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Effect of cold plasma generated with different gas mixtures on safety, quality and nutritional aspects of fresh sea bream fillets

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ABSTRACT

Cold atmospheric plasma (CAP) is a non-thermal technology, successfully used to decontaminate and extend the shelf-life of various foods. However, since CAP is highly oxidative, it can cause quality deterioration in sensitive matrices, such as fish products. This research aimed to evaluate the effect of CAP treatment with a surface dielectric barrier discharge (SDBD) with different gas mixtures (80% Ar/20% O_2, or 80% N_2/20% O_2) on the decontamination of inoculated pathogens (*E.coli* and *L.innocua*; log 4 CFU/g inoculum) and endogenous spoilage microbiota and on the main quality indices of seabream (*Spaurus aurata*) fillets. For selected appropriate treatments, the impact on the nutritional value of the products was investigated through in vitro digestion, bioaccessibility of fatty acids and the degree of protein hydrolysis.

The use of CAP resulted in a decrease in the bacterial load in the fresh sea bream fillets up to 1 log CFU/g obtained with Ar/O_2 gas mixture for 20 min, affected by the treatment duration, but not by the gas mixture. Although a slight increase in lipid oxidation was observed (from 0.5 mg MDA/kg to a maximum of 4 mg MDA/kg), the digestibility of the products was not affected.

Industrial relevance: From an industrial point of view, increasing shelf-life of perishable products such as fish fillets with an environmentally friendly and non-thermal technology could represent a great advantage; however, maintaining quality is of paramount importance for the industrial use of this novel processing technology. The results of the present study show negligible effects on the nutritional quality of seabream fillets, which encourages further research.

1. Introduction

Fish is worldwide considered as healthy food for human consumption due to its significant content of proteins, minerals (mainly iodine and selenium), and n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA). High fish consumption is related to a reduced risk of cardiovascular diseases and many dietary guidelines recommend to consume it at least twice a week (Nestel, Beilin, Clifton, Watts, & Mori, 2021).

Unfortunately, pathogen growth and bacterial spoilage are frequent and fast in fish due to its high water content, high pH and presence of various substrates. Fish is a very perishable commodity, and increasing its shelf life and maintaining its qualitative and nutritional characteristics is still a challenge for the food industry. In this scenario, non-thermal processing technologies, able to sanitize food at low temperature without using chemicals and low environmental impact, are of great interest. Among them, cold plasma has emerged as a promising strategy

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for the processing of food products (Thirumdas, Sarangapani, & Annapure, 2014).

Plasma is an ionized gas composed by a mixture of ions, free electrons, neutral particles (atoms, molecules), activated and metastable species (NO_x^-), pro-oxidants like reactive oxygen species (ROS), reactive nitrogen species (RNS) and photons. The microbial inactivation by plasma has been attributed to different mechanisms, including oxidative damage to membranes or intracellular components, DNA damage by UV radiation, disruption of cell membranes (Zhao, de Alba, Sun, & Tiwari, 2019).

Non-thermal or cold atmospheric plasma (CAP), which is characterized by temperature close to room temperature, has been investigated for the treatment of various food products. Several operative parameters can affect the decontamination efficacy of CAP. Among them, the technologies used for its generation (e.g., dielectric barrier discharge – DBD, corona discharge, plasma jet etc.), the input power (applied voltage and current), the composition of the gas mixture and the flow rate, the time of exposure, and the type (direct or indirect) of treatment. Moreover, intrinsic factors such as the type of food matrix and the type, load and growth phase of the microorganisms may influence the final result.

The use of CAP as decontamination strategy has been investigated on different fish products, such as seabream fillets (Giannoglou, Panagiotis, Efthimiadou, Gogolides, & Katsaros, 2021), mackerel (Albertos et al., 2017), herring (Albertos et al., 2019) and sea bass (Olatunde, Benjakul, & Vongkamjan, 2019; Singh & Benjakul, 2020). Results generally showed a reduction of various classes of microorganisms, naturally present or artificially inoculated, ranging from 1 to 3 log CFU/g depending on treatment parameters and microorganism type. This microbial reduction often led to an increase in the microbiological shelf life of the product. However, due to the highly oxidative power of plasma reactive species and to the presence of highly unsaturated fatty acids in fish, an increase of primary (e.g., peroxide value) and secondary (e.g., TBARs) lipid oxidation indexes was often observed (Gavahian, Chu, Mousavi Khaneghah, Barba, & Misra, 2018), generally proportional to exposure time and applied energy (Albertos et al., 2019).

These results underline the need of a global evaluation of the effects of new processing techniques that should increase the microbiological safety of the product without altering its nutritional characteristics. The present work aims at deepening the knowledge on the effect of cold plasma on fish products by considering a variety of quality index evaluated also after in vitro digestion.

In the present work, the effect of CAP generated using different gas mixtures on the decontamination of inoculated pathogens, on the endogenous spoilage microflora and on the main qualitative indexes of seabream (*Spaurus aurata*) fillets was evaluated. A novel surface dielectric barrier discharge (SDBD) was used to produce an ozone rich atmosphere in a closed chamber, and different gases mixtures (argon 80% + oxygen 20%, or nitrogen 80% + oxygen 20%) were used in the closed volume.

After selection of the most suitable treatments, the impact on the nutritional value of the products was investigated. To this aim, seabream fillets underwent in vitro digestion, and the fatty acid (FA) bioaccessibility and degree of protein hydrolysis were evaluated.

2. Material and methods

2.1. Raw material preparation

Seabream fillets were obtained from Kefalonia Fisheries S.A., Greece. Seabreams were beheaded, filleted and skinned at Economia del Mare (Cesenatico, Italy), filleted and fast frozen at −40 °C and stored in the laboratory of CIRI-Agro for a maximum of two weeks. Experimental procedures were carried out after thawing at 4 °C overnight. The average weight of fillets was 53 ± 6.5 g.

From the superior part of each fillet, two square samples (2 × 2 cm, average weight 7.5 ± 0.6 g) were obtained manually with a sharp knife. Samples were placed on stainless steel racks in the treatment chamber and subjected to the CAP treatment. Untreated samples were considered as control (C).

2.2. CAP system

The CAP system used was previously described (Capelli et al., 2021) and it consisted of a surface dielectric barrier discharge (SDBD) plasma source posed on the top of a plexiglass box to create a closed volume. The box was equipped with two optical quartz windows to allow Optical Absorption Spectroscopy (OAS) analysis. In addition, the box had two gas connections, one used as an inlet and the other as an outlet when flushing the chamber with the treatment gas. The SDBD was air cooled to ensure safety of operation and repeatability of results; the plasma source was connected to a high voltage microsecond pulsed generator. A schematic representation of the CAP system is shown in Fig. 1 highlighting the different components.

2.3. Plasma treatments

Gas mixtures were obtained using a gas quaternary mixer (mod. KM100–4, Witt-Gasetechnik, Witten, Germany) connected to compressed gas cylinders and a gas-flushing welding machine (mod. Multiple 315, Orved Srl, Venezia, Italy) as following:

- Air: 80% N_2, 20% O_2
- Argon: 80% Argon, 20% O_2

The treatment chamber was saturated with the gas mixture and the treatment lasted for 10 and 20 min. Longer treatment times were discarded as they did not improve the microbiological decontamination while promoting a detrimental effect on the visual quality of the product. Samples were coded accordingly as Air-10, Air-20, Argon-10, and Argon-20 (Minekus et al., 2014). Each treatment was repeated in two independent replicates, and 8 samples were prepared for each treatment.

2.4. Analytical determinations

2.4.1. Setup for optical absorption spectroscopy (OAS) and data processing

Optical Absorption Spectroscopy (OAS) was setup according to (Simoncelli et al., 2019a).

