Effects of novel modified atmosphere packaging on lipid quality and stability of sardine (Sardina pilchardus) fillets

International Journal of Food Science +Technology

Institute of Food Science +Technology

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Journal:	*International Journal of Food Science and Technology*
Manuscript ID	IJFST-2020-29507.R2
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	n/a
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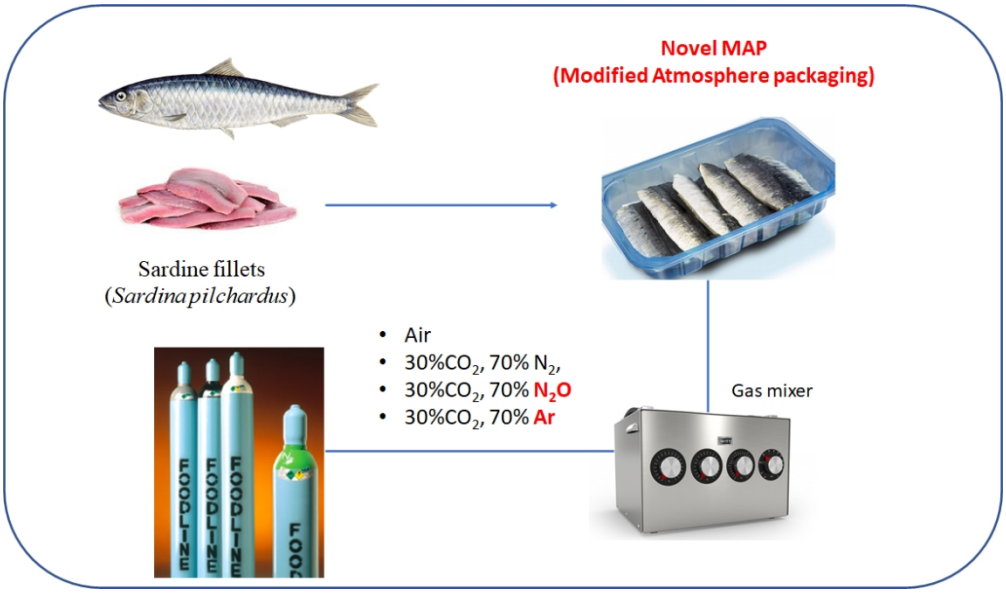
Keywords:	Sardine, Modified Atmosphere Packaging (MAP), argon, nitrous oxide, Lipid Oxidation



Graphical abstract

309x181mm (96 x 96 DPI)

Effects of novel modified atmosphere packaging on lipid quality and stability of sardine (*Sardina pilchardus*) fillets

Running title: Novel modified atmosphere packaging for fish

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Abstract

Modified atmosphere packaging (MAP) is an efficient method to increase shelf-life of fishery products by inhibiting bacterial growth and oxidative reactions. Beside the traditional gases used for MAP, novel gases such as argon (Ar) and nitrous oxide (N_2O) were approved for food use in the European Union. The present research investigates the effect of MAP with unconventional gas mixtures, that previously positively affected microbial shelf-life, on colour, lipid oxidation and sensorial characteristics of sardine fillets during storage. Four atmosphere conditions were tested: Air (20.8% O_2/79.2% N_2), N_2 (30% CO_2/70% N_2), N_2O (30% CO_2/70% N_2O) and Ar (30% CO_2/70% Ar). Samples were stored for 12 days at 3 °C. Results showed that the removal of oxygen significantly inhibited the oxidation process; however, most of the investigated parameters related to fat oxidation

did not show any improvement, except for a slight decrease in lipid hydrolysis and improvement in sensory properties in the packaging containing Ar.

