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Descontaminação fúngica pelo ozônio gasoso de cavala (*Acanthocybium solandri*) fresca, seca e salgada

Fungi decontamination by gaseous ozone of fresh, dried and salted mackerel (*Acanthocybium solandri*)

Descontaminación fúngica por caballa fresca, seca y salada (*Acanthocybium solandri*) ozono gaseoso

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Resumo

O objetivo desse estudo foi investigar o efeito antifúngico do ozônio gasoso (O_3) (agente de descontaminação verde) em peixes cavala (*Acanthocybium solandri*) em diferentes tipos de preservação (frescos, secos e salgados). Os parâmetros de umidade das amostras [umidade / a_w] antes do tratamento com gás foram de 80,7% / 0,98, 55,55% / 0,74 e 49,5% / 0,70, respectivamente. Os peixes foram contaminados com gêneros de fungos capazes de crescer em substratos com alto teor de umidade (*Fusarium*) e baixo teor de umidade (*Aspergillus / Penicillium*), depois gases tratados com 50 μmol de O_3 / mol, expostos por 10, 20 e 30 min, e incubados (25ºC, 7 dias) para avaliar a inativação de fungos. As amostras tratadas com O_3, quando expostas ao gás por mais tempo (30 min / Dia 7), tiveram o crescimento de fungos totalmente inibido (100%), enquanto as demais apresentaram apenas efeito de crescimento reduzido, ou seja; 40 e 70% por 10 e 20 min, respectivamente. Os esporos de *Fusarium* não foram capazes de crescer em nenhuma amostra de proteína estudada (ambos, Grupo Controle e Tratado). Por outro lado, embora *Aspergillus* e *Penicillium* cresceram no controle e em algumas amostras tratadas, seu crescimento foi inibido pelo O_3, dependendo do tempo de

exposição. Esse gás mostrou (nas condições aplicadas) um controle eficaz de fungos para diferentes formas de conservação da cavala.

Palavras-chave: Antifúngico; Ozônio; Fungos; Esporos; Pescado, Peixe.

Abstract

The aim of this study was to investigate the antifungal effect of ozone gas (O_3) (green decontamination agent) on mackerel fish (*Acanthocybium solandri*) in different types of preservation (fresh, dry and salted). Samples humidity [mc / a_w] parameters prior gas treatment, were of 80.7%/0.98, 55.55%/0.74 and 49.5%/0.70, respectively. Fish were contaminated with fungi genera that are able to grow on high (*Fusarium*) and low (*Aspergillus / Penicillium*) moisture content substrates, then gas treated at 50 μmol O_3 /mol, exposed during 10, 20 and 30 min, and incubated (25ºC, 7 days) to evaluate fungal inactivation. The O_3 treated samples, when O_3 exposed during the longest time (30 min/Day 7^{th}) had the fungal growth totally inhibited (100%), while the others presented only reduced growth effect, i.e; 40 and 70 % for 10 and 20 min, respectively. *Fusarium* spores were not able to grow in any of the protein based sample studied (both, Control & Treated Group). On the other hand, *Aspergillus* and *Penicillium* although grew on Control and some treated ones, their growth was inhibited by O_3 depending on exposure time. That gas showed (under the conditions applied) to be fungi control effective for mackerel different forms of conservation.

Keywords: Antifungal; Ozone; Fungi; Spores; Fish.