OAS acquisitions were performed using a grating with a resolution of 150 mm^{-1} and setting a width of 10 μm for the inlet slit of the spectrometer. With the aim of achieving fast acquisition with a time resolution of 40 ms, a photomultiplier tube (PMT-Princeton Instruments PD439, US) connected to a fast oscilloscope (Tektronix MSO46) was used as detector. The PMT amplification factor was fixed at 565 and it was kept constants for all measurements.

The concentration of Reactive Oxygen and Nitrogen Species (RONS) in the closed chamber was calculated spectrally resolving the collected beam and taking the Lambert-Beer Law into account:

$$\frac{I}{I_0} = e^{(-L\sigma n)} \quad (1)$$

where the concentration of the absorbers (n), which has to be quantitatively evaluated, is correlated with the light absorbed after an optical path of length (L) and expressed as the ratio between the initial light intensity (I_0) and the residual light intensity (I) after passing thorough the absorbers region. The absorption cross-section σ is a function of the light wavelength ($\sigma = \sigma(\lambda)$). In the experiments, the optical path was 14 mm long.

The wavelengths selected to perform the acquisitions and the relative absorption cross-sections for the absorbers (O_3 and NO_2) are reported in

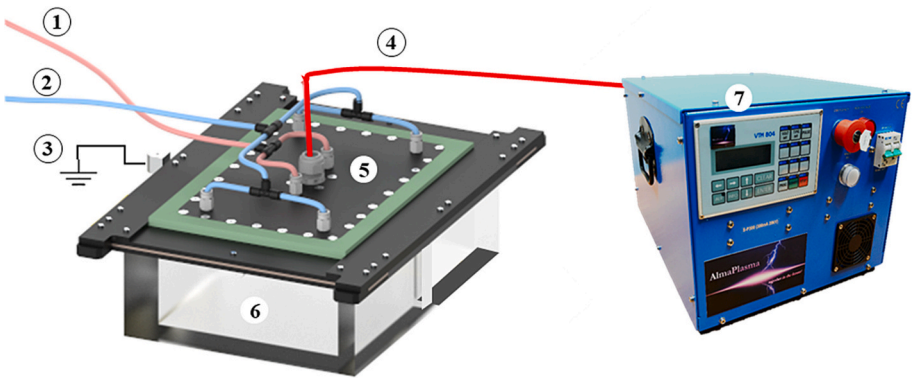

Fig. 1. Schematic representation of the CAP system. 1) air cooling outlet, 2) air cooling inlet, 3) ground connection, 4) high voltage connection, 5) SDBD, 6) treatment chamber, 7) high voltage generator.

Table 1
Selected wavelengths and absorption cross-sections in cm^2 for O_3 and NO_2.

Selected wavelength	O_3 cross-section	NO_2 cross-section
253 ± 1,2 nm	(1,12 ± 0,02)E-17	(1,1 ± 0,3)E-20
400 ± 1,2 nm	(1,12 ± 0,08)E-23	(6,4 ± 0,2)E-19

Table 1. These wavelengths were defined, in accordance with (Moiseev et al., 2014), to maximise the absorption of the molecules relevant to our study while minimising the contribution, and thus the disturbance, of other absorbing molecules. The contribution of background radiation was subtracted from the measured values of light intensity.

2.4.2. Temperature measurements

A temperature fiber optic sensor (OpSens MultiSens) was used to perform measurements inside the chamber. It was placed 4.5 cm from the plasma source, in the same position of the seabream filets during treatments. The probes monitored the temperature during 20 min of plasma treatment, in both conditions of gas mixture inside the chamber (Argon and Air). Every measure was repeated three times.

2.4.3. Microbiological test

2.4.3.1. Inoculum refresh and propagation. Pure cultures of *Escherichia coli* 555 and *Listeria innocua* (as a surrogate for L. *monocytogenes* (Rød, Hansen, Leipold, & Knøchel, 2012) were obtained from the microbial collection of the Department of Agricultural and Food Sciences of the University of Bologna (Italy). Bacteria were revived from glycerol stocks at −80 °C by two successive growths at 37 °C for 48 h in TSB broth (Oxoid, Thermo Fisher Scientific, Waltham, MA, USA) and BHI broth (Oxoid, Thermo Fisher Scientific, Waltham, MA, USA) for *L. innocua* and *E. coli*, respectively. Subsequently, for each bacterial strain, a log 4 CFU/ml inoculum was used for the fillets.

For each different thesis, double samples of seabream fillet were prepared. The manipulation took place under aseptic conditions in a microbiological hood and the fillets were kept under ice. Each fillet was cut with a sterilized blade in the center and equal portions of approximately 10 g each were prepared. Based on the exact weight recorded, each sample was inoculated with 1 ml of a suspension of log 4 CFU/ml of *E. coli* or L. *innocua*. The inoculum was distributed on the fillet and kept at 1 h at 4 °C to be absorbed on surface. Control samples (not inoculated) were prepared for each thesis and all samples were prepared in duplicate.

2.4.3.2. Microbial quantification of survived bacteria. Microbial enumeration on foods were made according to the ISO 4833-2:2013, with little modifications for *E. coli* and L. *innocua* that were incubated at 37 °C. Briefly, the samples were homogenized in a 1:10 (w / v) dilution for 2 min in a Stomacher 3500 paddle mixer (Seward Ltd., Worthing, UK), and decimal dilution tubes were prepared. Subsequently, 0.1 ml of the appropriate dilutions was plated onto the PCA for enumerating the total mesophilic aerobic bacteria, onto the BHI plates for enumerating *E. coli*, and onto the TSB plates for enumerating L. *innocua*. Thereafter, the PCA plates were incubated for 48 h at 30 °C prior the enumeration, and the BHI and the TSB plates were incubated for 48 h at 37 °C before counting.

All plates were run in duplicate, and the results were expressed as log colony forming units (CFU) per gram of product (log CFU/g).

2.4.4. Quality determination

pH was determined on the homogenate obtained mixing 5 g of fish tissue and 5 g of distilled water using a pH-meter (Crison, Barcellona, Spain). Moisture content was determined gravimetrically by drying in an oven at 70 °C until constant weight.

Colour parameters lightness (L*), redness (a*) and yellowness (b*) were measured with a spectrophotocolorimeter mod. ColorFlex™ (Hunterlab, Reston, Virginia). The tristimulus L*, a*, b* measurement mode (CIE, 1987) was used. The colour was measured on both upper and lower part of the fillets.

The 2-thiobarbituric acid-reactive substances (TBARS) analysis was performed in the fish fillets according to (Bao & Ertbjerg, 2015) to evaluate the oxidation changes during storage at 2–4 °C. A standard curve of 1,1,3,3-tetraethoxypropane was used to calculate the amount of malondialdehyde produced. TBARS content was expressed as mg of malonaldehyde (MA) per kg of fillet.

2.4.5. In vitro static digestion

In vitro static digestion was performed according to the INFOGEST protocol (Minekus et al., 2014). Briefly, 7 g of each sample were chopped to simulate chewing, and then mixed with 5.6 ml of simulated salivary fluid (SSF), 35 µl of calcium chloride 0.3 M and 1.365 ml of distilled water for two minutes at 37 °C. Then, 11.2 ml of simulated gastric fluid (SGF), pepsin (2000 U/ml) and 7 µl of 0.3 M calcium chloride was added. The pH was lowered to 3 using HCl 37% and the flask was kept stirring at 37 °C for 2 h in a thermostatic water bath. At the end of the gastric phase, pancreatin (100 U/ml), bile (10 mM), 12.4 m ml l of simulated intestinal fluid and 56 µl of 0.3 M calcium chloride were added and the pH was raised to 7 using NaOH 35%. The flask was kept stirring for 2 h at 37 °C. At the end of the duodenal phase, the digesta was collected and the enzymes were inactivated by lowering the pH to 3. Samples were centrifuged at 4500*g* for 10 min at 4 °C.