Keywords:

Sardine; modified atmosphere packaging; argon; nitrous oxide; oxidation

1. Introduction

Seafood products play an important role in a nutritionally balanced diet, however, fresh products are highly perishable due to endogenous enzymes and metabolic activities of microorganisms (Sivertsvik et al. 2002). One of the major deteriorative process that occurs during the storage of fish and which has a major influence on quality is lipid oxidation. Lipid oxidation may affect both odour and flavour of fish and, in severe cases, its nutritional value (Pacetti et al. 2015). Fish and shellfish are excellent sources of polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA, 20:5v3) and docosahexaenoic acid (DHA, 22:6v3). PUFAs are known to be markedly susceptible to peroxidation and to be readily incorporated into the mechanism of lipid peroxidation to yield free radicals and lipid peroxy radicals. Therefore, their protection against oxidation by use of novel packaging technologies as well as storage conditions is essential.

Modified atmosphere packaging (MAP) can extend the shelf-life of most fishery products by inhibiting bacterial growth and oxidative reactions. The achievable extension of shelf-life depends on species, fat content, initial microbial population, gas mixture, the ratio of gas volume to product volume, and storage temperature (Sivertsvik et al. 2002). Traditional MAP uses different concentrations of O_2, CO_2 and N_2, generally removing or reducing oxygen and increasing the concentration of CO_2 (Erkan et al. 2006), while N_2 is used as filler gas. In fish products, the aim is to inhibit lipid oxidation and microbial growth.

However, since Directive 95/2/CE and further amendments the EU has also approved argon (Ar) and nitrous oxide (N_2O) for food use. They are generally used to replace N_2, an inert gas, in packaging, hence as fillers. However, both Ar and N_2O were observed to increase the sensitivity of different microorganisms to anti-microbial compounds (Qadir and Hashinaga 2001). Moreover, some reports indicate that Ar is able to interfere with receptor sites of enzymatic oxygen (Spencer and Humphreys 2003; Rocculi et al. 2005). Choubert et al. (1988) and Randell et al. (1977) found contrasting results regarding the increase of shelf-life due to the use of Ar in rainbow trout and herring fillets packaging. On the other side, positive effects of N_2O have been reported to mainly for fruit packaging, on account of its ability to partially inhibit respiration and senescence (Rocculi et al. 2005).

Sardine (*Sardina pilchardus*) is an important Mediterranean commercial fish species; its high fat content makes it very susceptible to oxidation. In a previous study of our group, we investigated the effect of Ar and N_2O in MAP of sardine fillets on microbiological quality and freshness indicators during storage. Results indicated that Ar allowed to inhibit bacterial spoilage and freshness decay, increasing the shelf-life of the product (Pinheiro et al. 2019).

The present research is focused on the evaluation of the effect of MAP with unconventional gas mixtures (Ar and N_2O) on the colour and different lipid oxidation indicators of sardine fillets during refrigerated storage.

2.Material and methods

2.1 Fish Samples

Sardines (*Sardina pilchardus*), fished in the Adriatic Sea (Cesenatico, Italy), were mechanically filleted (8.5 ± 0.5 cm average length and 10 ± 1 g average weight). A total of 35 kg were packed in different MAP conditions.

2.2 Modified atmosphere packaging (MAP)

250 g of fillets were placed in polypropylene trays and sealed with a PET/PP high barrier film (permeability to O_2 <123.2 cm^3/m^2 day bar and water vapor transmission rate, WVTR< 4.8 g/m^2 day) with a volume ratio product:gas of 1:1. A gas quaternary mixer mod. KM100-4 (Witt-100 Gasetechnik, Witten, Germany) combined to a gas-flushing welding machine mod. Multiple 315 (Orved Srl, Venezia, Italy) was used to obtain different combinations of selected gases: Air (20.8% O_2 + 79.2% N_2), N_2 (30% CO_2 + 70% N_2), N_2O (30% CO_2 + 70% N_2O) and Ar (30% CO_2 + 70% Ar). For each atmosphere, 24 packages were prepared and stored at 3 ± 1°C for 12 days. For the analytical determinations, 4 trays for each MAP condition were collected after 0, 1, 2, 5, 6, 8, 12 days of storage. Sampling times were chosen to be representative of the shelf life of the fillets according to previous experiments.