Resumen

El objetivo de este estudio fue investigar el efecto antifúngico del gas ozono (O_3) (agente de descontaminación verde) en el pescado caballa (*Acanthocybium solandri*) en diferentes tipos de conservación (fresca, seca y salada). Los parámetros de humedad de las muestras [humedad / aw] antes del tratamiento con gas fueron 80.7% / 0.98, 55.55% / 0.74 y 49.5% / 0.70, respectivamente. Los peces se contaminaron con géneros de hongos capaces de crecer en sustratos con alto contenido de humedad (*Fusarium*) y bajo contenido de humedad (*Aspergillus / Penicillium*), luego gases tratados con 50 μmol de O_3 / mol, expuestos durante 10, 20 y 30 min, e incubado (25ºC, 7 días) para evaluar la inactivación de hongos. Las muestras tratadas con O_3, cuando se expusieron al gas durante más tiempo (30 min / día 7), tuvieron el crecimiento de hongos totalmente inhibido (100%), mientras que las otras mostraron solo un efecto de crecimiento reducido, es decir; 40 y 70% durante 10 y 20 min, respectivamente. Las esporas de *Fusarium* no pudieron crecer en ninguna muestra de proteína

estudiada (tanto en el grupo de control como en el tratado). Por otro lado, aunque *Aspergillus* y *Penicilliun* crecieron en el control y en algunas muestras tratadas, su crecimiento fue inhibido por O_3, dependiendo del tiempo de exposición. Este gas mostró (bajo las condiciones aplicadas) un control efectivo de hongos para diferentes formas de conservar la caballa.

Palabras clave: Antifúngico; Ozono; Hongos; Esporas; Peces.

1. Introduction

Fish is a high nutritional value protein food, with essential fatty acids, vitamins and minerals, apart from of low cholesterol content (Hosomi et al., 2012; Dhaneesh et al., 2012). It is a healthier consumption option than the red meats (Gonçalves, 2011) being, a quite important product world trade wise including for jobs and income in several countries (Brabo et al., 2016). Brazil produced 722,560 tons of fish in 2018, an increase of 4.5% over the 691,700 tons of the previous year (PEIXE BR, 2019). With such a large production and consumption, it is necessary to monitore its quality and safety. Fish exposure, to environmental contaminants (living organisms: bacterias, yeast, fungi, parasites) comes from either the extractive fishing and/or aquaculture production as well less poor storage conditions of fresh and prosseced final products (dried/salted) (Aquino et al., 2019). Keeping its shelf life condition (time from production to the point where fish becomes unacceptable for consumption), is one of the most important attributes of quality (Forsythe, 2002). To determine the useful meat products shelf life, it is common to study its microbiological, chemical and sensorial parameters, despite whether, fresh or dried / salted (ICMSF, 2015).

Fresh fish are more susceptible to deterioration and contamination, as there is no barrier that prevents the microorganisms from acting immediately on them. The only evaluation made by the consumer, before the purchase is the sensory, in order to detect fish Deterioration (Franco & Landgraf, 2008; Icmsf, 2015). The visual evaluation cannot detect other alterations that are only possible to be perceived with the aid of equipment and analysis, such as the microscope, microbiological tests and chemical analysis.

On the other hand, *dried* (dehydrated) fish comes from the most primitive, yet effective, way of preserving fish. It consists of taking off as much moisture as possible from the food, thus decreasing its amount available for microorganisms to development (Nespolo et al., 2015; Augusto, 2017). It can be carried out in a more rustic way, with the use of the sun as the heat agent. However that method (although cheaper) is not feasible for large productions, as it is not possible to control the hygienic-sanitary conditions of the open environment

(Fellows, 2006). Fish becomes susceptible to dust, animal attacks, among other problems. The most used drying method is by utilizing oven, where the fish is submited to controlled temperature (heat) and time exposure (Fellows, 2006; Augusto, 2017).

Apart from that, *salted* fish comes from one of the oldest methods of preserving food. The process (salting) comprises of three steps: fish salt application, brine-fish system formation and maturation (with changes in taste and aroma). Salting can be conducted in different forms such as dry salting, brine, wet salting and concomitant salting and fermentation. Small and flat fish (such is swordfish, sardine and mackerel) may be whole salted. On the other hand, medium or large fish (such is tuna fish, sea bass and salmon) need to be eviscerated, opened or cut into fillets before salting (otherwise salt will not enough penetrate to prevent deterioration). In the case of fatty fish (high lipid content) salted, their contact with the air must be avoided to prevent oxidation during and after salting (Ordóñez, 2005). Regarding regulation for moisture content (mc) and total mineral residue levels, salted fish must have higher than 35 and 25%, respectively (BRASIL, 2017). The Codex Alimentarius establishes that the dried and salted fish should not have last than 12% sodium chloride (WHO, 1989).

