Eco-innovative possibilities for improving the quality of thawed cod fillets using high-power ultrasound.

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ABSTRACT

In order to improve the quality of thawed cod fillets and minimize the impact of processing, an extended hydration phase is applied in the fishery product industry in order to recover the water lost during freezing and thawing. Such long phases not only compromise productivity, but increase the chances of microbial growth in fish. Ultrasound (US) is a technology that could reduce these long hydration times, thanks to its capacity to improve mass-transfer processes, thereby limiting the development of fish microbiota.

This investigation studies the effect of different US intensities (25 kHz, 29.4 W/kg to 2.9 W/kg, 113.7 to 15.3 W) on weight gain (WG) in the hydration process of cod fillets. The influence of the hydration medium's pH (from pH 8.5 to 10.5) in combination with US was likewise evaluated. Microbiological and sensory analyses were carried out at the end of the hydration process in order to evaluate its impact.

The higher the applied US power, the lower was the WG. US intensities of 2.9 W/kg produced the highest increments in WG (18.6%), reducing hydration time by 33% and thereby achieving the same hydration values as in control samples. The combination of US with a controlled pH of 8.5 permitted to shorten hydration time by an additional day, and also led to improved microbial quality in comparison with control samples. Sensorial analyses indicated that after 5 d of hydration, Quality Index Method (QIM) values were better than those obtained for control samples after 5 and 7 d. Specifically, color and gaping were the sensorial attributes of cod fillets better protected with the application of US.

Keywords: Ultrasound; Hydration; Cod fillets; Freezing-thawing; Microbiological quality; Sensorial quality.

1. Introduction

Food preservation through the reduction of temperature below the freezing point is a method commonly applied to maintain the quality of fishing products and maximize their shelf-life. The principal goal of the freezing process is to preserve the nutritional and organoleptic characteristics of food by slowing down chemical, enzymatic, and microbiological reactions (FAO and WHO, 2012).

When a product is frozen, ice crystals can cause cell damage, even when optimal freezing conditions are applied. Ice crystal size mainly depends on the speed of the freezing process. Fast freezing rates result in small ice crystals that are evenly distributed inside and outside the cells. Slow freezing rates lead to ice crystal formation mainly in the extracellular areas, and in such a way that they cause tissue damage, producing higher rates of water loss during the thawing phase (Alizadeh et al., 2007). Therefore, the final quality of fish fillets, including texture, water retention capacity, drip loss, and microbial quality, is directly related to freezing parameters and conditions, storage, and thawing (Uyar et al., 2015). Fish meat is remarkably sensitive to changes during these phases when compared to other food products (Schubring et al., 2003).

In order to allow fishery products to recover from those damages, the food industry usually includes a hydration step after thawing, mainly for products that are later sold under Modified Atmosphere Packaging (MAP). This hydration process allows for the recovery of water lost during thawing, thereby helping to improve texture, flavor, juiciness, and the appearance of freshness (Huff-Lonergan and Lonergan, 2005). In the case of fishery products such as fish fillets sold under MAP, this type of treatment can last up to 7 d, which can allow or encourage growth of spoilage microbiota, leading to a decrease in the shelf-life of the fillets when they are commercialized. To control microbial growth, it is therefore necessary to include additives. However, since consumers and the industry prefer that additives be reduced or eliminated, it would be of great interest to find strategies that help shorten hydration times without affecting the quality of the final product. Ultrasound (US) could optimize this process by reducing the hydration time for thawed fish fillets. This is even more interesting in the case of cod, since cod can only be fished during a short period of the year (from December to February); in order to ensure a constant supply of cod throughout the entire year, freezing and subsequent thawing are essential steps.

It is worth noting that US is included within the technologies considered as "Green Food Processing" (Chemat et al., 2017a). This new concept covers those technologies (pulse electric fields, microwaves, supercritical fluid extraction and processing, controlled pressure drop process, ultrasound) which, compared with traditional processes, allow to reduce processing time, water and energy consumption, thereby resulting in more sustainable food processing.

Several researchers have pointed out that high-intensity US (> 1 W/cm^2), which works within a frequency range of 20-150 kHz, is thoroughly effective in facilitating and promoting mass transfer in liquid-solid systems, mainly with the purpose of extracting components and facilitating their entry into the solid (Chemat et al., 2017b; Rodrigues et al., 2017; Tao and Sun, 2015). The main physical mechanism associated with high-intensity US in a liquid medium is attributed to the phenomenon of cavitation (Esclapez et al., 2011; Chandrapala et al., 2012). Cavitation is the consequence of asymmetric implosions of gas bubbles that are formed during a series of compressions and decompressions caused by sound waves. When this collapse occurs close to a solid surface, it generates a microjet with determined characteristics of pressure (100 MPa), temperature (5000 K), and speed (400 km/s) (Hemwimol et al., 2006). The consequence is an improvement of mass-transfer, and even the formation of pores that could facilitate the exit or entry of certain components (Mason and Lorimer, 2002). In addition to microjets, US might also generate micro-agitations in the liquid that could affect mass transfer (Liang, 1993). The predominance of one or the other effect depends on cavitation intensity and, therefore, on the amount of applied US power.

The mass transfer effectiveness of US has already been demonstrated for several other food products, but hardly at all in fishery products. Given the need to optimize the hydration process in the fishing industry, ultrasound could provide an opportunity to shorten processing times while obtaining greater weight gains, thereby increasing the quality of the final product and reducing the use of additives. This study aimed to investigate the influence of the application of varying ultrasound treatments during the hydration phase of thawed cod fillets, and to evaluate their impact on product quality from a sensorial and microbiological point of view.

2. Methodology

2.1 Preparation of the raw material and reactives

Frozen skinless cod fillets (*Gadus morhua*) of approximately 550 ± 35 grams per fillet supplied by Scanfisk Seafood S.L. (Zaragoza, Spain) were stored in a freezing room at -18 °C until use. Prior to the experiment, the cod fillets were subjected to an air-thawing process in a cold room at 4 ºC for 36 h. For each determination, cod fillets were submerged in a water-based solution for a maximum of 7 d of hydration. The liquid solution used to apply the treatments during the hydration phase was a commercial solution based on distilled water (4 ºC), along with a combination of two food additives: E450 (a mixture of diphosphates of sodium, potassium, and calcium) and E451 (a mixture of triphosphates of sodium and potassium) (Carnal 2110, Budenheim Iberica SLU, Spain) NaCl (Panreac, Spain), and Aquactive 3S, which is a solution based on citrates and hydrogen peroxide (Aquactive 3S, Budenheim Iberica SLU, Spain) in the proportions of 3.2 g/L, 1.8 g/L, and

0.3 mL/L, respectively. The proportion of cod fillets versus hydration solution was 6:11 (2.4 kg of fillets and 4.4 L of water solution). All hydration experiments were carried out inside a cold chamber at 4 ºC.

When indicated, the influence of the hydration medium's pH was evaluated. To investigate this point, three pH values (8.5, 9.5 and 10.5) were studied when either applying or not applying US. To control pH during the hydration phase, a pH-meter (pH-Meter Basic 20+, Crison Instruments, Spain) was used to monitor the pH, and different quantities of a 1 N solution of NaOH (Merck KGaA, Germany) were added to adjust it. The monitoring and adjustment of pH was carried out every 24 h.

2.2. Ultrasound equipment and treatment conditions

The hydration process with the application of US was carried out in an ultrasound bath with a capacity of 15 L and with a nominal power of 200 W (Bandelin, M1003, Berlin, Germany). To investigate the influence of US during the hydration phase, 3 US powers were investigated: 29.4 W/kg (100%), 14.7 W/kg (50%) and 2.9 W/kg (10%) compared to control, in which the process was applied without the use of US. Considering that the actual input power from the device is converted to heat which is dissipated in the medium, calorimetric measurements were performed to assess actual ultrasound power similarly to the manner described by Both et al. (2014). Based on these measurements, the transmitted powers were 113.7, 64.3 and 15.3 W when using 100 (29.4 W/kg), 50 (14.7 W/kg), and 10 % (2.9 W/kg), respectively.

In order to avoid temperature increase, and since long hydration times were being investigated (up to 7 d), US was applied at the indicated intensities during 20 min, interrupted by 100-min intervals without US. Under these conditions, the temperature of water and fillets in all treatments was always lower than 14º C, which is the maximum recommended temperature for a thawing process (Chourot et al., 1996).