2.3 Physico-chemical analyses

pH values were assessed in samples homogenized with distilled water (1:2 sample:water ratio) with a pH meter (Crison, Barcellona).

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, 1976) was used. The hue angle (H^0) was calculated as follow:

$$H = \arctan {}^{b*}/_{a*}.$$

For each sample and storage time, the average of at least 15 measurements was calculated.

2.4 Lipid Oxidation indexes

2.4.1 Total Lipid extraction

Lipids were extracted with a method previously described by Bligh and Dyer (1959) according to the modification of Vernocchi et al. (2007).

Peroxide value (PV), conjugated diene (CD) and trienes (CT) measurements were used for the determination of primary lipid oxidation. Thiobarbituric acid reactive substances (TBARS) was used to determine the secondary oxidative products and lipid hydrolysis was measured by free fatty acids (FFA) contents. Moreover, fatty acid composition was determined by gas chromatography (GC).

2.4.2 Peroxide value (PV)

The value of PV was determined by the ferrothiocyanate method (Chapman and McKay 1949). Results were expressed as millimoles of O_2 per kg of lipid.

2.4.3 Conjugated diene (CD) and triene (CT) hydroperoxides

CD and CT were measured according to the method of Abdalla and Roozen (1999). Results were expressed in terms of absorbance (232 nm and 268 nm for dienes and trienes respectively) relative to 50 mg of fat. The measurement was performed in duplicate for each extract.

2.4.4 Thiobarbituric acid reactive substances (TBARS)

The 2-thiobarbituric acid-reactive substances (TBARS) were measured according to the method described by Bao and Ertbjerg (2015). Results were expressed as mg of malondialdehyde (MA) per kg of fillet, calculated using a standard curve of 1,1,3,3-tetraethoxypropane.

2.4.5 Free fatty acid (FFA)

FFA content was determined using the method of Lowry and Tinsley (1976) modified according to Bernárdez et al. (2005). The absorbance was read at 710 nm and the amount of FFA determined, using a standard curve prepared from oleic acid. Results were expressed as g oleic acid/100 g lipids.

2.4.6 Fatty acid composition

Lipid purification and methyl ester synthesis

The lipid fractions were obtained using an aminopropyl bonded sorbent columns (SPE-NH2) ISOLUTE (Biotage, Milan, Italy). Columns were equilibrated with 9 mL of hexene and loaded with 20 mg of total lipid extract. FFAs fractions were recovered using 2% formic ethyl conveyed in diethyl ether. After purification samples were dried at room temperature under nitrogen flux and the methyl ester synthesis was carried out using diazomethane. Fatty acids methyl esters were dried under nitrogen flux, resuspended into hexane and stored at -40°C until the GC-MS analysis.

GC–Mass Spectrometry analysis and fatty acid identification

GC–MS analysis were carried out on an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) coupled to an Agilent 5970 mass selective detector operating in electron impact mode (ionization voltage, 70 eV). A Chrompack CP-Wax 52 CB capillary column (50 m length, 0.32 mm i.d., 1.2 μm df) was used (Chrompack, Middelburg, The Netherlands). The temperature program was 50 °C for 2 min, then programmed at 1 °C min^{-1} to 65 °C and finally at 5 °C min^{-1} to 220 °C, which was maintained for 22 min. Injector, interface, and ion source temperatures were 250, 250, and 230 °C, respectively. Injections were performed in triplicate with a split ratio of 1:30 and helium (1 mL min− 1) as the carrier gas.

FFAs methyl esters samples were prepared by adding 100 ppm of undecanoic acid methyl ester as internal standard. Injections were performed in triplicate. FFAs were identified using the NIST library. The amount of each FFA (mg/kg) were determined by the comparison of each peak area with the peak area of the internal standard (C11:0). The results are means of three independent experiments.