Among products of animal origin, fish represents the most susceptible to the deterioration process, due to the association of intrinsic and extrinsic factors. The intrinsic factors, are of greater relevance: the high tissues nutrients (substrate that can easily be used by microorganisms) and water activity (a_w) and the rapid destructive action of the naturally occurring enzymes (fish natural microbiota), the large amount of unsaturated lipids and the pH values close to neutral (Soares et al., 1998; Gonçalves, 2011). In order to avoid / prevent and/or control living organisms, decontamination methods have been studied, such as oxidative compounds (peracetic acid, sodium hipocloride, ozone (O_3) among others (Freitas-Silva et al., 2013; Christ et al., 2016; Soares et al., 2018).

O_3 gas is considered a green decontamination agent for living organisms, among them fungi. Its application on fish (high protein content food) has been little studied though. Despite that, some studies have been reported in the fish industry against some microorganisms (Silva et al., 2011; Silva & Gonçalves, 2014; Luiz et al., 2017). That gas has proved to be effective against bacteria (gram-negative and positive), fungi, yeasts, viruses, protozoa, including the sporulated forms and protozoan cysts, which are more resistant (Alexandre et al., 2011; Silva et al., 2011).

Considering the economic and nutritional importance of fish on diets worldwide, there is a need to guarantee its sanitary safety (by green methods), and to implement measures to

avoid or reduce the presence of living organisms hazards - the objective of this work was to investigate the antifungal effect of O_3 mackerel (*Acanthocybium solandri*), sold under different forms of preservation (fresh, dry and salted fish).

2. Methodology

The mackerel, sold under different preservation conditions i.e., fresh, dried and salted, from the retail market of Fortaleza city, Ceará state (CE), Northeastern Brazil.

Fungi strains: *Aspergullis, Penicillium* and *Fusarium* genera, from the Laboratory of Mycotoxicology and Food Contaminants mycoteca, Food Science and Technology Department.

Samples collection and preparation: portions of mackerel samples (50g each) were coleted (fresh, dried and salted) in polyethylene bags (300x300 mm) from the Fish Market of Fortaleza city, CE, then sent to LABMICO in thermal box. Samples fillets/tissues were assepticaly sectioned into cubes to proceed the decontamination study.

Humidity determination: (b.1) moisture content (mc) – performed by direct drying the sample by heating (105 ºC) for 3 h in anoven, cooling in a desiccator for half an hour and weighing until reaching constant weight (AOAC, 2002) and **(b.2) a_w** – by utilizing an a_w meter that provides the sample (2.a) reading at a temperature close to 25 °C (Decagon, 2016).

Fungi inoculation: spores of *Fusarium* (> mc) and *Penicillium* & *Aspergillus* (< mc) fungi were inoculated into 50g portions of each sample (n=3) and let them set for 24h prior treatment. Two Controls were prepared (n=3) assepcticaly with each (fresh/dried/salted) fish fish samples: GC_1 (no O_3 treated - no inoculum) and GC_2 (no O_3 treated - inoculated) (Mukhopadhyay et al., 2019).

Ozone application: the gas (concentration: 50 μmol/mol, flow: of 5 L/min) was applied during 10, 20 and 30 min on the fish samples (previously inoculated with fungi) following the methodology described by Soares et al., (2018). Samples were incubated at 25 ^oC in a biological oven and observed for 7 days (Silva et al., 2007).