2.2 Analysis

2.2.1 Weight gain of cod fillets

Percentage of weight gain (WG) of cod fillets was determined every 24 h. Weight gain was calculated as follows: WG (%) = [(D1 − D2) / D2] × 100, where D1 is the weight of the sample after hydration, and D2 the weight of the sample before hydration (day 0). Fillets were weighed in an analytical balance (Sartorius, TE3102S, Germany).

2.2.2 pH and electrical conductivity of the hydration solution

Water samples were taken every 24 h during treatment, and were left to stand at room temperature. Once tempered, electrical conductivity was measured with a conductivity meter (Conductivity Probe FY A641LFP1/LFL1, Ahlborn, Germany). As previously indicated, the pH of the hydration solution was monitored with a pH-meter (pH-Meter Basic 20+, Crison Instruments, Spain) every 24 h.

2.2.3 Total Volatile Basic Nitrogen (TVB-N) analysis

TVB-N content of cod fillets at time 0 and after 5 and 7 d of hydration was evaluated. TVB-N determination was carried out in a Kjeltec unit UDK 130 D (Velp Scientifica, Italy) by direct steam distillation over boric acid according to European Regulation CE 1022/2008. Results were reported as the average of two replicates per sample.

2.2.4 Microbiological analysis

To evaluate the evolution of microbiota in the hydration water, Total Aerobic Mesophilic (TAM) counts were determined using LH agar (Long and Hammer Agar) (Broekaert et al., 2011). Previous results showed no statistical differences among counts when recovery was carried out at either 25 ºC or 7 ºC (Antunes-Rohling et al., 2019). Samples (1 mL) of hydration medium were surface-plated, and plates were then incubated for 48-72 h at 37 ºC. Longer incubation times did not modify the obtained counts (Antunes-Rohling et al., 2019).

Cod fillet microbiota was evaluated at time 0 and after 5 and 7 d of hydration by investigating several microbial groups: Total Aerobic Mesophilic (TAM), Seafood Spoilage Organisms (SSO), *Enterobacteriaceae*, and Proteolytic Bacteria (PB). Fish samples (25 g) were transferred aseptically inside a laminar flow cabinet to sterile Stomacher bags (Stomacher ® 400 classic, Seward), where 225 ml of previously sterilized Buffered Peptone Water (BPW, Oxoid, Hamphsire, UK) was added before homogenizing for 30 seconds using a mechanical homogenizer (Stomacher Lab Blender 400, Seward). For microbiological enumeration, ten-fold dilution series of sample homogenates were prepared, and 0.1 or 1 mL volumes were spread on agar Petri dishes. The different bacterial groups were enumerated as described in Table 1. After incubation, colony-forming units (CFU) were counted with an improved automatic colony-counting image analyzer (Protos, Synoptics, Cambridge, UK), previously described in detail by Condón et al. (1987). Results were expressed as Log_{10} N_t/N_0, where N_t is the count after a treatment time and N_0 is the initial count (untreated or control samples).

2.2.5 Sensory analysis

Sensory evaluations were carried out using the Quality Index Method (QIM) scheme for thawed cod fillets described by Seafish (2010). The QIM score was based on texture, odor, color, blood stains, gaping, and parasites of raw cod fillets. The attributes evaluated and the demerit scores (0-3 points) are included in the supplementary material. The QIM score was the sum of the scores given by the sensory panel on individual quality parameters on a scale from 0 to 16 (the higher the value, the worse the fish freshness). Sensory evaluation was carried out with a panel of 10 expert sensory assessors who had been previously trained according to ISO 8586-2: 2008.

2.3 Statistical data analysis

Experiments were carried out in triplicate on different days, and the displayed results are the mean values. Standard deviation ($p=0.05$) was used to show the variability of results. One-way ANOVA and t-test analyses were performed to analyze results using GraphPad PRISM® 5.0 software (GraphPad software, Inc., San Diego, CA, USA). Statistical significance was assigned to comparisons with $p < 0.05$. The results of the sensory analysis were evaluated in the XLSTAT program, Version 2016 (Addinsoft©). First, the data were evaluated according to a "Panel Analysis", in order to verify how the judges behaved in response to the parameters and treatments. The results were then statistically evaluated by ANOVA ($p <0.05$) to verify significant differences between the studied treatments.

3. Results

In order to study the effect of ultrasound on the hydration process, the investigation was carried out in several stages. In a first step, the effect of different ultrasound intensities was evaluated. Secondly, the combined effect of ultrasound and pH of the hydration medium on the weight gain of fillets was investigated. As discussed below, weight gain is highly dependent on the pH of the hydration medium due to the interaction of water with fish proteins. Once the ultrasound power and the pH of the hydration medium had been determined, results of the hydration process were investigated in a 7-day hydration process simulating the industrial process. In this posterior set of experiments, apart from weight gain, we also evaluated different quality parameters (TVB-N), microbial counts, and sensorial quality.

3.1 Influence of the application of different US intensities during hydration of cod fillets

In the first part of this study, the effect of different ultrasound intensities was evaluated during a 3-day hydration of cod fillets. Figure 1 shows the weight gain (WG) across time of thawed cod fillets hydrated in a commercial hydration solution, applying US at different intensities (29.4, 14.7, and 2.9 W/kg) or not applying US at all. As observed, WG increased with time, and attained the maximum values after 48 h under all investigated conditions. The effect of US varied with its intensity. Thus, after 48-72 h, the higher the US intensity (29.4 W/kg; 100%), the lower the weight gain, even in values lower than control samples: 14.8 vs 12.0%, for control and US treated fillets (29.4 W/kg; 100%), respectively. When the lowest intensity was applied (2.9 W/kg; 10%), weight gain increased to 18.6% compared to control samples. At medium intensity (14.7 W/kg; 50%), weight gain was very similar to control: a WG of around 14.6% after 3 d of hydration was observed. Similar trends were observed with cod fillets from different batches (data not shown). Therefore, an intensity of 2.9 W/kg with a frequency of 25 kHz and an application protocol of 20 min with US and 100 min without US would be the most effective treatment to obtain greater weight gains for the same hydration time, or to shorten the process with the purpose of achieving a certain hydration percentage. Based on these results, US of 25 kHz and 2.9 W/kg (10%) was used for subsequent experiments.

Figures 2A and 2B show the evolution of pH in the hydration solution (2A) and its increase in electrical conductivity (2B) during the process. The hydration solution that contained Carnal 2110, Aquactive 3S and NaCl had an initial pH of 8.35 ± 0.15 and an electrical conductivity of 38.75 ± 1.22 mS/cm. As observed, pH decreased with time, falling to levels between 7.0 and 7.5 after 3 d of hydration, with no statistically significant differences observed among treatments. Electrical conductivity values increased with time and US intensity: after 72 h, electrical conductivity increments of 68%, 22% and 20% for 29.4, 14.7, and 2.9 W/kg, respectively, were measured. The electrical conductivity of the control hydration solution varied negligibly (5.7%) after 3 d of hydration.

3.2 Influence of pH control during the hydration of cod fillets

Since pH varied during the hydration process, and since the interaction of water with proteins is pH-dependent, the effect of pH was investigated. Figure 3 shows the WG during the hydration process when applying US (25 kHz, 2.9 W/kg, 10%) or not applying US, in hydration media of varying pH (8.5, 9.5 and 10.5). In this case, the hydration process was extended to 5 days. Control samples (non-controlled pH and without the application of US) showed similar WG than those observed in Figure 1, with final hydration values of 10.2% observed after 5 d, which would already be achieved after 2-3 d of hydration with any of the evaluated procedures. When controlling the pH solution (dashed lines) without the application of US, the final weight gains were of 16.5, 19.8, and 23.2% in media with pH of 8.5, 9.5, and 10.5, respectively. When US was applied (continuous lines), the gains were 19.8, 28.0, and 27.1%, respectively, thereby representing a 3 to 7% increase compared to non-US-treated samples hydrated at the same pH level. According to these results, the maximum weight gain percentages attained by the control treatment (10.2%) after 5 d would be achieved in 24 h or even less with any of the evaluated processes. The highest effect of US was observed when pH was controlled at 9.5. This process could have been of interest in terms of WG. However, this pH level was discarded from future investigations, since it is too distinct from that of cod fillets (pH 6.5 ± 0.2), and it affected sensorial properties during shelf-life (data no shown). Treatments at a pH level of 10.5 were discarded for the same reason. Based on the obtained results, it can also be concluded that the application of US in a hydration medium with pH controlled at 8.5 could reduce hydration time by 2-3 d while achieving the same WG compared with the control process.

