sixty fillets with skin of CP (weighing 550g each) were vacuum-packed individually in low-density polyethylene and heat-welded using a sealing machine (TECMAQ, AP450; Rio de Janeiro, Brazil). Under these conditions the fillets were submitted to two treatments: T1 (no UV radiation) and T2 (55.83 mJ/cm²). The UV equipment had 12 UV-C lamps (6 of 30W and 6 of 55W; OSRAMHNS, OFR, Munich, Germany) were placed longitudinally around the chamber's inner surface using a balanced pattern. Samples were placed at the geometrical center of the chamber using nylon net[7]. Radiation intensity was determined using a UV radiometer (MRUR-203, Instrutherm Ltda., São Paulo, Brazil). The UV-C radiation dose (mJ/cm²) was changed by altering intensities (switching some lamps on/off) while keeping the same total exposure time (60s). After the UV treatment, samples were stored at 4ºC for 6 days and submitted to microbiological biogenic amines analyses on days 0, 2, 3, 4, 5 and 6. All analyses were performed with experimental and analytical duplicates.

Total aerobic mesophilic (TAMB) and psychrophilic bacteria (TAPB) were evaluated using serial dilutions of 25 g of sample homogenized with 225 mL of 0.1% peptone water. Plate count agar was used to determine the bacterial count. Results were expressed as log cfu per gram. Analysis of biogenic amines was conducted with 5 g of fish meat, extracted with perchloric acid 5% and derivatized with benzoyl chloride (40 µL). The chromatographic system consisted of a Shimadzu Prominence UFLC apparatus (Shimadzu, Kyoto, Japan), a C18 Spherisorb ODS2 (15 × 0.46 cm i.d., 5 µm, Waters) column equipped with a Supelco Ascentis C18 (2 × 0.40 cm, i.d. 5 µm) guard column, under isocratic conditions. The mobile phase consisted of 42:58 (v:v) of acetonitrile (Tedia) and ultrapure water (Simplicity-Millipore, Molsheim, France). Chromatography conditions were 1 mL/min of flow rate, 20 µL of injection volume, column temperature of 20°C, UV absorption at 198 nm, run time of 15 min and cleaning step of 10 min between injections with 100% acetonitrile[8].

Bacterial growth parameters (log and stationary phase) were obtained using the statistical software DMFit 2.0 (IFR, Norwich, United Kingdom), based on predictive microbiology model[9]. A One-way ANOVA was carried out to identify differences between treatments during the storage time. When a significant F was found, additional post-hoc tests with Tukey adjustment were performed. All analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA).

3. Results and discussion

Previous to treatment, high initial mesophilic and psychrophilic (5.4 and 4.1 log CFU/g respectively) were detected. Different factors, including water conditions, preservation temperature, transportation and hygienic and sanitary conditions[10] may influence this counts. No lag phase was observed in both bacterial groups assessed, indicating that bacteria adapted quickly to cellular damage caused by UV-C light. The efficacy of this technology for disinfection of food products varies according to a variety of factors, such as characteristics of bacterial strain and species, growth rate[11], initial bacterial population density, composition and food type[12]. Furthermore, the mode of action of this preservation method works only on the food's surface and irregularities in the surface of the matrix may act as physical protection against UV-C rays contributing to bacterial survival. Moreover, it is well known that UV-C radiation promotes biochemical changes such as protein degradation, increasing nutrient bioavailability for the remaining bacteria[13]. This set of factors may have resulted in rapid bacterial adaptation and entry into log phase less than 24 hours, with no identifiable lag phase.

No significant count reduction ($p > 0.05$) was observed in samples subjected to UV-C when compared to the control group (Table 1), indicating that the largest UV-C radiation dose was not efficient in reducing initial microbial contamination of the fillets. Furthermore, longer doubling time and a smaller number of colonies were observed during the stationary phase in samples subjected to UV-C radiation in comparison to the control group, showing that this technology was also efficient in retarding microbial growth. These results may be linked to the well-established and variable action of UV-C, which may cause damages that affect DNA transcription and replication, leading to cell death and a lower number of microbial cells at the end of the growth curve[12, 13].

Table 1. Count reduction and growth parameters of total aerobic mesophilic and psychrophilic bacteria in samples of hybrid *Colossoma macropomum x Piaractus mesopotamicus* fillets stored at 4°C.