2.4.6. Lipid content and fatty acid composition

In not digested samples, total lipid content was evaluated using the official ISO 1443:1991 standard method (ISO, 1990).

To evaluate the FA composition, lipids were extracted from not

digested and digested seabream fillets according to (Bligh & Dyer, 1959) with slight modifications. The methylation of FA was performed adding 500 µl of hydrogen chloride solution 0.5 M in methanol (Sigma-Aldrich, 07607) at 100 °C for 1 h. At the end of the methylation step, 2 ml of hexane and 2 ml of distilled water were sequentially added. The hexane layer was transferred in a test tube and dried under nitrogen infusion. The resulting fatty acids methyl-esters (FAMEs) were suspended in 100 µl of hexane.

The analysis of FAMEs was performed by fast-GC (GC-2030, Shimadzu, Kyoto, Japan) using a capillary column (30 mt, 0.2 µm film thickness) with a programmed temperature gradient (50 °C–250 °C, 10 °C/min). The peaks were identified based on their retention time, which was predetermined using a standard mix solution (Supelco, CRM47885), and quantified using Lab Solution Software (Shimadzu, Kyoto, Japan) (Di Nunzio et al., 2022).

Since the digestive fluids contained FAs from added bile, in digested samples the FA content of blank digestion, i.e., without food, was subtracted.

2.4.7. Protein hydrolysis during digestion

In not digested samples, protein content was evaluated using the official ISO 937:1991 standard method (Benedict, 1987). To evaluate the extent of protein hydrolysis during digestion, digested samples were centrifuged at 50.000 *g* for 20 min at 4 °C and then filtered on 0.22 µm syringe filter. Protein concentration was assessed spectrophotometrically by o-phthaldialdehyde (OPA) assay and measuring the absorbance at 280 nm using L-glutamic acid and non-fat dry milk as standard, respectively. The protein content from the enzymes added during in vitro digestion was subtracted, and values were standardized for the dilution factor due to the addition of digestive fluids.

2.4.8. ^1H NMR evaluation of protein digestibility

Digested samples were centrifuged at 14 k rpm for 10 min at 4 °C, 750 µl of supernatant was added 120 µl of DSS-d_6 (3-(Trimethylsilyl)-1-propanesulfonic acid-d_6 sodium salt) 10 mM buffer standard solution in D_2O and pH adjusted to 7.01. samples were then centrifuged at 14 k rpm for 10 min at 4 °C to remove salt precipitate and 700 µl of supernatant were placed in a standard 4.25 mm NMR tube and refrigerated until analysis.

^1H NMR spectra were acquire following the method described by (Urbinati et al., 2021) and recorded at 300 K on a Bruker US+ Avance III spectrometer operating at 600 MHz, equipped with a BBI-z probe and a SampleCase™ sampler for automation.

2.5. Statistical analysis

All analyses were performed using samples from two independent treatment replicates. All statistical analyses were performed using the software TIBCO Statistics 8.0 (Tibco Inc., Palo Alto, CA, USA). Normality was verified with the Shapiro-Wilk test, while homoscedasticity was evaluated with the Levene test. Differences between the samples within a bacterial group were assessed with Student's test, set at $p < 0.05$. Data were normalized using the mean centering method. Differences in the FA composition and relative release and in protein hydrolysis during digestion were verified by the one-way ANOVA using Tukeys' as post-test and assuming $p < 0.05$ as significant. Averages are given as mean ± SD.

3. Results and discussion

3.1. Electrical characterization

To evaluate the power density (PD) delivered to the plasma source, voltage and current waveforms were recorded by means of a high voltage probe (mod. Tektronix P6015A) and a current probe (mod. Pearson 6585) connected to an oscilloscope (mod. Tektronix DPO40034). The power density was evaluated using the following formula:

$$PD = 1 \Big/ T \int V \bullet I \, dt$$

Applying a maximum peak voltage of 6.6 kV with a repetition frequency of 5 kHz and a maximum current of 594 mA, the calculated PD was 36.5 ± 0.33 W over three repetitions.

3.2. ROS-RNS concentrations

The kinetics of ozone and nitrogen dioxide inside the treatment chamber, in which different gas mixtures have been previously fluxed until full volume saturation, were observed.

The mean ozone concentration values are reported in Fig. 2 for the 10 and 20 min treatments for both gas mixtures.

Using the Air mixture, ozone reached about 3100 ppm after 10 min and significantly increased to about 4600 ppm after 20 min. Conversely, using the Argon mixture, ozone concentration reached the maximum level of about 49,000 ppm already after 10 min, without any further increase at the longer treatment time.

NO_2 concentration was not detectable, which is in good agreement with the literature (Eliasson & Kogelschatz, 2023; Moiseev et al., 2014; Shimizu, Sakiyama, Graves, Zimmermann, & Morfill, 2012; Simoncelli et al., 2019b). As no trace of NO_2 was found, it was decided not to investigate the presence of other nitrogen-derived reactive species (NO, NO_3, N_2O, N_2O_5); as shown by Kogelschatz, nitrogen oxides are produced in a chain of reactions, it is therefore not possible to produce NO_x in the total absence of NO_2. When parameters are set to achieve small values of PD, ozone generation reactions are favored instead of nitrogen oxides production, which are, for this reason, negligible in ozone regime. For higher PD values, the production of nitrogen oxides is favored, contributing to ozone destruction. In this study, PD was always lower than 0.1 W/cm^2, which is considered a good approximation of the threshold between the two different regimes, as reported by the above-mentioned papers.

3.3. Temperature measurements

Temperature profiles inside the chamber were measured at 4.5 cm from the plasma source, where the seabream filets were placed during the plasma treatment. In all the investigated cases, the maximum temperature reached after 20 min of treatment never exceed 35 °C, thus excluding in both cases the thermal effect on the food matrices during treatments.

3.4. Microbiological results

The initial loads of total aerobic mesophilic bacteria, *E. coli* and L. *innocua* were of 3.02 ± 0.03 log CFU/g, 2.24 ± 0.05 log CFU/g and < 1.0 log CFU/g, respectively. Values of total mesophilic bacteria are comparable to those reported in the literature ranging from 3.0 to 5.0 log CFU/g on filleted fish (Albertos et al., 2017). In inoculated samples, treatments caused growth inhibition of bacteria in a time dependent manner (Fig. 3**)**. Based on the results and the statistical analysis, the most effective plasma treatment seemed to be Argon-20, showing the highest mortality rate for all microorganisms. In this condition, *E. coli* resulted the most sensitive microorganism to this treatment with a reduction equal to 1.05 ± 0.12 log CFU/g. This agreed with (Bartis, Graves, Seog, & Oehrlein, 2013) which showed that lipopolysaccharides, essential elements of the cell wall of Gram-negative bacteria, underwent significant changes during treatment with argon plasma. The increased plasma sensitivity of Gram-negative bacteria was also reported by (Mai-Prochnow, Clauson, Hong, & Murphy, 2016), where a much greater reduction in cell number was achieved with plasma

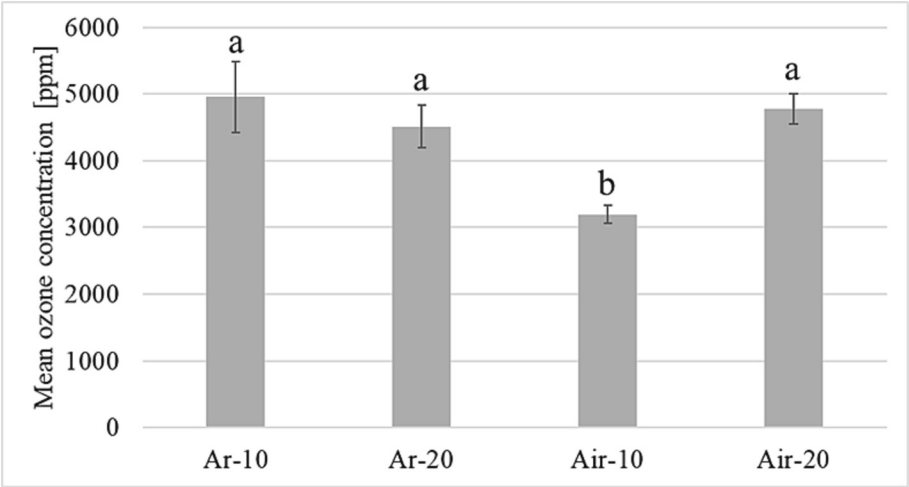

Fig. 2. Ozone concentration values in 10 and 20 min of plasma treatment for both argon and air atmospheres. Data are mean ± SD of 4 biological replicates in each condition. Statistical analysis was by paired Student *t*-test assuming $p < 0.05$ as significant. Different letters indicate statistical significance.