2.5 Sensory evaluation

Sensory evaluation was carried out according to the quality index method (QIM) described in detail by Stamatis and Arkoudelos (2007) for sardine fillets. The attributes examined were: (1) the development of slime on the surface of the fillet; (2) muscle incision and firmness; (3) odour; and (4) overall appearance of the fish fillet. Each assessment was carried out by a minimum of six trained

panellists with a long-term training and experience in fish evaluation. Four categories were ranked: highest quality or excellent (E), good quality (A), fair quality (B), and unacceptable quality (C).

2.6 Statistical analysis

Significant differences (p-level <0.05) between means at different storage times were explored by the analysis of variance (ANOVA with post-hoc Tukey HSD); Kruskal-Wallis test was used if significant differences emerged by the Levene's test.

3. Results and Discussion

3.1 Physico-chemical parameters

The initial pH values of sardine samples was 6.24 and, after 12 days of storage, a slight increase up to 6.52 was observed for all samples. Similar results were previously reported for sardine fillets (Erkan et al. 2006). However, the observed changes were not statistically significant (data not reported).

Changes in the L*, a* and H° colour parameters of sardine fillets packed in air and MAP during storage are shown in **Table 1**. During storage, L* (lightness) showed a slight decrease in all samples, but at the end, sardine fillets packaged in N_2O showed a significantly lower L* value compared to the N_2 and air samples ($p < 0.05$). On the contrary, a*(redness) values increased in all samples but after the fifth day until the end of storage, in fillets packed in N_2 and Ar, it was significantly higher compared to samples in air.

The hue angle (H°) decreased during storage showing differences among the tested atmospheres. From day 5 until day 8, hue values in Air samples were significantly higher than the MAP samples. However, at the end of the storage, no significant differences were observed among the different MAP samples ($p > 0.05$).

As shown by Thippareddi and Phebus (2002), the concentration of oxygen in the package determines the oxidative state of the myoglobin in fresh meats (**Fig. 1**). From a macroscopic point of view, this

phenomenon is evident considering the images of sardine fillets acquired in conditions of standardized illumination reported in **Fig. 2**. After 12 days of storage, while sample packed in air (b) evidenced a yellow/brown colour, the N_2, N_2O and Ar samples (c, d, e) evidenced an evident purple-red colour component, even more evident also respect to the fresh sample (a). The yellow-brown tint and the inhomogeneous distribution of colour in the surface of the sample packed in air is indicating an advanced state of oxidation. On the contrary, the absence of oxygen or an oxygen concentration very close to 0% promotes deoxymyoglobin (DMb) development, that is probably the reason for the increase of the redness observed in fillets packed in N_2, N_2O and Ar.

3.2 Lipid Oxidation indexes

Primary lipid oxidation was evaluated by PV. Evolution in the PV during storage is shown in **Fig. 3A**. At the beginning, the value was rather low (1.56 meq O_2/kg fat), indicating a good oxidative quality of the raw material. In the sample packed in air, the increase in PV began just on day one, following the typical trend of peroxide formation. At the end of the 12 days, PV was significantly greater (8.34 meq O_2/kg fat) in Air samples compared to the MAP samples, in which PV remained almost constant during storage with values that never exceeded 4 meq O_2/kg fat.

The low peroxide values in the MAP samples are probably due to the low concentration of this gas in the headspace of the packages. According to the classification reported by Pinheiro et al. (2019), samples in air could be considered 'spoiled' (PV=8-10) at the end of the storage, while the other samples were still 'good' (PV=2-5).

Fig. 3B and **3C**, shown the absorbance at 232 nm and 268 nm of the conjugable oxidation products, dienes and trienes respectively. For all samples, no difference in CD and CT of sardine fillets was observed within the first 8 days of storage ($p > 0.05$). Similarly, Chaijan et al. (2006) reported that in the sardine muscle (*Sardinella gibbosa*), no difference in CD was observed within the first 12 days of iced storage.

After day 8, the CD and CT values in the Air samples increased significantly ($p < 0.05$) compared to the MAP samples that instead remained almost constant. At the end of storage, the CD and CT values were highest in the Air samples compared with the MAP samples.