3.3 Microbiological analysis

In order to rapidly and dynamically evaluate the total microbiota present in the hydration solution featured in the experiments shown in Figure 3, the evolution of the total aerobic mesophilic bacteria count when applying the different treatments (US of 25 kHz and 2.9 W/kg or no US, using hydration media of pH 8.5, 9.5, and 10.5) was studied (Figure 4). As observed, the pH level allowed to control microbial growth, even producing a decrease of the final microbial loads by 1.3 and 1.8 Log_{10} cycles in media of pH 8.5 and 9.5, respectively, when compared to control after 5 d of hydration. No counts were detected at pH 10.5. However, the application of ultrasound limited the effect of pH 8.5, leading to microbial counts (2.2 Log_{10} cycles) similar to those of the control process (without US or pH control).

3.4. Evaluation of the industrial process at lab scale

Once the ultrasound power and the pH of the hydration medium had been determined, results of the hydration process were investigated in a 7-day hydration process simulating the industrial process, in the course of which quality parameters such as TVB-N, microbiota of cod fillets and impact on sensorial parameters were evaluated, along with weight gain. Considering the number of cod fillets that fit inside the US bath and in order to use fillets of the same batch for all investigated conditions, samples at day 0, 5, and 7 were evaluated. Figure 5 shows the evolution of WG, pH, and TVB-N of cod fillets hydrated in the previously described commercial solution with and without pH control (adjusted to pH 8.5), and in commercial solution with pH controlled to 8.5 and the application of US (USpH, 25 kHz, 2.9 W/kg, 20 min US on, 100 min US off). As observed, weight gain values similar to those shown in Figures 1 and 3 were obtained when comparing the same treatment conditions. In the case of control samples, a maximum WG of 10.7% was achieved after 7 d of hydration. For the same hydration time, pH control or the application of US made it possible to increase WG, or to reduce hydration time to achieve the same WG, obtaining better results when US was applied. Thus, pH control or pH control coupled with the application of US (USpH) made it possible to obtain an extra 4.5 and 7.7% WG compared to control samples after 7 d of hydration. On the other hand, pH control with or without the application of US reduced hydration time from 7 d to 2 and 5 d, respectively, to obtain the same WG as with the industrial process.

As observed in Figure 5B, the pH of the cod fillets varied depending on the process. In the industrial process, pH was significantly lower than in the others. When pH of the hydration medium was controlled to 8.5, the pH of the fillets increased: this increment was lower when US was applied. In the case of TVB-N, all fillets showed values lower than the maximum limit (35 mg/100g) legally permitted by European Regulation 1022/2008. After 5 d of hydration, no statistically significant differences among treatments were observed. Only fillets hydrated with the control process resulted in significantly higher values compared with the other processes.

Figure 6 shows the counts of different groups of microorganisms (TAM, SSO, BP and *Enterobacteriaceae*) present in cod fillets at days 0, 5, and 7 of hydration for the different evaluated processes. As observed, microbial counts increased along with hydration time, but in a different manner for each microbial group and process. In control, after 7 d of hydration, the microbial count of TAM, SSO and *Enterobacteriaceae* lay over the established or recommended limits: 6 Log_{10} CFU/g for TAM (CE 2073/2005), 7 Log_{10} CFU/g for SSO (IFST, 1999; Gram & Daalgard, 2002). and 3 Log_{10} CFU/g for *Enterobacteriaceae* (CE 2073/2005). In the other processes with pH control, better values were observed; however, after 7 d of hydration, TAM were also over the limit, and SSO were close to it. It is remarkable that the addition of NaOH made it possible to control *Enterobacteriaceae* counts to the point of non-detectability. Finally, when US was applied, the lowest counts were obtained after 5 days for all investigated microbial groups with the sole exception of BP.

Finally, a sensory analysis was carried out in order to quantify the extent to which pH control and the application of US interfered in sensory parameters as assessed through the QIM evaluation method. The results of day 5 and day 7 of hydration are represented in Figure 7. Table 2 shows the mean values and the statistically significant differences between the treatments for the evaluated parameters as characterized with the QIM evaluation method (texture, odor, color, blood stains, and gaping). In the evaluated samples, no presence of parasites was detected because the raw material had been preselected. Therefore, no statistical evaluation thereof was carried out in the sensorial analysis phase. A higher QIM signifies low product quality, with the maximum QIM score being 16 points. As observed, QIM values increased with hydration time. At the end of the day 5 of hydration, the compared results of the different processes were not statistically different, whereas, on day 7, hydration without pH control and without applying US

(CONTROL7) resulted in higher QIM values, thereby indicating worse quality when compared with other treatments. However, this treatment was not statistically different, on the whole, from the treatment that controlled pH at 8.5 and applied US (USpH7) after 7 hydration days. These QIM quality results could reflect the high microbiological counts observed.

Based on the data presented in Table 2, the poorer quality scores of cod fillets hydrated with the industrial process after 7 d (CONTROL7) were due to effects in texture, color, blood stains, and gaping. Cod fillets hydrated after 5 d with the application of US (USpH5) achieved one of the best QIM values; no differences with respect to other treatments in terms of texture, odor, color, blood stains or gaping were observed.

4. Discussion

The process of hydration of thawed cod fillets is an additional step used by companies to improve the product's sensory quality and texture (Barat, Rodríguez-Barona, Andrés, & Visquert, 2004). Traditionally, to achieve these goals, industries have been using food additives such as phosphates (Reddy and Finne, 1986; Tenhet et al., 1981) and/or NaCl (Sutton et al., 2007; Kin et al., 2009).

The water gains incurred by the muscle proteins of the meat are directly related with the space or the extant volume among the muscular filaments. That is to say, capillary forces keep the water of the muscles inside the myofibrils; when the latter are more open or more closed, more or less water is retained (Offer and Knight, 1988). The degree of opening is conditioned by the repulsion or attraction among muscle fibers, and by the changes that can be intrinsically or extrinsically induced by biochemical or chemical processes (Sikorski, 2001; Ofstad and Hermansson, 1997). Extrinsic factors that affect WHC, are, for example, the characteristics of the solution, the type of solute used, the temperature, and the solution's pH (Sikorski, 2001).

Regarding the hydration solution's composition, additives are commonly used in the process, and they play an important role. The main purpose of the phosphate family, with its strong anionic properties, is to increase the water retention capacity of the fish muscle (Hamm, 1971; Sutton and Ogilvie, 1968). The effect of phosphates has already been described in the literature: Lindkvist et al. (2008) obtained positive effects on appearance and weight gain in the processing of cod. On the other hand, the Cl^- anions pertaining to salt have the capacity of uniting with the muscular filaments, thereby causing rejection among them, which, in turn, results in a greater diffusivity of the water in the muscular structure, which increases muscular swelling and, consequently, the water's retention capacity (Ruusunen and Puolanne, 2005; Bocker et al., 2008). However, it is worth noting that saline solutions with concentrations greater than 1 M (approximately 6%) could exert the opposite effect (Barat et al., 2000). These specific characteristics of phosphates and salt would explain the fact that the control treatments used in this investigation resulted in a weight gain of around 10-15% in 2-3 d of hydration, as shown in Figures 1, 3, and 5. Some authors have investigated the influence of different types of phosphates and their concentration with or without the addition of salt during the process of marinating chicken meat; they have concluded that the combination of the two additives could synergistically benefit the weight yields (Xiong and Kupski, 1999). Preliminary experiments in cod fillet hydration using only water indicated that weight gain was minimal or almost non existent (data not shown). In other words, when phosphates and salt were added to the solution, WG increased significantly.

Apart from the above-mentioned additives, this study also proposed the use of US to improve hydration and to reduce processing times with the final objective of opening up the possibility of reducing or eliminating additives. Ultrasound technology is commonly used to optimize processes based on the increase of mass transfer, such as that which takes place in extraction, curing, cleaning, brining, pickling, and marinating (Chemat et al., 2017b; McDonnell et al., 2014).