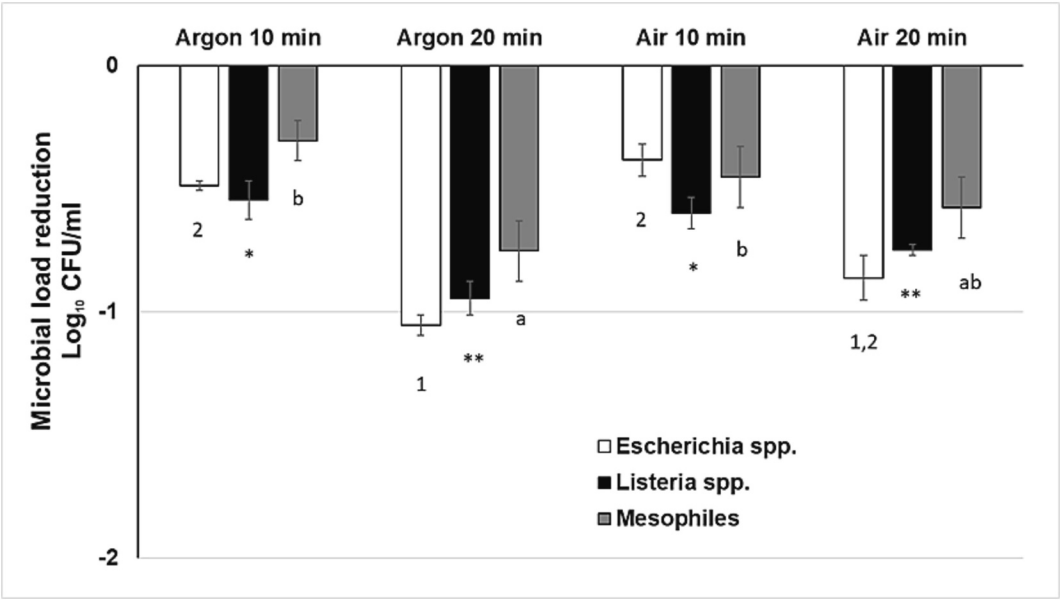

Fig. 3. Reduction of bacteria on inoculated samples by plasma treatment in respect to untreated and control (log CFU/g). Data are mean ± SD of 3 biological replicates in each condition. Statistical analysis was by the one-way ANOVA using Tukeys' as post-test and assuming $p < 0.05$ as significant. Different symbols within a bacterial group indicate statistical significance.

treatment for Gram-negative bacteria compared to Gram-positive bacteria.

Using Argon-20 treatment, *L. innocua* also underwent a reduction of about 1 log cycle, equal to 0.94 ± 0.06 log CFU/g. Similar results were observed for treatment with Air-20, with which *E. coli* and L. *innocua* loads were reduced by 0.86 ± 0.09 log CFU/g and 0.74 ± 0.02 log CFU/g, respectively. In agreement with our results, (Rød et al., 2012) reported the efficacy of cold plasma treatment with argon, especially when mixed with oxygen, on the reduction of L. *innocua*. Therefore, based on the results of the present study, the greatest abatement was obtained with argon for both *E. coli* and *L. innocua*. In the not inoculated samples (control), mesophiles appeared to be more sensitive to Argon-20, registering a reduction of 0.75 ± 0.12 log CFU/g, while their reduction was 0.57 ± 0.12 log CFU/g, with treatment with Air −20, although differences were not statistically significant.

Many studies report that Gram-negative bacteria are more sensitive to plasma treatment than Gram-positive ones (Ermolaeva et al., 2011; Liang et al., 2012; Lunov et al., 2016; Yong et al., 2015; Ziuzina, Patil, Cullen, Keener, & Bourke, 2014), probably due to the different composition of the bacterial cell walls and cell membranes. Indeed, Gram-negative bacteria are characterized by a thin cell wall and with complex structures including peptidoglycans, lipopolysaccharides and phospholipids. In contrast, the cell wall of Gram-positive bacteria is thicker and more solid, composed of many layers of peptidoglycan and a unique structure composed mainly of the acids, which strengthens the cell wall and increases its mechanical strength (Zhang et al., 2019). Maximum microbial mortality for L. *innocua* was obtained with both atmospheres (Air-20 and Argon-20) after 20 min of plasma treatment but it was slightly lower than corresponding *E. coli* samples. Due to the slight mortality effects of plasma treatments, our results did not represent a robust confirmation of previous findings, but suggested a different efficacy of treatment duration on Gram-positive and Gram-negative bacteria.

3.5. Qualitative measurements

Moisture of untreated seabream fillets was 72.3 ± 0.9% and it was not modified by CAP treatment for the gas mixtures and treatment time considered (data not shown).

pH of untreated samples was 6.57 ± 0.01, and significantly decreased after all treatments, as shown in Fig. 4. This effect was more evident when using the Argon gas mixture compared to the air one, and increasing treatment time from 10 to 20 min.

The lowest measured pH value was 6.34 ± 0.04 in the Argon-20 sample. Similar results were already observed by (Albertos et al., 2019; Giannoglou et al., 2021), and attributed to the formation of nitric acid (HNO_3) and nitrous acid (HNO_2) caused by the dissociation of H^+ during treatment. Although reduction of pH might lead to a lower water holding capacity (WHC) with a consequent higher drip loss, we speculate that it is not probable in our conditions since pH reduction was quite small (from 6.6 to 6.4) and the isoelectric point of fish protein is around 4.5–5.5. Nevertheless, the effect on drip loss should be further investigated during the shelf life.

Figs. 5 shows the colour parameters of luminosity (L*), red index (a*) and yellow index (b*), measured in the upper and lower sides of plasma treated fillets compared to the untreated sample. An increase of L* value was observed in all treated samples both on the upper and lower side of the fillets but was significant only for the longest treatment with both atmospheres (Air-20 and Argon-20 samples). The a* value was slightly reduced in lower side of the Air samples compared to the untreated ones, while other samples were unchanged. Similarly, the yellow index was mostly unchanged in both sides of the fillet.

Changes in colour after plasma exposure of fish fillets are widely reported and often associated with the oxidation of lipid and pigments, and with the denaturation of proteins (Rathod et al., 2021). However, the variation of colour parameters seems to be related to the considered fish species. An increase of colour lightness was also observed on seabream fillets subjected to plasma generated through a SDBD at 3 kV, 45 kHz for 15 min at 25 °C (Giannoglou et al., 2021). Similarly, Olatunde et al. (2019) observed an increased L* in sea bass fillets treated with high voltage CAP as treatment time increase.

On the contrary, (Albertos et al., 2019) and (Albertos et al., 2017) found a decrease in L* values in herring and mackerel respectively. The difference might be due to the type and concentration of pigment and haeme-protein in the tissue.