Mycological tests (e.1) total fungi count – the total fungi count was performed using the method of Samson et al. (2006) by plating serial dilutions (10^{-1} to 10^{-3}) in PDA medium with 50-ppm chloramphenicol. **(e.2) genera identification:** it was performed using the micro cultivation technique (Koneman et al., 2001) where each colony from (b.1) was spiked into culture medium Czapek agar in duplicate and incubated at 22-25 ºC for 5 days, followed by identification by light microscopy. This, the morphological structures were evaluated by

observing the mycelium septation, there produtive structures that give rise to conidia, and even its surface from the macro and microscopic observation, the identifications of the fungal genera were carried out according to the Weber e Pitt (2000) identification keys: Frisvad et al., (2004).

Statitics: data obtained for the analysis of mc and a_w were statistically evaluated using mean values, standard deviation, ANOVA and Tukey's test at 5% probability for comparison of means using *Statistica* 7.0 software (Statsoft, 2007). All analyzes (except the identification step) were performed in triplicate.

3. Results and Discussion

As expected, results obtained by applying the antifungi agent - O_3 gas (at different times) on the mackerel (fresh / dried / salted) samples, showed some variations. Those occurred either, on fungi spores development and/or genera suscebility - related to the gas application length of time: (a) within the same fish preservation Type or when compared its effect (b) between them. Data on prior-treatment humidity (mc and a_w) and post-treatment O_3 effect are shown on Tables 1, 2 and Figures 1, 2.

3.1 Mackerel humidity prior-treatment

The results obtained for humidity (mc and a_w) are described in Table 1.

Table 1. Humidity parameters of mackerel (*Acanthocybium solandri*) fresh, dried and salted samples prior ozone tratament

Humidity*	**Mackerel preservation Type**		
	Fresh	**Dried**	**Salted**
Moisture content (%)	80.70±0.36^a	55.53±0.40^b	49.53±0.42^c
Water activity	0.9800±0.00^a	0.7400±0.00^b	0.7000±0.00^c

*mean ± standard deviation (n=3) the averages followed by the same letter in the line did not differ statistically from each other (p> 0.05) by the Tukey test at the 5% probability level. Source: Authors.

The way of preserving the samples is directly related to the parameters mentioned above. It is known that the greater the a_w of a food, the greater the possibility of developing deteriorating microorganisms and / or pathogens. Moisture, on the other hand, is directly

connected to the way in which the product is preserved, and a smaller amount of this also favors a longer shelf life.

MC: *fresh*- as expected, the mc present in the whole mackerel fresh sample was higher than the processed (dried & salted) ones, reaching 80.7%. That result was corrobated by studies carried out for trout (*Oncorhynchus mykiss*) with 87.3% mc and for the fish mapara (*Hypophthalmus edentatus*) fillet with 76.9% (López et al., 2017; Maciel et al., 2016). Those high mc values obtained were considered adequate, as the sample is at its *in natura* state (expected ↑mc), althoug they allow microorganisms proliferations. Regarding the *dried* and *salted* mackerels – in the current study, the samples mc werelower than the fresh ones, reaching 55.5 and 49.5%, respectively. In the literature, authors have published similar mc for those dehydrated (dried and salted) fish preservation Types. Baltazar et al., (2013) reported mc values ranging from 49.14 to 55.71% in salted cod (*Gadus morhua*) samples; Lima and Sant'Ana (2011) obtained values of 52.3 and 49.6% when comparing Atlantic and Pacific cods (both salted), respectively.