Several studies have pointed out the effectiveness of US in favoring the transport of solutes to a solid in treatments that use a hypotonic liquid medium, as in meat processing (Alarcon-Rojo, et al., 2015) and cheese brining (Sánchez et al., 2000). These achievements are related to the mechanical effects induced mainly by “microjets” (Ozuna et al., 2013) and by the “sponge effect” (Cárcel et al., 2007), which can encourage the formation of micro-channels in the liquid-solid interface that facilitate the entry or exit of components (Fernandes et al., 2008; Yao, 2016). The effectiveness of the application of US is conditioned by process variables such as frequency and ultrasonic intensity (Vajnhandl and Marechal, 2005). In the latter case, several authors have reported that the higher the ultrasonic power applied, the higher the extraction yields (Zou et al., 2010). However, other authors have indicated that when certain values of ultrasonic power are exceeded, extraction yields remain constant and, in some cases, even decrease (Lou et al., 2010).

In this investigation, the highest ultrasonic intensity (29.4 W/kg, 100%) was not effective in achieving greater weight gain. This phenomenon could be due to the intense cavitation brought about by the “microjets” created by US, which could limit the movement of water towards the surface of the product, or even produce the exit of components from the cellular interior to the exterior (Antunes-Rohling et al., 2018). The latter circumstance could explain the decreases in pH (Figure 2A) and the increase in electrical conductivity (Figure 2B) of the hydration medium observed in this study. This effect, together with the considerable mechanical action exerted by US, could be responsible for the lower weight gain of fish fillets at high US intensity.

When 2.9 (10%) and 14.7 W/kg (50%) were applied, similar increases in electrical conductivity were observed, but different variations in pH drop and distinct weight gains were likewise noted. In the case of 14.7 W/kg, an interaction might be taking place between the mechanical effect of US, the release of components, and the variation in pH, which would result in WG similar to the control treatment and a lower pH drop when compared with other treatments. However, the mechanical effect of US would limit the flow of water to fish meat, as occurred when 100% US was applied. Based on the obtained results, the application of US at the lowest intensities (2.9 W/kg) resulted in higher WG or reduced hydration times to achieve the same level of hydration. Therefore, it was possible to improve the hydration of cod fillets, which involves a mass transfer process from the external liquid medium to the interior of the cod fillets. As mentioned above, US generates microstreaming and microjets (mechanical effects) in the surrounding liquid: they produce microchannels that enhance the penetration of the solution inside the fillets. Besides, when US is applied to a solid medium, contractions and expansions occur in the solid ("sponge effect") which favor the intake of water to the cod fillets (Cárcel et al., 2007; Ozuna et al., 2013).

As indicated above, the WHC of meat/fish is thoroughly dependent on a series of extrinsic factors, including the hydration solution's pH (Sikorski, 2001). The ability to capture water is due to the interaction of water with proteins and, more specifically, to the proportion of hydrophilic amino acids that are exposed to the water molecules with which they interact. This interaction is conditioned by the pH of the medium, so that when the pH moves away from the isoelectric point of the proteins (approximate pH of 4.5-5.5 in fish meat), the net charge becomes increasingly positive (pH more acidic) or negative (pH more basic), thereby producing repulsion among the filaments of proteins that form the fish and thus leaving more space for water molecules, which, in turn, increases the meat's water retention capacity (Sikorski, 2001). Due to these circumstances, the variation in pH of the hydration liquid was evaluated along with weight gain, as shown in Figure 3. There was an additive effect between the pH control of the hydration medium and the application of US, and this effect that had not been previously observed. The combination of both processes would therefore facilitate the interaction of water with proteins, which could have more space or more bond points to hold more water. The consequence is the possibility of reducing the hydration process by 2 or 3 d to achieve the same WG.

This reduction in time also results in an improved microbial quality of fillets as compared with customary industrial processes. As observed in Figure 6, the application of US and the control of pH enabled to reduce the counts of the different investigated microbial groups. Moreover, no *Enterobacteriaceae* were detected. This would also explain the higher TVB-N values of fillets from the control (industrial) process after 7 d of hydration, and their lower quality properties as measured with QIM. Similarly to the hydration process, the combination of US and pH control would act additively to control the microbial loads. The NaOH added to the hydration media to control the pH would allow to limit or even inactivate microbiota released from the fillets to the hydration media, as observed in Figure 4. This effect was stronger when pH was higher. Many studies have reported the use of NaOH for the inactivation of microorganisms, mainly spores, during the sanitation, cleaning, and elimination of biofilms in the food chain (GE-Healthcare, 2001). According to Santoro et al. (2011), hydration solutions with alkaline media could serve as a bactericidal agent and could therefore lengthen hydration times without affecting microbiological quality. Therefore, the use of NaOH for controlling the pH of the hydration medium could serve as an alternative to help limit microbial growth, but only up to a certain concentration, in order not to affect sensorial parameters as observed at pH 10.5 and, to a lesser degree, at 9.5.

When US is applied, it can exert a bactericidal effect in itself, or it can improve the effect of NaOH by facilitating or increasing the contact between bacteria and the chemical agent. Several previous studies have well described the lethal effect of US due to cavitation, which results in mechanical shocks, the production of free radicals, and localized heating, all of which can alter cellular structural and functional components to the point of causing sublethal or lethal injuries, thereby reinforcing the effect of other antimicrobials (Bermúdez-Aguirre et al., 2011; Nguyen et al., 2009). For example, it has been shown that the application of US at an intensity of 20.96 W/cm^2 during 120 min was effective in lowering the microbial loads of *Escherichia coli* O157: H7 and vegetative cells of *Bacillus cereus* in the meat-curing process (Hajmeer et al., 2006). This could likewise occur in the case of microorganisms present on the surface of the fillets and, to a lesser extent, in the case of microorganisms released to the hydration medium when applying US (Figure 4). In the latter case, the mechanical effect of cavitation through the action of "microjets" is effective in dragging microorganisms from the fillet to the medium, which could result in a higher microbiological count in the hydration solution, as has been described in literature (Gao et al., 2014; Barukčić et al., 2015). In addition to this effect, US could also promote the release of components into the environment (Figure 2B) and consequently increase the availability of macromolecular nutrients for microorganisms (Alarcon-Rojo et al., 2015; Feng et al., 2008), which could, in turn, reduce the effect of antimicrobial compounds (NaOH, hydrogen peroxide). The main limitation inherent in the release of microorganisms from the surface of the fish to the hydration media is that a specific hygienization process (i.e. UV-C light) for the hydration solution would be required in order to limit the re-contamination of fillets in an industrial application when introducing new fillets in re-used solution. In any case, and although this effect can indeed occur, the microbial loads of cod fillets were similar or lower than those observed for the control process as shown in Figure

6. This is remarkable when microbial loads are compared for hydrating times of similar weight gain: 7 d for the control samples with 2 d for the US-assisted process with pH regulation (Figure 5).

The obtained results demonstrate that the combined application of US at low intensities and the control of pH at 8.5 during the hydration process would allow to reduce process length by 2-3 d, as compared to the industrial process, thus resulting in a product of better quality from a microbiological point of view – and also of similar or even better sensorial quality. As can be observed in Figure 7 and in Table 2, quality parameters of cod fillets after 5 or 7 d of hydration when using US and pH control, evaluated through the QIM index, were similar to fillets hydrated with the industrial process during 5 d (CONTROL5) and better than those hydrated for 7 d (CONTROL7). These results would indicate that the use of US and the control of pH would also act synergically with the additives present in the hydration solution, leading to an improvement in the sensory quality properties of cod fillets. Phosphates are commonly used to improve the textural properties of meat products, as well as to assist in the stabilization of color, taste, and other sensory characteristics (Unal et al., 2004). In addition, certain studies have proposed that their use improves color stability, resulting in less yellow discoloration and higher luminosity by leaving the protein chains more open, thereby reflecting more light (Kin et al., 2009; Nguyen et al., 2013). Apart from this, the application of US in this study did not have a negative impact on the final quality of the product. However, this is not always the case, since the degradation of food has indeed been observed when applying US, due to physicochemical effects (Pingret et al., 2013). Pedrós-Garrido et al. (2017) evaluated the final quality of salmon, mackerel, cod, and hake fillets after applying US surface decontamination treatment (30 kHz, 51.41 W/l) during 45 min. Hardly any degradation was observed in cod and mackerel in terms of total lipid values, thiobarbituric acid reactive substance (TBARS) values, and color measurements. In contrast, significant reductions of TBARS and lower red and yellow index values were observed in salmon samples. Hake fillets only showed significantly lower values of yellow index compared to controls. Li et al. (2020) conducted a study of ultrasound-assisted thawing (28 kHz, 135 W/L) of bighead carp fillets. They did not observe any effect of US on thawing loss, cooking loss, or texture parameters (hardness, chewiness, and resilience) compared to water immersion thawing. Likewise, color index, TBARS values and volatile compounds were similar in control and treated samples. This means that, depending on the product or the US conditions, there might be impact on food quality or not, thereby indicating the need for further research regarding quality-impact-effect of US.