Fig. 6 shows the TBARS values measured in sea bream samples after cold plasma treatments compared to the control sample. Untreated sample showed a low value of 0.5 ± 0.2 mg MDA/kg, confirming the good oxidative status of the raw material, even after freezing and thawing. After exposure to CAP, TABRS concentrations significantly increased up to about 3.5–4.0 mg MDA/kg, without significant differences among samples. The enhancement of lipid oxidation has been observed in the majority of fish products subjected to CAP treatments (Gavahian et al., 2018). Indeed, compared to other food products, the presence of highly unsaturated fatty acids, renders fish products particularly susceptible to oxidation. (Pérez-Andrés et al., 2020) found no increase in lipid oxidation of mackerel fillets treated at 80 kV for 5 min, although an acceleration of carbonyl formation related to protein oxidation was observed. According to (Olatunde et al., 2019), both lipid and protein degradation were proportional to treatment time in Asian seabass slices.

The increase in lipid oxidation might decrease the sensorial quality of the product, that should be further evaluated by a sensorial analysis. A possible strategy to inhibit lipid oxidation, beside the optimization of processing, is the application of some antioxidant compounds before treatment (Olatunde, Benjakul, & Vongkamjan, 2020; Singh & Benjakul, 2020).

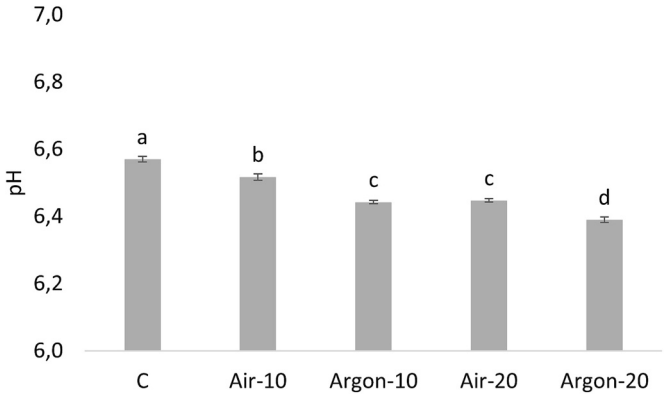

Fig. 4. pH values of seabream fillets treated with CAP compared to the untreated sample (C). Data are mean ± SD of 3 biological replicates in each condition. Statistical analysis was by the one-way ANOVA using Tukeys' as post-test and assuming $p < 0.05$ as significant. Different letters indicate statistical significance.

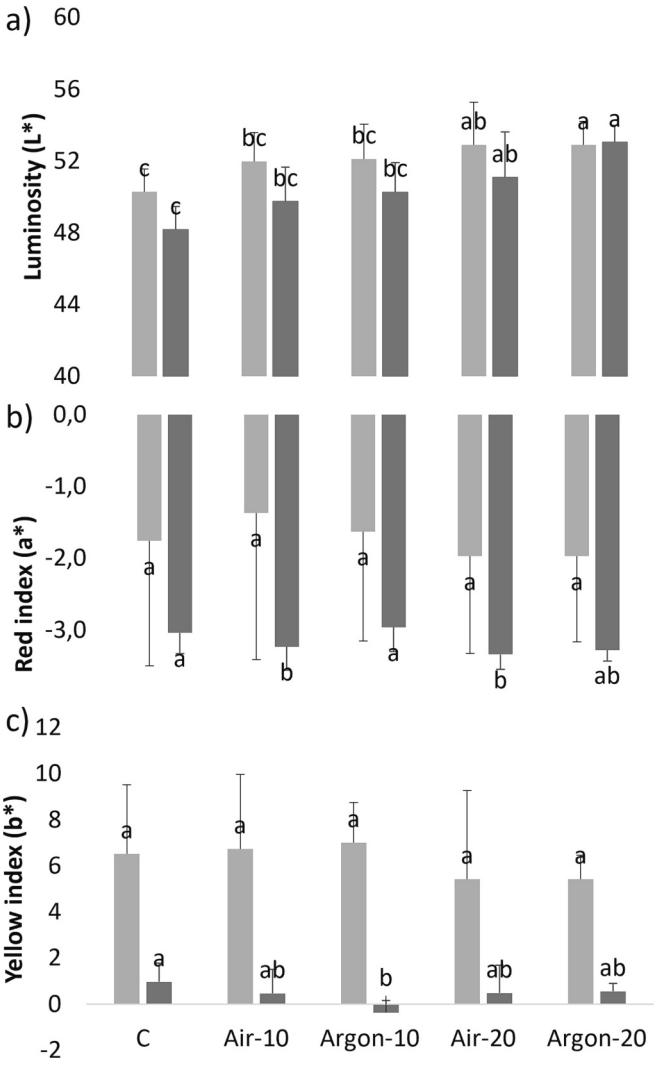

Fig. 5. Colour parameters of Lightness-L* (a), red index-a* (b) and yellow index -b* (c) measured in upper (light grey bars) and lower (dark grey bars) side of seabream fillets treated with CAP compared to the untreated sample (C). Data are mean ± SD of 3 biological replicates in each condition. Statistical analysis was by the one-way ANOVA using Tukeys' as post-test and assuming $p < 0.05$ as significant. Different letters indicate statistical significance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

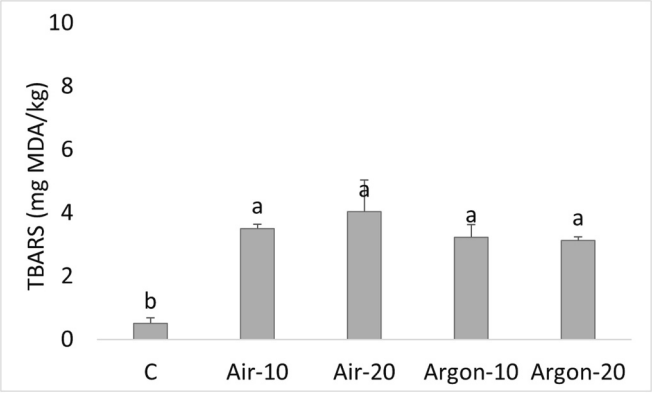

Fig. 6. TBARS values of seabream fillets treated with CAP compared to the untreated sample (C). Data are mean ± SD of 3 biological replicates in each condition. Statistical analysis was by the one-way ANOVA using Tukeys' as post-test and assuming $p < 0.05$ as significant. Different letters indicate statistical significance.

3.6. Fatty acid composition and relative release

Since the longest treatment time (20 min) was verified as the best one, the qualitative and quantitative FA composition was evaluated in the control, Air-20 and Argon-20 samples.

In not digested samples, the plasma treatment did not significantly affect the FA composition (Table 2). Large within-group variability was observed in the argon-treated fillets. Because it could mask significant differences between these and other samples, replicate technical analyses were performed on each biological sample, which confirmed within-group variability. Similar lipid content in control and treated samples was also confirmed using the official ISO 1443:1991 standard method (ISO, 1990).

Not only the quantity, but also the FA composition of the fats consumed is of great nutritional importance. In particular, fish products provide long-chain n-3 polyunsaturated fatty acids (n-3 LC-PUFA), the consumption of which is associated with a reduced risk of cardiovascular disease (Nestel et al., 2021). A more relevant picture of the nutritional value of foods can be obtained if one considers not only the composition of FAs but also their digestibility. Digestibility studies can provide insight into the bioaccessibility of FAs, i.e., the percentage of ingested FAs that is released from the food matrix during digestion and is available for absorption (Fernández-García, Carvajal-Lérida, & Pérez-Gálvez, 2009). Therefore, the FA content and composition of the sea bream fillets were re-evaluated after the in vitro digestion. As in the undigested samples, regardless of treatment no differences in fatty acid composition were detected between control and treated samples (data not shown).