A_w: *fresh* - similar behavior of mc for the three preservation Types, occurred for a_w. The fresh whole mackerel, presented the higest a_w (0.9800). It is known that medium to high a_w favors development of microorganisms and enzymatic reactions. In studies with different fresh fish species, several results were repported for a_w, from 0.9630 to 0.9900 (López et al., 2017; Nuwanthib et al., 2016; Santos et al., 2017). On the other hand, the *dried* and *salted* – presented a_w values (mean: 0.7400 and 0.7000, respectively) close to those considered not adequate to allow microbial growth (Franco & Landgraf, 1999). Baltazar et al., (2013) registered values of 0.7500 for salted cod, while Lima and Sant'Ana, (2011) studying different fish species found a_w values from 0.7420 to 0.7500. The higher the a_w, the faster the microorganisms growth; so the importance of a_w in the food conservation (Molina-Filho et al., 2006).Values of a_w between zero and 0.20 indicate that the water is strongly bound, while when from 0.70 to 1.00, most of the water is free (for chemical reactions / enzymes catalyses/ microorganisms development) (Franco & Landgraf, 1999).

MC *VERSUS* A_w: the values found were proportional when comparing the two parameters, as the mc decreased, the a_w also reduced. Statistically speaking, the samples showed that there are differences in the parameters regarding to the Types of mc fish preservation. Both parameters are related to the sorption isotherms, which are water sorption curves that represent equilibrium (between the mc and a_w) at a given temperature and pressure. Information on sorption isotherms is important on planning a dehydration (drying) process application for microbiological safety. The sorption characteristics of the fish meat

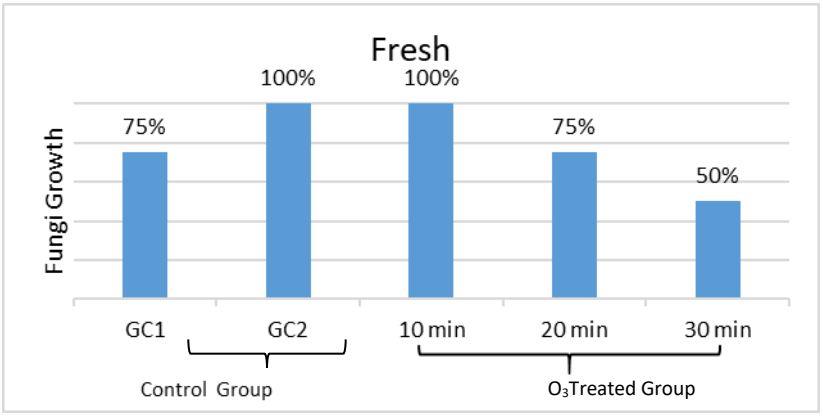
can be affected by different factors including dissolved solutes (salt) presence and so temperature (Fellows, 2006).

3.2 O_3 antifungal effects on fresh, dried and salted mackerels

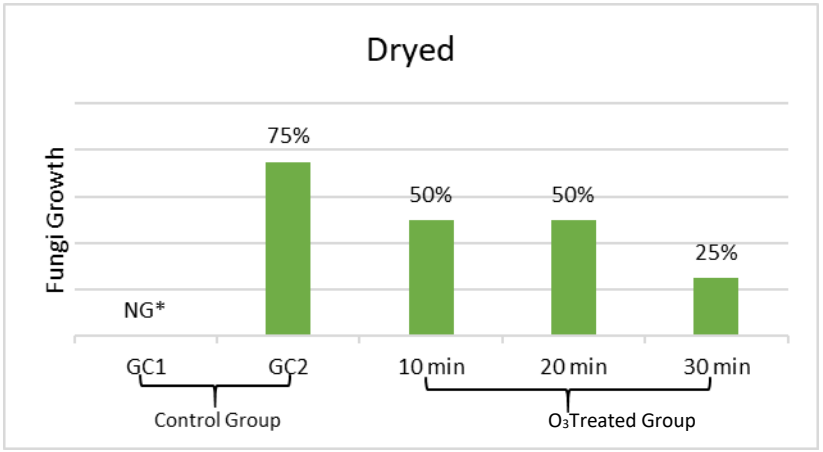
By applying the O_3 gas (concentration: 50 µmol O_3/mol; exposure: 10/20/30 min) on the fresh, dried and salted fish samples, it was possible to register its fungi spores inactivation effect during the 7 days incubation (Figure 1 a, b, c).