5. CONCLUSIONS

In this investigation, the combined effect of the application of ultrasound in conjunction with the control of the pH of the hydration medium during the industrial hydration process of cod fillets was evaluated. Traditional procedures require up to 7 d of hydration, thereby necessitating the use of additives to facilitate the hydration process and to control microbial loads. The application of US at intensities of 2.9 W/kg enabled to increase weight gains by 5-7% with respect to the industrial process, or to reduce the hydration time of thawed cod fillets from 3 d to 2 d, achieving the same weight gain.

When the influence of the pH control of the hydration medium was studied, it was observed that weight gain improved when pH of the hydration solution was more basic, i.e. up to pH 9.5. The combination of pH control (8.5) and US (25 kHz, 2.9 W/kg, 20 min on and 100 min off) increased the hydration of cod fillets by 4% compared to the pH-control process and by 17% compared to the industrial process after 5 d of hydration. For a similar weight gain (10%), the hydration process assisted with US and with pH control at 8.5

reduced the hydration process by 5 d, and by 2 d when US was not applied. This not only led to the highest weight gain, but allowed to control microbial growth, while not impairing the sensory quality properties of cod fillets. Apart from these results, the application of US and/or the control of pH during the hydration process could serve as an interesting strategy to reduce the use of additives in the process, which would be of great interest for consumers and for the industry. However, more research on the specific effect and on the interaction of US with different additives would be required.

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Highlights

- 17% hydration improvement of cod fillets by ultrasound.
- Synergistic hydrating effect resulting from mass transfer between pH and ultrasound.
- Sensorial quality of cod fillet maintained with ultrasound treatment.
- Potential additive-free hydration of cod fillets.

*Credit Author Statement

Credit author statement

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Javier Raso: Supervision, Writing - Review & Editing.

Guillermo Cebrián: Conceptualization, Writing - Review & Editing, Funding acquisition.

Ignacio Álvarez: Conceptualization, Methodology, Writing - Review & Editing, Funding acquisition, Supervision.

Figure

Figure 1. Influence of ultrasound intensity (25 kHz) on the evolution of the weight gain of cod fillets during hydration. Ultrasound treatments: (□) 29.4 W/kg, (Δ) 14.7 W/kg, (○) 2.9 W/kg and (♦) 0 W/kg (control).

Figure 2. Evolution of the pH (2A) and the electrical conductivity (2B) of the hydration solution of cod fillets hydrated when applying ultrasound (25 kHz) at different intensities: (□) 29.4 W/kg, (Δ) 14.7 W/kg, (○) 2.9 W/kg, (♦) 0 W/kg (control). .

Figure 3. Evolution of the weight gain of cod fillets hydrated in media of different pH (■, □: pH 8.5; ▲, Δ: pH 9.5; ●, ○ pH 10.5) with (solid lines and black markers) and without (discontinued lines and white markers) US (25 kHz, 2.9 W/kg). Control samples (♦): fillets hydrated in the commercial solution.

Figure 4. Evolution of the TAM counts of the hydration solution during the hydrating process of cod fillets under different conditions: (□) pH controlled at 8.5, (Δ) pH controlled at 9.5, (■) US-assisted process (25 kHz, 2.9 W/kg) with pH control at 8.5. (♦) Control process: fillets hydrated in the commercial solution.

Figure 5. Weight gain (5A), pH (5B), and N-BVT (5C) of cod fillets hydrated in commercial solution (CE, ♦, grey bars), in commercial solution with pH controlled at 8.5 (pH, □, white bars), and in commercial solution with pH controlled at 8.5 and the application of US (USpH, ■, black bars) (US treatment: 25 kHz, 2.9 W/kg, 20 min US, 100 min US pause). Capital letters indicate statistically significant differences for the same hydration day and different treatments ($p \leq 0.05$). Lower-case letters indicate statistically significant differences between days for the same treatment ($p \leq 0.05$).

Figure 6. Total aerobic mesophilic (TAM) (6A), spoilage seafood organism (SSO) (6B), proteolytic bacteria (PB) (6C) and *Enterobacteriaceae* counts (6D) of cod fillets hydrated in commercial solution (CE, ■, gray bars), in commercial solution with pH controlled at 8.5 (pH, □, white bars), and in commercial solution with pH controlled at 8.5 and the application of US

(USpH, ■, black bars) (US treatment: 25 kHz, 2.9 W/kg, 20 min US, 100 min US pause). Capital letters indicate statistically significant differences for the same hydration day and different treatments ($p \leq 0.05$). Lower-case letters indicate statistically significant differences between days for the same treatment ($p \leq 0.05$).

Figure 7. Results of the Quality Method Index (QIM) for cod fillets hydrated during 5 d (white bars) and 7 d (black bars) in a commercial solution without pH control (Control), and in a commercial solution with pH controlled at 8.5 (pH) and the application of US (USpH) (US treatment: 25 kHz, 2.9 W/kg, 20 min US, 100 min US pause). Capital letters indicate statistically significant differences for the same hydration day and different treatments ($p \leq 0.05$). Lower-case letters indicate statistically significant differences between days for the same treatment ($p \leq 0.05$).