Since the bioaccessibility of specific FAs could be affected by the food matrix and by processing, we evaluated total and single FA digestibility and referred it as relative release (RR), as suggested by (Tormási & Abrankó, 2021). The RR was calculated as mg FAMEs in digested sample/mg FAMEs in corresponding not digested samples *100, and it is reported in Table 3.

The RR of single and total FAs was similar in all conditions and in most cases, it was >100. It was already reported that in vitro digestion can be more comprehensive than chemical extraction (Danesi et al., 2020), and our results suggest that the use of chemical extraction for the assessment of the FA content of certain food could lead to an underestimation. Although this hypothesis deserves further attention, for the purpose of this study the important result was the absence of significant differences in the RR of FA between the control and treated fillets.

3.7. Protein hydrolysis during digestion

In not digested samples, the protein content was similar in control and treated samples (control: 20.58 ± 0.28 g/100 g; treated: 20.46 ± 0.19 g/100 g, n.s.). Protein hydrolysis during digestion was evaluated measuring the concentration of amino acids/peptides in digested samples by two different methods having a different ability to detect protein fragments with different molecular mass (Urbinati et al., 2021). Regardless of the method used, no significant differences were found between the control and treated fillets (Table 4).

To further investigate on the impact of CAP treatment on protein hydrolysis after digestion, nuclear magnetic resonance (NMR) spectroscopy has been applied to the same samples analysed with the spectrophotometric and the OPA assays. The advantage of using this further technique is associated to its universal detection capability, without the requirement of an external standard to calculate an instrumental response factor, provided that the molecules under investigation contain at least one atom of hydrogen and are soluble in the solvent of the

Table 2
Fatty acid composition (as FAME) of not-digested fillets (T0).

	Control	Air-20	Argon-20
14:0	121.04 ± 14.01[a]	90.74027 ± 8.49[a]	158.25 ± 124.32[a]
16:0	586.00 ± 61.00[a]	446.92 ± 32.18[a]	729.84 ± 516.08[a]
16:1 n-7	191.05 ± 18.13[a]	146.52 ± 27.14[a]	239.99 ± 183.77[a]
18:0	117.74 ± 11.77[a]	86.35 ± 3.17[a]	143.87 ± 98.21[a]
18:1 n-9	1046.92 ± 120.48[a]	784.42 ± 109.14[a]	1358.82 ± 1026.06[a]
18:2 n-6	382.51 ± 41.61[a]	295.09 ± 32.43[a]	495.95 ± 361.31[a]
18:3 n-3	92.02 ± 10.09[a]	71.11 ± 9.93[a]	121.77 ± 91.35[a]
20:1 n-9	149.86 ± 16.72[a]	112.67 ± 11.29[a]	192.79 ± 147.31[a]
20:4	28.68 ± 2.55[a]	24.40 ± 2.89[a]	36.07 ± 20.9[a]
20:5 n-3	170.80 ± 13.96[a]	132.19 ± 13.20[a]	205.19 ± 127.09[a]
22:5 n-3	85.41 ± 8.12[a]	69.60 ± 8.88[a]	108.46 ± 70.40[a]
22:6 n-3	364.45 ± 33.17[a]	303.96 ± 26.21[a]	441.62 ± 245.80[a]
ΣSFA	824.78 ± 86.36[a]	624.01 ± 42.93[a]	1031.96 ± 738.43[a]
ΣMUFA	1387.83 ± 155.11[a]	1043.61 ± 146.40[a]	1791.60 ± 1357.12[a]
ΣPUFA	1123.87 ± 107.39[a]	896.36 ± 92.26[a]	1409.06 ± 916.85[a]
ΣPUFA n-3	712.69 ± 63.81[a]	576.87 ± 57.35[a]	877.03 ± 534.59[a]
ΣPUFA n-6	411.19 ± 44.14[a]	319.49 ± 35.30[a]	532.02 ± 382.26[a]
Σ n-6/Σ n-3	0.57 ± 0.02[a]	0.55 ± 0.01[a]	0.56 ± 0.11[a]
Total	3336.49 ± 348.00[a]	2563.98 ± 280.73[a]	4232.61 ± 3011.88[a]

Data are expressed as mg FAME/100 g sample and are mean ± SD of 3 biological replicates in each condition. Statistical analysis was by the one-way ANOVA using Tukeys' as post-test and assuming $p < 0.05$ as significant. Different letters indicate statistical significance. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 3
Relative release of fatty acids after in vitro digestion.

	Control	Air-20	Argon-20
14:0	136.24 ± 46.84[a]	117.35 ± 15.68[a]	132.18 ± 60.09[a]
16:0	126.88 ± 39.77[a]	104.32 ± 18.14[a]	116.07 ± 36.90[a]
16:1 n-7	139.30 ± 42.09[a]	125.77 ± 10.78[a]	130.40 ± 51.11[a]
18:0	104.22 ± 34.43[a]	58.67 ± 24.99[a]	95.46 ± 27.44[a]
18:1 n-9	137.35 ± 37.12[a]	123.57 ± 15.05[a]	124.34 ± 47.85[a]
18:2 n-6	132.19 ± 36.92[a]	119.94 ± 14.85[a]	117.77 ± 40.69[a]
18:3 n-3	138.41 ± 36.71[a]	120.21 ± 13.08[a]	122.32 ± 48.32[a]
20:1 n-9	139.61 ± 39.45[a]	131.21 ± 15.73[a]	132.74 ± 57.12[a]
20:4	115.48 ± 28.30[a]	98.87 ± 11.31[a]	93.29 ± 14.03[a]
20:5 n-3	112.51 ± 28.03[a]	101.32 ± 11.33[a]	94.31 ± 17.40[a]
22:5 n-3	132.02 ± 35.52[a]	120.93 ± 12.41[a]	116.25 ± 35.85[a]
22:6 n-3	113.93 ± 28.67[a]	108.90 ± 16.21[a]	98.88 ± 16.70[a]
ΣSFA	125.01 ± 40.00[a]	99.86 ± 19.02[a]	114.87 ± 37.67[a]
ΣMUFA	137.85 ± 38.04[a]	124.68 ± 14.56[a]	126.01 ± 49.19[a]
ΣPUFA	123.39 ± 32.74[a]	112.97 ± 14.40[a]	106.35 ± 26.41[a]
ΣPUFA n-3	118.95 ± 30.52[a]	109.99 ± 14.29[a]	102.03 ± 21.59[a]
ΣPUFA n-6	131.03 ± 36.34[a]	118.34 ± 14.58[a]	115.49 ± 37.77[a]
Total	129.81 ± 36.78[a]	114.50 ± 15.64[a]	115.89 ± 37.32[a]

Data are mean ± SD of 3 biological replicates in each condition. Statistical analysis was by the one-way ANOVA using Tukeys' as post-test and assuming $p < 0.05$ as significant. Different letters indicate statistical significance. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 4
Protein release after in vitro digestion.

	Control	Air-20	Argon-20
A 280 nm	13.32 ± 2.25[a]	12.33 ± 1.73[a]	14.16 ± 1.97[a]
OPA	15.32 ± 1.77[a]	15.28 ± 2.15[a]	14.73 ± 2.34[a]

Data are mean ± SD of 3 biological replicates in each condition and are expressed as g protein in 100 g digested sample. Statistical analysis was by the one-way ANOVA using Tukeys' as post-test and assuming $p < 0.05$ as significant. Different letters indicate statistical significance.

sample. All molecules released during digestion satisfy these requirements, including amino acids, peptides, and larger soluble fragments of proteins. Thus, the area of diagnostic signals in specific regions of the NMR spectrum is directly proportional to the concentration of hydrogen atoms belonging to the molecule to be quantified (either single amino acids, short peptides, small or large protein fragments). As only the soluble molecules are detected, the NMR technique provides the condition necessary to evaluate the accessibility of nutrients upon digestion.