Group	*Total aerobic mesophilic bacteria**			*Total aerobic psychrophilic bacteria**		
	Log phase (h)	Stationary phase	Count reduction (Log cfu.g^{-1})	Log phase (h)	Stationary phase	Count reduction (Log cfu.g^{-1})
T1	0.39	9.78	0.00 ± 0.14^{a}	0.19	9.14	0.00 ± 0.00^{b}
T2	0.53	8.90	0.26 ± 0.17^{a}	0.47	8.13	0.25 ± 0.01^{a}

* No lag phase was observed; Log phase expressed in hours; stationary phase expressed in log cfu g^{-1}; Count reduction (Log cfu.g^{-1}): Bacterial load before UV-C treatment - Bacterial load after UV-C treatment. T1 (No UV-C dose) and T2 (55.83 mJ/cm²). Different lower case letters in the same column are significantly different ($p < 0.05$).

The microbiological upper limit for fresh fish of 7 log CFU/g was used to determine shelf life of fillets during storage (ICMSF, 1986). For total aerobic mesophilic bacteria, the values reached the upper limit of 7 log CFU/g on the 2nd day of storage in the control group and on the 3rd day for T2. The shelf life of fillets was thus extended by at least 50% in samples subjected to T2.

Results for the concentration putrescine, cadaverine and histamine throughout the storage time are represented in Table 2. Great initial biogenic amine concentrations were found in the present study, which agrees with high initial bacterial load in fillets. A significant increase ($p < 0.05$) in putrescine values was observed from the 4th day of storage for T1 and T2. Both treatments showed increases in cadaverine values ($p < 0.05$) from the 2nd day of storage, however values for T1 were higher when compared to T2 during the whole storage time from the 2nd day ($p < 0.05$). Regarding histamine production, an increase ($p < 0.05$) was verified from the 5th day of storage for samples subjected to T1, from the 2nd day for those subjected to T2. Histamine concentrations exceeded the maximum limit of 50 mg/kg (50 ppm) established by the American Food and Drugs Administration[14] and considered in this study on the 1st day of storage for T1 and 2nd day for T2.

Table 2. Biogenic amine concentrations (mg/Kg) in hybrid *Colossoma macropomum x Piaractus mesopotamicus* fillets stored at 4ºC for 6 days.

	Storage time (days)	
Group	0	6
Putrescine		
T1	$560.38 \pm 327.77^{b, A}$	$1941.70 \pm 525.72^{a, B}$
T2	$262.81 \pm 16.31^{b, A}$	$3711.85 \pm 496.71^{a, A}$
Cadaverine		
T1	$27.39 \pm 9.74^{b, A}$	$2630.53 \pm 622.15^{a, A}$
T2	$16.56 \pm 5.28^{b, A}$	$1369.25 \pm 197.47^{a, B}$
Histamine		
T1	$361.33 \pm 187.55^{b, A}$	$5877.83 \pm 561.24^{a, A}$
T2	$28.36 \pm 7.10^{b, B}$	$290.61 \pm 70.06^{a, B}$

Values followed by different lower case letters in the same row and different capital letters in the same column are significantly

different ($p < 0.05$). T1 (No UV-C dose) and T2 (55.83 mJ/cm²)

Overall, UV-C radiation (T2 and T3) lead to higher biogenic amine production throughout the storage time, corroborating to Lázaro et al.[7], which also found an increase in biogenic amine values in rainbow trout fillets and chicken fillets subjected to UV-C. These results may be related to biochemical changes caused by the action of UV-C radiation, since it promotes degradation of proteins and essential amino acids[13], providing substrate for microbial metabolism by increased availability of free amino acids that leads to greater biogenic amines production[15].

4. Conclusion

UV-C enhanced the shelf-life of *Colossoma macropomum* x *Piaractus mesopotamicus* fillets by at least 50% by retarding microbial growth, presented lower growth rate and number of colonies in the stationary phase and increase the biogenic amines production

Acknowledgements

The authors thank the Research Foundation of the State of Rio de Janeiro (grants E-26/111.673/2013, E-26/201.185/2014, E-26/010.001911/2014 and E-26/110.094/2014, FAPERJ, Brazil) and the National Council of Technological and Scientific Development (grants 313917/2013-2, 401922/2013-8 and 311361/2013-7, CNPq, Brazil) for financial support. Bottino, F.O. and Lázaro, C.A. were supported by the National Council of Technological and Scientific Development (CNPq, Brazil).

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