Figure 1

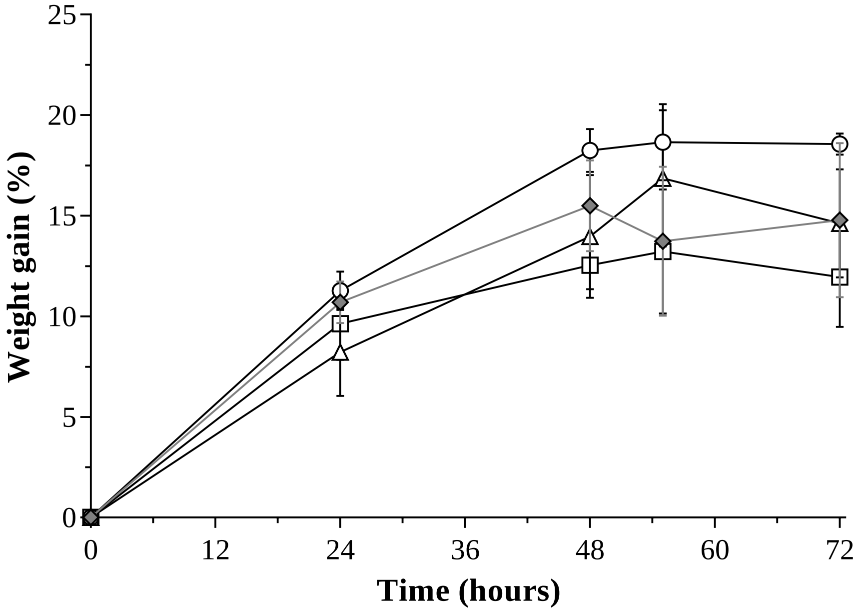


Figure 2

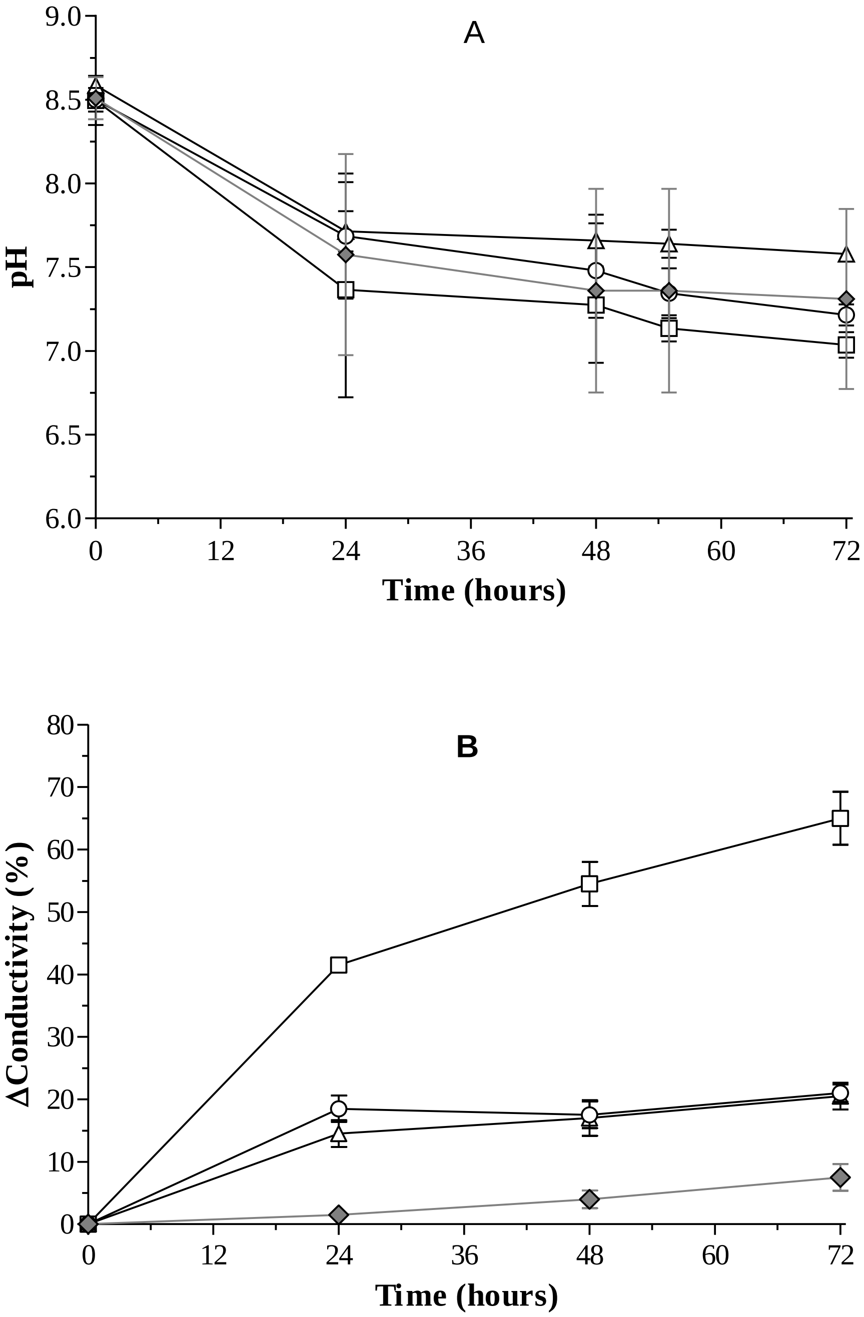

A
9.0
8.5
8.0
7.5
7.0
6.5
6.0
pH
0
12
24
36
48
60
72
Time (hours)
B
80
70
60
50
40
30
20
10
0
ΔConductivity (%)
0
12
24
36
48
60
72
Time (hours)

Figure 3

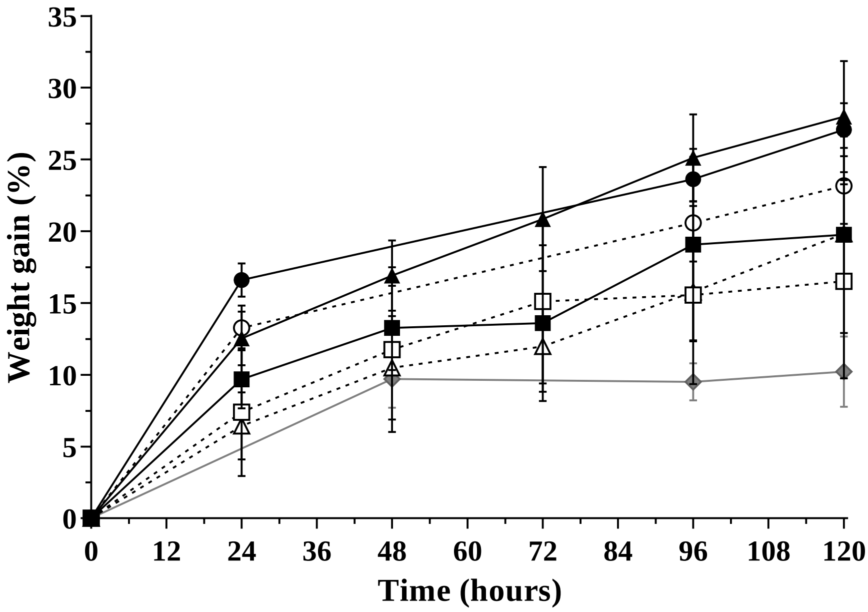

35
30
25
20
15
10
5
0
Weight gain (%)
0
12
24
36
48
60
72
84
96
108
120
Time (hours)

Figure 4

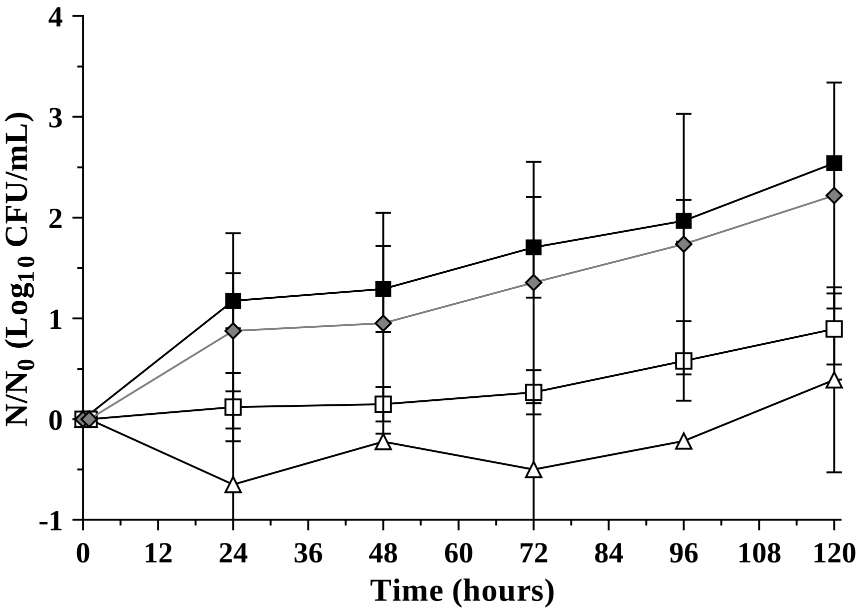
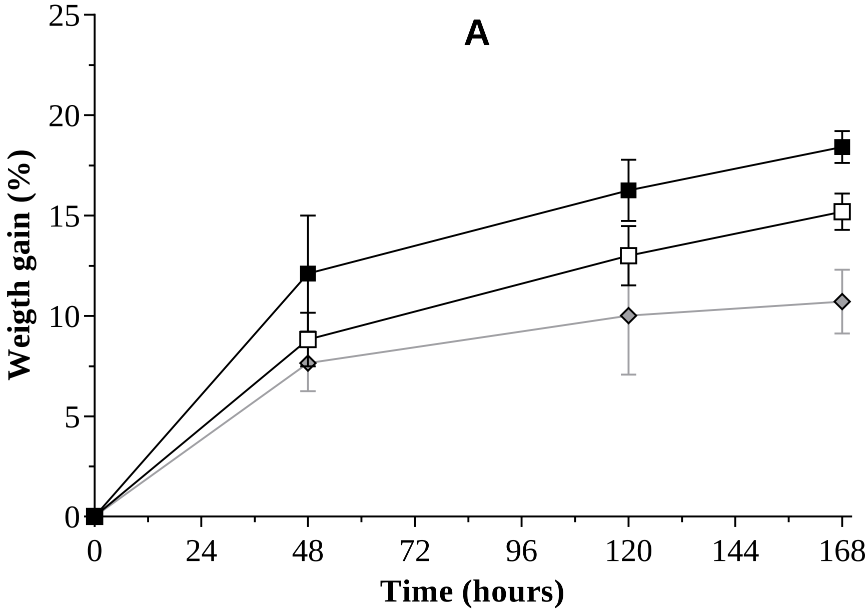
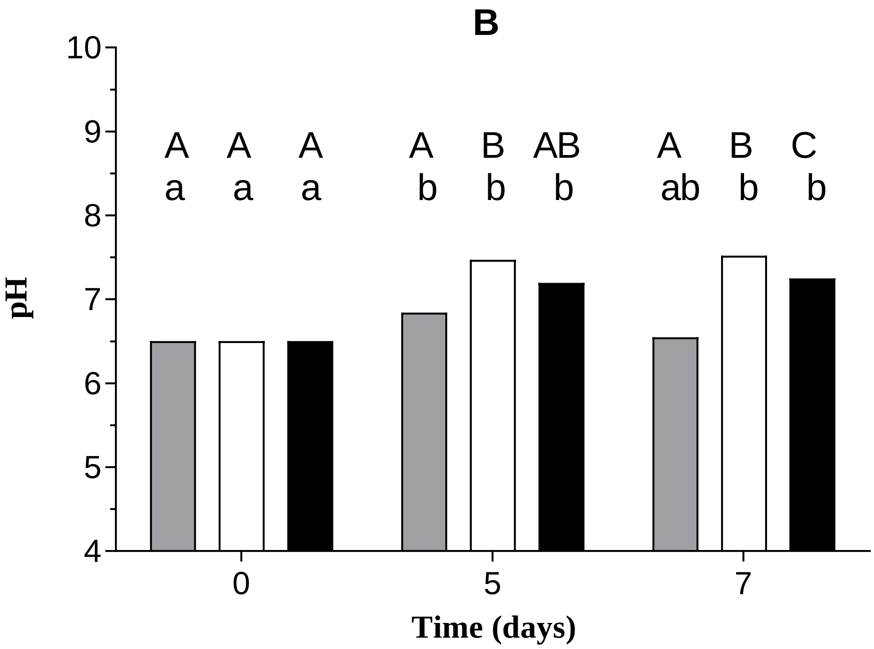