Five diagnostic regions were examined in the NMR spectra of digestates, each of which represented a specific category of hydrolysis products: i) the hydrophobic amino acid region collecting the signals generated by the hydrogen atoms belonging to alanine, valine, leucine and isoleucine; ii) the hydrophilic amino acid region collecting the signals generated by serine, cysteine and threonine; iii) the spectral region collecting the hydrogen atoms in the alpha position, which is directly related to the total amount of amino acids in the digestion fluid, as they all contain this specific atom; iv) the region of aromatic amino acids collecting signals from phenylalanine, tyrosine, histidine and tryptophan. These four regions provide information of the amino acid composition of the oligopeptides and small fragments released during digestion. The fifth region collected the signals belonging to peptide hydrogen atoms that were not accessible to water because they were encumbered in a larger protein fragment made soluble by detachment from the insoluble myofibrillar protein. As shown in Table 5, the only difference emerging from NMR spectral data was an overall 10% reduction of protein hydrolysis in Air-20 fillets compared to Argon-20 and controls. Since the lowering is of the same extent in all NMR spectral regions, it is argued that the digestion profile is not affected by the treatment. Since this phenomenon does not reproduce in argon-plasma treated samples, further investigations are needed to exclude that the underestimation of digestibility is notably due to a different fillet composition which is reflected in a lower amount of protein to be digested.

4. Conclusions

Our study clearly showed that the use of CAP in a closed chamber decreases the bacterial load in fresh seabream fillets. Interestingly, the effect was strongly dependent on treatment time, while no differences were found using argon or nitrogen gas mixtures. The quality of fillets was slightly affected by the CAP treatment, which had no effect on FA bioaccessibility and protein hydrolysis after in vitro digestion.

These data highlight the possibility of sustaining the plasma treatment in a nitrogen/oxygen mixture, that results more efficient on the economic point of view if compared to the argon/oxygen mixture, since it can be sustained avoiding the use of noble gases. Future steps will be devoted at the evaluation of the effect of CAP treatment generate in ambient air, in order to evaluate a more realistic and applicable approach in the food sanitation, and to protect the product from lipid oxidation tailoring the use of natural antioxidant treatments.

Author contributions

Conceptualization, F·C. and P.R.; Methodology, S.T.; R.L., G.P., and E.C.; Validation, F·Ca and R.L.; Investigation, S.T, L.N, F·C, G.A., G.P.,C. M and E.C.; Data curation, S.T, F·C., F.Ca.; Writing – original draft, S.T. and L.N, G.A., R.L.; Writing – review & editing, P.R., A.G., A.B., F.C and Å.E.; Supervision, P.R., A.G., F.C., M.G. and A.B.; Funding acquisition, Å. E, F.C. All authors have read and agreed to the published version of the manuscript.

Table 5
Relative concentrations, assessed by NMR spectroscopy, of molecular species released by in vitro digestion of fillets proteins, classified according to the spectral regions where signals resonate.

	Control	Air-20	Argon-20
Hydrophobic Amino Acids Region (0.20–2.00 ppm)	104.45 ± 2.60 [a]	94.62 ± 5.15 [b]	106.18 ± 6.48 [a]
Hydrophilic Amino Acids Region (2.00–3.00 ppm)	36.90 ± 0.76 [a]	33.13 ± 1.85 [b]	37.41 ± 0.71 [a]
Total Amino Acids (α-CH) Region (3.20–4.70 ppm)	76.70 ± 2.04 [a]	69.41 ± 3.72 [b]	77.39 ± 1.95
Aromatic Amino Acids Region (6.40–7.70 ppm)	9.82 ± 0.18 [a]	9.15 ± 0.37 [b]	9.74 ± 0.31 [a]
Total Soluble Proteins Region (7.70–9.60 ppm)	4.40 ± 0.16[a]	3.89 ± 0.21[b]	4.31 ± 0.33 [a]

Data are mean ± SD of 3 biological replicates in each condition and are expressed as arbitrary integral units/5 g of digested sample/100 g. Statistical analysis was by the one-way ANOVA using Tukeys' as post-test and assuming $p < 0.05$ as significant. Different letters indicate statistical significance.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

References

Albertos, I., Martin-Diana, A. B., Cullen, P. J., Tiwari, B. K., Ojha, K. S., Bourke, P., & Rico, D. (2019). Shelf-life extension of herring (*Clupea harengus*) using in-package atmospheric plasma technology. *Innovative Food Science and Emerging Technologies, 53*(June 2017), 85–91. https://doi.org/10.1016/j.ifset.2017.09.010

Albertos, I., Martín-Diana, A. B., Cullen, P. J., Tiwari, B. K., Ojha, S. K., Bourke, P., … Rico, D. (2017). Effects of dielectric barrier discharge (DBD) generated plasma on microbial reduction and quality parameters of fresh mackerel (*Scomber scombrus*) fillets. *Innovative Food Science and Emerging Technologies, 44*(July), 117–122. https://doi.org/10.1016/j.ifset.2017.07.006

Bao, Y., & Ertbjerg, P. (2015). Relationship between oxygen concentration, shear force and protein oxidation in modified atmosphere packaged pork. *Meat Science, 110*, 174–179. https://doi.org/10.1016/j.meatsci.2015.07.022

Bartis, E. A. J., Graves, D. B., Seog, J., & Oehrlein, G. S. (2013). Atmospheric pressure plasma treatment of lipopolysaccharide in a controlled environment. *Journal of Physics D: Applied Physics, 46*(31). https://doi.org/10.1088/0022-3727/46/31/312002

Benedict, R. C. (1987). Determination of nitrogen and protein content of meat and meat products. *Journal of the Association of Official Analytical Chemists, 70*(1), 69–74.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911–917.

Capelli, F., Tappi, S., Gritti, T., De Aguiar Saldanha Pinheiro, A. C., Laurita, R., Tylewicz, U., … Rocculi, P. (2021). Decontamination of food packages from SARS-COV-2 RNA with a cold plasma-assisted system. *Applied Sciences (Switzerland), 11*(9). https://doi.org/10.3390/app11094177

CIE. (1987). *Colorimetry*. In *Central Bureaux of the Commission* (2nd ed.). Wien, A: Internationale de l'Eclaraige.

Danesi, F., Calani, L., Valli, V., Bresciani, L., Del Rio, D., & Bordoni, A. (2020). (poly) phenolic content and profile and antioxidant capacity of whole-grain cookies are better estimated by simulated digestion than chemical extraction. *Molecules, 25*(12), 1–11. https://doi.org/10.3390/molecules25122792

Di Nunzio, M., Loffi, C., Chiarello, E., Dellafiora, L., Picone, G., Antonelli, G., … Bordoni, A. (2022). Impact of a shorter brine soaking time on nutrient bioaccessibility and peptide formation in 30-months-ripened Parmigiano Reggiano cheese. *Molecules, 27*(3). https://doi.org/10.3390/molecules27030664

Eliasson, B., & Kogelschatz, U. (2023). Ozone: Science & Engineering: The journal of the international ozone association ozone generation with narrow – Band UV radiation. *March 2013*, 365–373.

Ermolaeva, S. A., Varfolomeev, A. F., Chernukha, M. Y., Yurov, D. S., Vasiliev, M. M., Kaminskaya, A. A., … Gintsburg, A. L. (2011). Bactericidal effects of non-thermal argon plasma in vitro, in biofilms and in the animal model of infected wounds. *Journal of Medical Microbiology, 60*(1), 75–83. https://doi.org/10.1099/jmm.0.020263-0

Fernández-García, E., Carvajal-Lérida, I., & Pérez-Gálvez, A. (2009). In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. *Nutrition Research, 29*(11), 751–760. https://doi.org/10.1016/j.nutres.2009.09.016

Gavahian, M., Chu, Y.-H., Mousavi Khaneghah, A., Barba, F. J., & Misra, N. N. (2018). A critical analysis of the cold plasma induced lipid oxidation in foods. *Trends in Food Science and Technology, 77*, 32–41. https://doi.org/10.1016/j.tifs.2018.04.009

Giannoglou, M., Panagiotis, D., Efthimiadou, A., Gogolides, E., & Katsaros, G. (2021). Comparative study on the effect of cold atmospheric plasma, ozonation, pulsed electromagnetic fields and high-pressure technologies on sea bream fillet quality indices and shelf life. *Food Engineering Reviews, 13*, 175–184.