Figure 1. Fungi growth percentage of reduction (spores inactivation) by ozone (O_3) gas of treated mackerel (*Acanthocybium solandri*) – *FRESH*, *DRIED* and *SALTED* (7^{th} Day incubation) [NG: no growth, GC: control group].

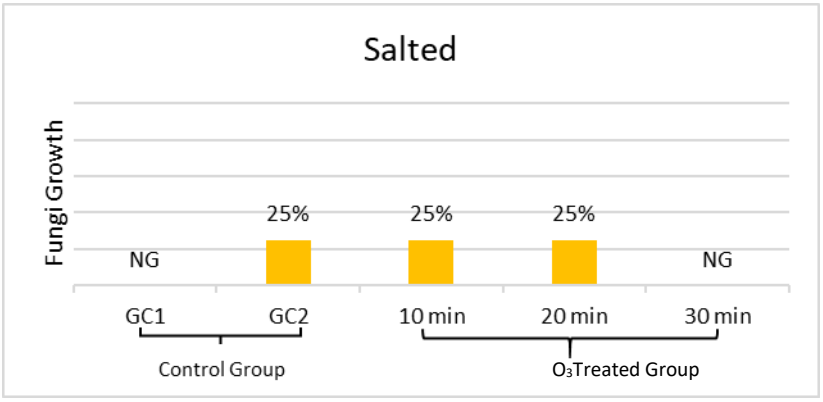
(a)



(b)



(c)



Source: Authors

The figure above illustrates how the application of the gas at different times acted on fungal growth. It is possible to observe that the action of O_3 varied, depending on the way of preserving each sample.

FRESH*:* the O_3 treatment at the concentration applied in this experiment showed that there was a reduction on fungal development according to the exposure time of the samples to the gas (100, 75 and 50% fungal growth for 10, 20 and 30 min, respectively - Figure 1.a). However, even the longest O_3 exposure time (30 min), applied on the fresh mackerel, was not fully efficient, inhibiting only 50% of fungal growth at the end of the incubation period (7^{th} Day). As fresh fish is a product with high mc and a_w (Section 3.1) which favors microorganisms growth, that fungi growth characteristics could clearly be observed in the GC samples (except for *Fusarium* genera – to be discussion at Section 3.2). Fresh fish are very perishable and can deteriorate due to the microorganisms proliferation; its control is based on low temperature, and in some cases, combined with controlled atmosphere when packaged - vacuum or CO_2 (ICMSF, 2015). The life expectancy of fresh fish is determined mainly by a number of different microorganisms species, factors that depend on the natural microbiota and the management of the catch until storage whitch green gas (O_3) cold be of preference since conditions can be adjusted (Neiva, 2002).

DRIED: for the dried samples, exposed for 30 min to gas, fungal growth was inhibited by 75% while samples exposed for 10 and 20 min had a 50% inhibition at the end of 7^{th} Day (Figure 1.b). Regarding the Control groups (GC_1 and GC_2) as expected (low mc/a_w), GC_1 samples showed no growth over the period. The opposite occurred with GC_2 (inoculated with fungi spores) showing some fungal proliferation at Day 2 (despitits dehydration condition). Drying consists of two distinct physical phenomena: the evaporation of surface water and the passage of water from the center of the product to its surface which prevents high mc

(bacteria, yeasts and some fungi) microorganisms proliferation (Ferreira et al., 2002; Scussel et al., 2018). This method of gas preservation has been proved to be quite efficient in terms of fungal development.