4
3
2
1
0
-1
N/N0 (Log10 CFU/mL)
0
12
24
36
48
60
72
84
96
108
120
Time (hours)

Figure 5




B
pH
10
9
8
7
6
5
4
A A A A B AB A B C
a a a b b b ab b b
0
5
7
Time (days)

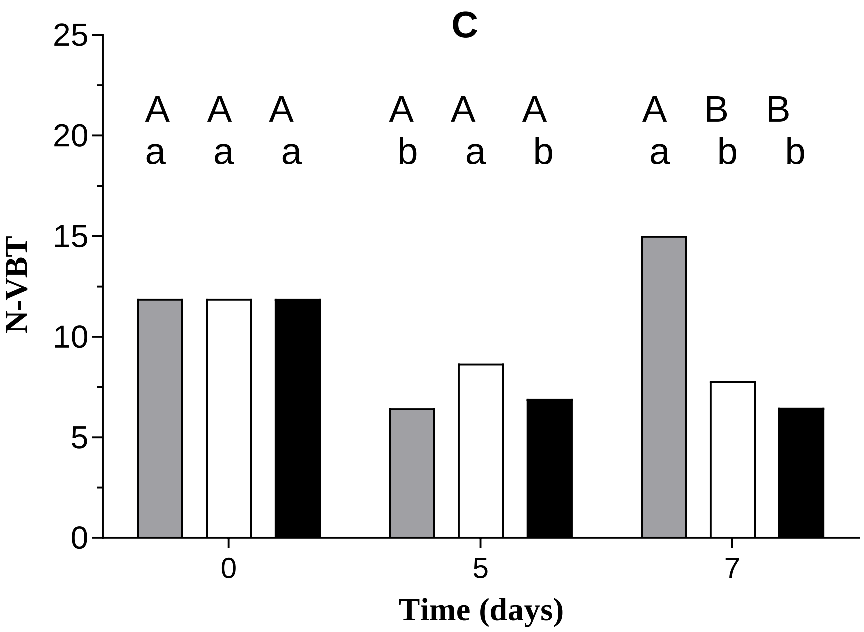
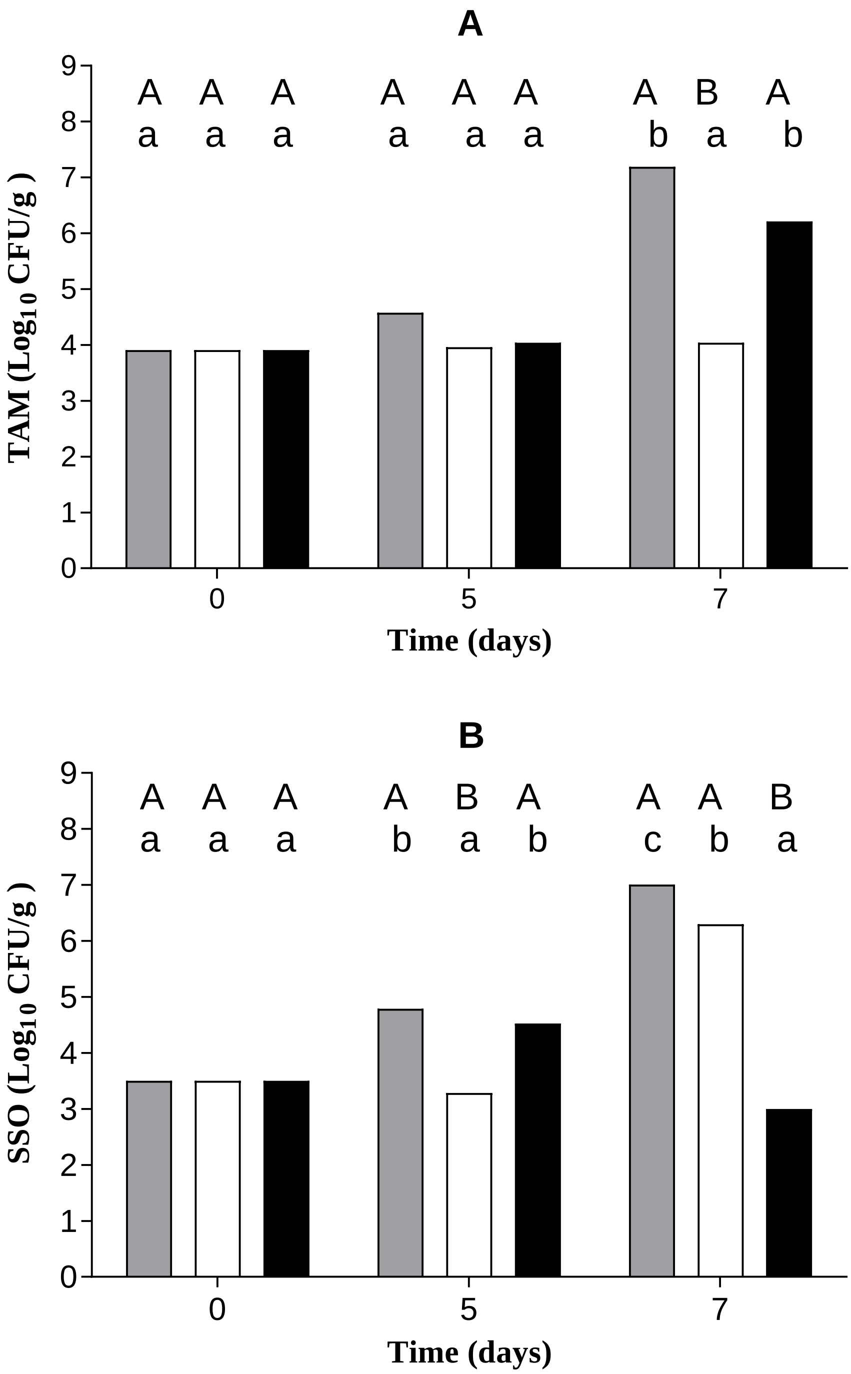
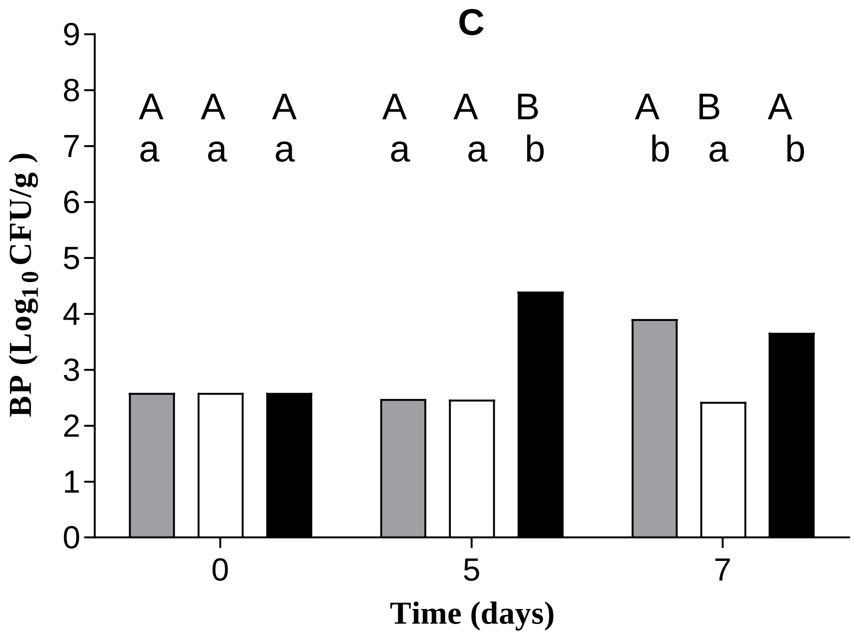