CD and CT are oxidation indexes for commonly used vegetable oils and for olive oils EU regulation (EC 796/2002) sets specific threshold values. However, for different oils and fats UV absorbance values should be evaluated individually due to their own chemical and physical characteristics. The CD and CT values in all MAP samples remained very low and did not increase with the storage time, indicating a protective effect of MAP on lipid oxidation.

According to Pinheiro et al. (2019), fish quality can be considered good until TBARS levels are below 5 mg malonaldehyde/kg, while the threshold for consumption is 8 mg malonaldehyde/kg.

TBARS values measured during storage of packed sardine fillets are reported in **Table 2**. Fresh samples showed values of about 0.33 mg MA/kg, similarly to values reported by other authors (Méndez et al. 2017), that increased during storage in all samples. However, for MAP samples values were far lower compared to sample packed in Air and were maintained low until the end of the storage. These results are in agreement with previous researches (Caglak et al. 2014; Messina et al. 2015). After 12 days, a value of 3.39 mg MA/kg was recorded for the Air sample, while in MAP samples the higher value was 1.09 mg MA/kg. Both values are lower than the reported critical values. Moreover, values are also lower compared to the ones reported by Erkan et al. (2006) for sardine fillets after 5 days of storage in two different MAP conditions. This difference may be explained by the fact that in the mentioned research the atmosphere composition included 5% of O_2 that probably allowed a faster lipid oxidation.

While Choubert et al. (2008) observed an improvement using Ar instead of N_2, that was attributed to its higher density that allows it to remove oxygen better than nitrogen, inhibiting the initiation and progression of oxidation, in the present research, although the three different tested MAP (containing N_2, N_2O and Ar) were effective in minimizing TBARS development, no significant difference ($p<0.05$) was observed among traditional and innovative gases.

Hydrolysis of ester bonds in lipids by enzymatic action or heating in the presence of water liberates FFAs (Bernárdez et al. 2005). In general, hydrolysis, induced by lipases and phospholipases, produces FFAs that can undergo further oxidation and production of low-molecular weight compounds responsible for the rancid off-flavour of fish products (Chaijan et al. 2006). Furthermore, FFAs and their oxidation products could impact muscle texture and functionality due to their ability to interact with myofibrillar proteins and to promote protein aggregation (Pacheco-Aguilar et al. 2000).

The evolution of lipid hydrolysis is presented in **Fig. 3D**. The initial fresh fillets value (0.09±0.01 g oleic acid/100g lipids) was similar to that obtained by Chaijan et al. (2006) for another sardine specie (*Sardinella gibbosa*). FFAs values for all samples increased slightly with storage time. In Ar samples, the higher value was observed at the day 8 (0.73 ± 0.22 g /100g lipids) followed by a decrease on day 12. At the end of the storage period, the samples packed on Ar gas mixture was lower than all samples. Chaijan et al. (2006) reported a high lipid hydrolysis (> 6 g FFA/100g lipid) in iced sardines at the end of the storage (15 days), probably because the authors used whole fish in which hydrolytic enzymes can derive also from internal organs. In this study, the highest FFAs value (0.96±0.44 g /100g lipids) was found on N_2 samples on day 12 and was similar to the values reported by Fagan et al. (2004) for mackerel and salmon fillets packed in MAP (60% N_2/40% CO_2) combined with freeze-chilling. However, no significant differences were found among Air, N_2 and N_2O samples throughout the storage period.

In order to understand the effect of the packaging atmosphere on the qualitative release of FFAs, gas-chromatographic analyses were performed, results are reported in **Table 3**. The data showed that the patterns of fat hydrolysis were different according to the atmosphere considered. The raw material was characterized by the release of mainly saturated FFAs such as C16:0, C18:0 and, to a minor extent, C14:1, respectively 92 and 8% of the total. After 8 days of storage, all samples were characterized by an increase of the lipolysis although slightly higher in the Ar samples with a major release of saturated and monosaturated fatty acids such including stearic (C18:0) and oleic (C18:1) ones. On the other hand, the sardine fillets packed in Ar, after 8 days of storage, presented high total

amount of polyunsaturated FFAs, related in particular to an increase content of 22:6 (n-3). However, this sample was characterized by the absence of C18:2n6c and C20:4n6, present both in the Air and N_2 samples. The highest amount of PUFA, after 8 days, was detected in Air samples, characterized also by the highest amount of TBARS (Table 2). The Air sample was characterized by the presence of C18:2n6c, C20:4n6, C20:5n3, 22:6 (n-3). Similar data were found by Chaijan et al. (2006) who found that the FFAs released in sardines were prone to oxidation as shown also by the marked increase in TBARS.