SALTED: this Type of mackerel preservation was the one that presented the best results regarding the fungal spores' inactivation when compared to Controls. Salted samples exposed to O_3 for 30 min showed no colonies formation during the incubation time – i.e., 100% development inhibition (Figure 1.c). Salting is one of the oldest and most efficient preservation methods known. Its principle is based on the use of salt that, in adequate concentration, decreases or even avoids the decomposition of the food by autolysis or by the action of microorganisms. In that case, the salt has a double function, both, of penetrating the fish and reducing the total amount of water, thus, reducing the water availability for microorganism's growth or enzymatic action (Ferreira et al., 2002). Only exposure to the O_3 for 30 min *Fusarium* and *Penicillium* were completely inactivated. GC_1 samples showed no proliferation over the whole incubation period. The GC_2 group and the GT samples exposed for 10 and 20 min to the gas showed formation of colonies from the 3th day, althogh that growth was not that considerable. Important to emphasize that, the gas to be efficient, care must be taken in their storage to avoid moisture, otherwise fungi will develop (Vieira et al., 2004).

FUNGI *VERSUS* O_3: regarding O_3 fish fungi decontamination reported in the literature, no data has been published to date to the authors' knowledge. The use of O_3 on food protein based has been applied for their storage either, in freezing chambers or cold warehouses (meat, fish, shellfish, cheeses, sausages, among others products). Its main aim is to reduce bacteria contamination that can occurs even in those storage systems (Vaz-Velho et al., 2006; Chawla et al., 2007). *Bacteria* - researches have been carried out on the use of O_3 in order to guarantee the quality and safety of fish, however taking into account its powerful effect (high oxidation capacity) against microbial. Any pathogen that can be disinfected, removed or altered by oxidation processes can be affected by O_3 (Gonçalves, 2009). To investigate the effectiveness of new O_3 application mechanisms, studies have been conducted specially for bacteria. Luiz et al., (2017) evaluated the efficacy of O_3 in *Salmonella* contaminated fish, and observed some efficiency to its eradication under the experimental conditions applied; Silva (2015) evaluated the efficiency of ozonated water as antimicrobial agent also in fish during processing, obtaining a 91.78% reduction of the initial populations of mesophilic bacteria. *Fungi* - **c**ontamination by fungi can cause numerous economic losses associated to nutrient reduction, palatability and the presence of mycotoxins, thus affecting human health (Scussel

et al., 2018). A number of studies has been carried out in order to develop fungi decontamination methods in foods including the O_3 (Mcdonough et al., 2011; Giordano et al., 2012; Beber-Rodrigues et al., 2015; Savi et al., 2014; Kreibich et al., 2016). Despite that, none of them was carried out for fungi contamination in fish**.** O_3 acts by the progressive oxidation of cellular components to destroy microorganisms, preventing their growth and the formation of mycotoxin (Guzel-Seydim et al., 2004; Savi et al., 2014).

3.3. Total fungi load and genera suscebility

The results obtained from the total fungi load at the end of the 7th day of incubation are shown in Table 2.

Table 2. Total fungi load of on the ozone treated mackerel (*Acanthocybium solandri*)

O_3 treatment (min)	**Mackerel preservation Type (CFU/g)***		
	Fresh	**Dried**	**Salted**
10	>100	5.3 x 10^3	1.0 x 10^2
20	2.7 x 10^4	5.2 x 10^3	0.7 x 10^2
30	2.1 x 10^4	2.5 x 10^3	NG**

* Day 7$^{th;}$ **NG: no growth; CFU: colony forming unit. Source: Authors.