C
N-VBT
25
20
15
10
5
0
A A A A A A A B B
a a a b a b a b b
0
5
7
Time (days)

Figure 6


A
9
8
7
6
5
4
3
2
1
0
A A A A A A A B A
a a a a a a b a b
TAM (Log10 CFU/g)
0
5
7
Time (days)
B
9
8
7
6
5
4
3
2
1
0
A A A A B A A A B
a a a b a b c b a
SSO (Log10 CFU/g)
0
5
7
Time (days)


C
BP (Log10 CFU/g)
Time (days)
0
5
7
A A A A A B A B A
a a a a a b b a b
0
1
2
3
4
5
6
7
8
9

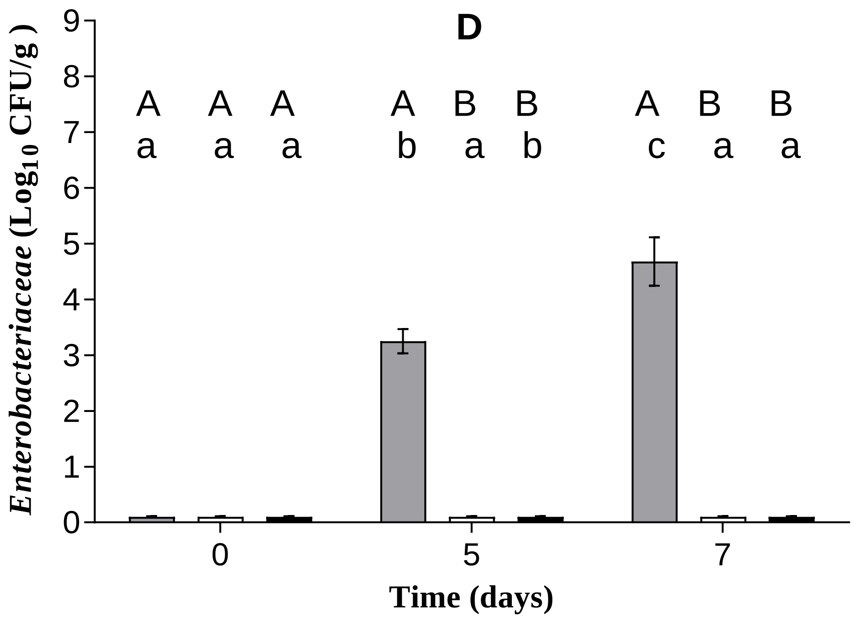
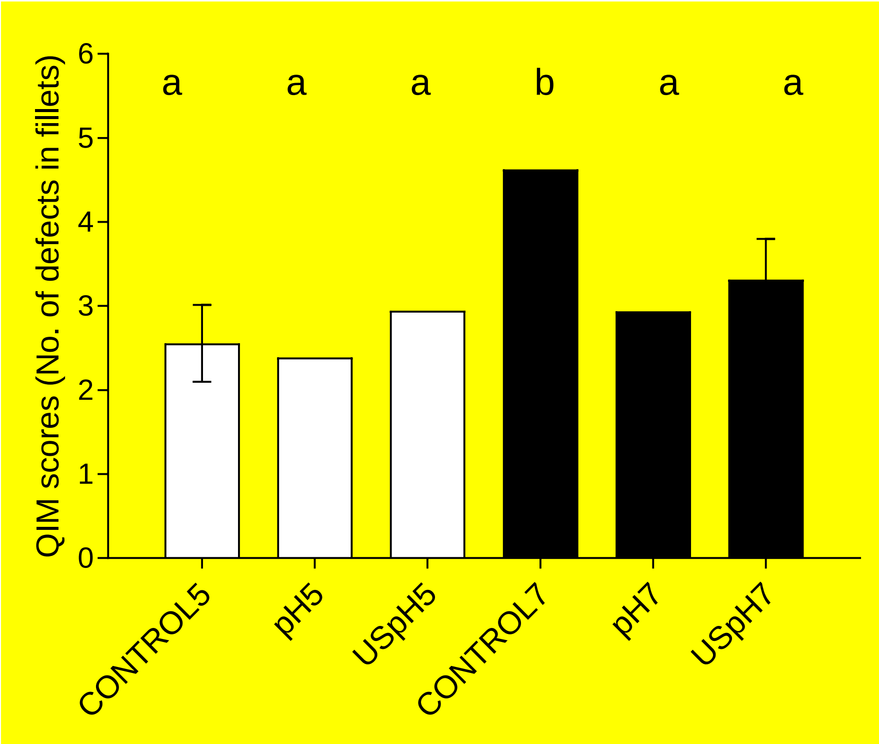

D
Enterobacteriaceae (Log10 CFU/g)
Time (days)
0
5
7
A A A A B B A B B
a a a b a b c a a
0
1
2
3
4
5
6
7
8
9

Figure 7



QIM scores (No. of defects in fillets)
0
1
2
3
4
5
6
a
a
a
b
a
a
CONTROL5
pH5
USpH5
CONTROL7
pH7
USpH7

Table 1: Recovery conditions for the different microbial groups investigated

Microbial Group	Agar	Temp	Time	Atmosphere	Plating
Total Aerobic Mesophilic	LH agar[1]	37 ºC	48 h	Aerobic	Spread
SSO[2]	Iron agar with L-cysteine[3]	20 ºC	72 h	Aerobic	Mass
Enterobacteriaceae	VRBG agar[4]	37 ºC	48 h	Aerobic	Spread
Proteolytic bacteria	MRS agar[5]	30 ºC	48 h	Aerobic	Spread

[1] Long and Hammer Agar (Broekaert et al., 2011).

[2] (Lougovois, Kyrana & Kyrana, 2003).

[3] (Gram, Trolle, & Huss, 1987).

[4] Violet Red Bile Glucose Agar (VRBG, Oxoid, United Kingdom).

[5] de Man, Rogosa and Sharpe (ISO 15214:1998).

Table 2. Results of the statistical analysis for sensory attributes evaluated by QIM: texture, odor, color, blood stains and gaping in a commercial solution without pH control (Control), and in a commercial solution with pH controlled to 8.5 (pH) and the application of US (USpH) (US treatment: 25 kHz, 2.9 W/kg, 20 min US, 100 min US pause).

		Treatments					
		CONTROL5	**pH5**	**USpH5**	**CONTROL7**	**pH7**	**USpH7**
Attributes (QIM)	**Texture**[NS]	0,83	0,78	0,83	1,19	1,31	1,25
	Odor[NS]	0,17	0,56	0,33	0,43	0,67	0,63
	Color*	0,22[ab]	0,17[a]	0,44[abc]	0,88[c]	0,50[abc]	0,63[bc]
	Blood stains**	0,50[a]	0,11[a]	0,39[a]	1,00[b]	0,35[a]	0,38[a]
	Gaping**	0,83[abc]	0,78[ab]	0,94[bc]	1,19[c]	0,88[bc]	0,44[a]

Different letters indicate significant differences among the treatments applied.

NS: Non significant

*: $p \leq 0.05$

**: $p \leq 0.01$

Supplementary material: QIM scheme for fillet from thawed cod

Quality Description	Scoring description	Points
Texture	Firm and stiff texture, no wateriness	0
	Slightly soft, initial wateriness	1
	Soft, wateriness noticeable	2
	Very soft and pronounced wateriness	3
Odour	Neutral	0
	Slightly sour, off odour	1
	Very sour off odour	2
Colour	Plain white	0
	Greyish	1
	Grey, starting yellow maybe slightly red	2
	Either yellow or very red, milky surfaces, freeze dried	3
Blood stains	No stains	0
	A single stain (diameter less than 3mm)	1
	Single small stains (1-2 with diameter less than 5mm)	2
	Very discoloured from many stains or totally red	3
Gaping	No gaping, coherent	0
	Slight gaping but still coherent	1
	Gaping noticeable, disrupted	2
	Gaping pronounced, disrupted	
Parasites	No parasites	0
	One parasites	1
	More than one parasite	2
	QIM SCORE	0-16