ISO. (1990). Meat and meat products. Determination of total fat content. In *Norm UNI ISO 1443:1991*. International Organization for Standardization.

Liang, Y., Wu, Y., Sun, K., Chen, Q., Shen, F., Zhang, J., Yao, M., Zhu, T., & Fang, J. (2012). Rapid inactivation of biological species in the air using atmospheric pressure nonthermal plasma. *Environmental Science & Technology, 46*(6), 3360–3368. https://doi.org/10.1021/es203770q

Lunov, O., Zablotskii, V., Churpita, O., Jäger, A., Polívka, L., Syková, E., Dejneka, A., & Kubinová, Š. (2016). The interplay between biological and physical scenarios of bacterial death induced by non-thermal plasma. *Biomaterials, 82*, 71–83. https://doi.org/10.1016/j.biomaterials.2015.12.027

Mai-Prochnow, A., Clauson, M., Hong, J., & Murphy, A. B. (2016). Gram positive and gram negative bacteria differ in their sensitivity to cold plasma. *Scientific Reports, 6* (December), 1–11. https://doi.org/10.1038/srep38610

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., … Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food–an international consensus. *Food & Function, 5*(6), 1113–1124.

Moiseev, T., Misra, N. N., Patil, S., Cullen, P. J., Bourke, P., Keener, K. M., & Mosnier, J. P. (2014). Post-discharge gas composition of a large-gap DBD in humid air by UV-vis absorption spectroscopy. *Plasma Sources Science and Technology, 23*(6). https://doi.org/10.1088/0963-0252/23/6/065033

Nestel, P. J., Beilin, L. J., Clifton, P. M., Watts, G. F., & Mori, T. A. (2021). Practical guidance for food consumption to prevent cardiovascular disease. *Heart, Lung & Circulation, 30*(2), 163–179. https://doi.org/10.1016/j.hlc.2020.08.022

Olatunde, O. O., Benjakul, S., & Vongkamjan, K. (2019). High voltage cold atmospheric plasma: Antibacterial properties and its effect on quality of Asian sea bass slices. *Innovative Food Science and Emerging Technologies, 52*(October 2018), 305–312. https://doi.org/10.1016/j.ifset.2019.01.011

Olatunde, O. O., Benjakul, S., & Vongkamjan, K. (2020). Cold plasma combined with liposomal ethanolic coconut husk extract: A potential hurdle technology for shelf-life extension of Asian sea bass slices packaged under modified atmosphere. *Innovative Food Science and Emerging Technologies, 65*(March), Article 102448. https://doi.org/10.1016/j.ifset.2020.102448

Pérez-Andrés, J. M., de Alba, M., Harrison, S. M., Brunton, N. P., Cullen, P. J., & Tiwari, B. K. (2020). Effects of cold atmospheric plasma on mackerel lipid and protein oxidation during storage. *Lwt, 118*(September 2019), Article 108697. https://doi.org/10.1016/j.lwt.2019.108697

Rathod, N. B., Ranveer, R. C., Bhagwat, P. K., Ozogul, F., Benjakul, S., Pillai, S., & Annapure, U. S. (2021). Cold plasma for the preservation of aquatic food products: An overview. *Comprehensive Reviews in Food Science and Food Safety, 20*(5), 4407–4425. https://doi.org/10.1111/1541-4337.12815

Rød, S. K., Hansen, F., Leipold, F., & Knøchel, S. (2012). Cold atmospheric pressure plasma treatment of ready-to-eat meat: Inactivation of *Listeria innocua* and changes in product quality. *Food Microbiology, 30*(1), 233–238. https://doi.org/10.1016/j.fm.2011.12.018

Shimizu, T., Sakiyama, Y., Graves, D. B., Zimmermann, J. L., & Morfill, G. E. (2012). The dynamics of ozone generation and mode transition in air surface micro-discharge plasma at atmospheric pressure. *New Journal of Physics, 14*. https://doi.org/10.1088/1367-2630/14/10/103028

Simoncelli, E., Schulpen, J., Barletta, F., Laurita, R., Colombo, V., Nikiforov, A., & Gherardi, M. (2019a). UV-VIS optical spectroscopy investigation on the kinetics of long-lived RONS produced by a surface DBD plasma source. *Plasma Sources Science and Technology, 28*(9). https://doi.org/10.1088/1361-6595/ab3c36

Simoncelli, E., Schulpen, J., Barletta, F., Laurita, R., Colombo, V., Nikiforov, A., & Gherardi, M. (2019b). UV-VIS optical spectroscopy investigation on the kinetics of long-lived RONS produced by a surface DBD plasma source. *Plasma Sources Science and Technology, 28*(9). https://doi.org/10.1088/1361-6595/ab3c36

Singh, A., & Benjakul, S. (2020). The combined effect of squid pen chitooligosaccharides and high voltage cold atmospheric plasma on the shelf-life extension of Asian sea bass slices stored at 4 °C. *Innovative Food Science and Emerging Technologies, 64* (February), Article 102339. https://doi.org/10.1016/j.ifset.2020.102339

Thirumdas, R., Sarangapani, C., & Annapure, U. S. (2014). Cold plasma: A novel non-thermal technology for food processing. In *, 10. Food biophysics*. https://doi.org/10.1007/s11483-014-9382-z. issue 1.

Tormási, J., & Abrankó, L. (2021). Assessment of fatty acid-specific lipolysis by in vitro digestion and gc-fid. *Nutrients, 13*(11). https://doi.org/10.3390/nu13113889

Urbinati, E., di Nunzio, M., Picone, G., Chiarello, E., Bordoni, A., & Capozzi, F. (2021). The effect of balsamic vinegar dressing on protein and carbohydrate digestibility is dependent on the food matrix. *Foods, 10*(2), 1–14. https://doi.org/10.3390/foods10020411

Yong, H. I., Kim, H. J., Park, S., Alahakoon, A. U., Kim, K., Choe, W., & Jo, C. (2015). Evaluation of pathogen inactivation on sliced cheese induced by encapsulated atmospheric pressure dielectric barrier discharge plasma. *Food Microbiology, 46*, 46–50. https://doi.org/10.1016/j.fm.2014.07.010

Zhang, Y., Wei, J., Yuan, Y., Chen, H., Dai, L., Wang, X., & Yue, T. (2019). Bactericidal effect of cold plasma on microbiota of commercial fish balls. *Innovative Food Science and Emerging Technologies, 52*(December 2018), 394–405. https://doi.org/10.1016/j.ifset.2019.01.019

Zhao, Y. M., de Alba, M., Sun, D. W., & Tiwari, B. (2019). Principles and recent applications of novel non-thermal processing technologies for the fish industry—A review. *Critical Reviews in Food Science and Nutrition, 59*(5), 728–742. https://doi.org/10.1080/10408398.2018.1495613

Ziuzina, D., Patil, S., Cullen, P. J., Keener, K. M., & Bourke, P. (2014). Atmospheric cold plasma inactivation of *Escherichia coli*, *Salmonella enterica* serovar typhimurium and *Listeria monocytogenes* inoculated on fresh produce. *Food Microbiology, 42*, 109–116. https://doi.org/10.1016/j.fm.2014.02.007