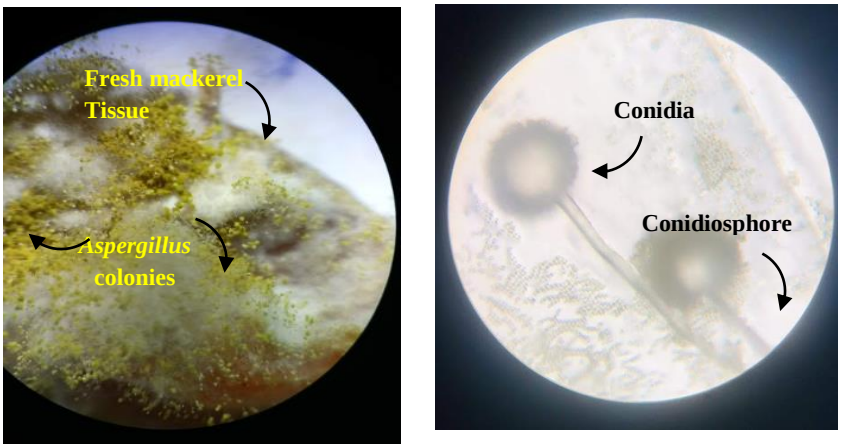
Most of the fish and derivatives at room temperature are perishable and can deteriorate fast due to the proliferation of microorganisms (ICMSF, 2015). Microorganisms use fish as a substrate to perform their metabolic activities, producing substances that impart an unpleasant aroma and taste, therefore necessary to be avoided/controled (Franco & Landgraf, 2008).

FUNGI LOAD: the data obtained corroborate with the results expressed previously for fungal growth. It could be observed that O_3 together with the processing of salted fish had an effect on fungal growth, which was completely inhibited with the application of gas for 30 min (<1x10^2 CFU/g - NG). On the other hand, the fresh fish that was under a minimal preservation process (*in natura*) had the O_3 lest influenced, i.e., not being able to inhibit fungi development on that type of tissue (↑mc) Type of sample. The total load for those samples could not be performed at dilution 10^4 due to high colonies development (>100). The dry samples had a relatively low fungal growth, with counts of 5.3x10^3, 5.2x10^3 and 2.5x10^3 when exposed to the gas for 10, 20 and 30 min, respectively. It is suggested that studies should be performed with higher O_3 concentrations and longer times of exposure than those

applied in the corrent work, to adjust efficiency.

FUNGI GENERA SUSCEBITY: regarding the identification of the O_3-resistant colonies, they were only from *Aspergillus* genera (Figure 2). Indeed, out of the strains (*Aspergillus*, *Penicillium*, and *Fusarium*) inoculated in this study, *Aspergillus* showed to be resistant under the conditions studied.

Figure 2. *Aspergillus* developement on mackerel (*Acanthocybium solandri*) fresh samples observed under: (a) stereo and (b): light microscopies [80x and 40x, respectively]



Source: Authors.

Species of *Aspergillus* are considered initiators of deterioration, being able to grow with low mc too. These fungi are potentially mycotoxigenic (Meronuck, 1987; Scussel, 2018). There is a lack of regulation related to fungi loadfor fish despite its species and or preservation Type (BRASIL, 2001). Therefore, further studies are needed to determine its resistance characteristics and so to effectively inactivate/destroy it. The most common sources of contamination of fish are the manipulator itself, incorrect storage, during the marketing process or even in its capture (Martins, 2006; Gonçalves, 2011).

4. Final Considerations

The courrent study showed that the longer the O_3 exposure, the better results on controlling/reducting fungal growth. The fresh mackerel samples when O_3 gas treated, showed the best fish inactivation efficiencyat 20 min exposure.

Dried and salted mackerels presented the most O_3 efficiency regarding fungi reduction, which was a combination of (a) low growth conditions (↓ mc↑sodium chloryde) and (b) O_3 oxidising effects.

The O_3 green method used to improve mackerel safety at different Types of preservation (fresh, dried and salted) showed to be adequate. Despite that there is need of further studies regarding bether concentration an time exposure in order to improve to fungi inactivation efficiency to reach the best results.

Indeed, as fish are among the most prone to microorganisms deterioration due to the high tissues humidity (fresh: mc / a_w) and nutritive substrate (fresh/dried/salted), a GRAS (no fungicide/chemical aplied) decontamination method could be adequate in order to control fungi proliferations without affecting there quality.

For future work, we suggest testing different conditions for applying O_3, such as concentration and time; in addition, different species of fish can be tested to see how they react to decontamination by O_3.

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