After 12 days of storage at 3 °C, the release of saturated and polyunsaturated fatty acids increased in all samples with the exception of the Ar sample that showed a slight decrease.

Regarding the monounsaturated fatty acids, for each considered sample, the total amounts found after 12 days was lower with respect the samples analysed at 8 d of storage.

Considering the relative quantities (%) of the three fractions on the total amount of FFA for each sample, it is possible to notice how in the Air sample, the release of PUFA was higher compared to the other samples at both considered sampling times, probably causing the higher oxidation level as shown by the previously considered indexes.

According to Choubert et al. (2008), the positive effect of replacing N_2 with Ar is related to its physical properties, particularly to its higher density, that allow for better inhibition of lipid oxidation. However, the authors did not specifically investigate lipid hydrolysis.

The accumulation of FFAs in fish muscle is mainly due to the enzymatic activity of lipase and phospholipase found in muscle, in digestive organs when present, but also deriving from microorganisms (Chaijan et al. 2006). In previous research, we showed that Ar was able to reduce microbial growth in sardine fillets compared to N_2, however, the effect on enzymatic activity was not investigated. It could be possible that the observed differences in FFAs release are related to an effect of enzymatic reactions in the fish tissue, however, specific research should be carried out to clarify this aspect.

3.3 Sensory evaluation

Samples packed in air reached the unacceptable condition (C) at the 5th day of storage (data reported in the supplementary material), while for samples packed in MAP the sensorial shelf-life was significantly improved. N_2 and N_2O samples were considered unacceptable at the 8th day, while sample packed in Ar maintained the "fair" score (B) until the end of the storage. These results are in agreement with previous data on microbial spoilage (Pinheiro et al., 2019), confirming that the use of Ar allowed to increase shelf life of sardine fillets because of improved microbiological status and sensorial characteristics.

4.Conclusions

As extensively known, the use of modified atmosphere in fish packaging with the removal of oxygen has confirmed to significantly inhibit the oxidation process during refrigerated storage.

Although previous studies have shown that Ar allowed to increase microbial shelf-life of sardine fillets most of the investigated parameters related to fat oxidation did not show any improvement during storage, except for a slight decrease in lipid hydrolysis in the packaging containing Ar. However, sensorial analysis showed that Ar sample was considered acceptable until the end of the storage.

Considering that the two investigated novel gases are at present more expensive compared to nitrogen, before a possible utilization at industrial level, a careful consideration of benefits versus costs should be carried out. Moreover, further studies are in due course in our lab in order to consider the reversibility of the visual quality improvement promoted by MAP after packaging opening, and in simulated preparation and consumption operations.

Conflict of interest

The authors declare no conflict of interest.

Data availability statement

Research data are not shared.

Acknowledgments

The authors acknowledge the financial support of the project "PRIZEFISH - Piloting of eco-innovative fishery supply-chains to market added-value Adriatic fish products" funded by the Call 2014 - 2020 Interreg V-A Italy - Croatia CBC Programme - Priority Axis: Blue innovation. Subsisdy Contract N° 10043583.

Ethical guidelines

Ethics approval was not required for this research

References

Abdalla, A. E., & Roozen, J. P. (1999). Effect of plant extracts on the oxidative stability of sunflower oil and emulsion. *Food chemistry*, **64**(3), 323-329.

Alasalvar, C. (2002). Seafoods: Quality, Technology and Nutraceutical Applications - an Overview. In C. Alasalvar, & T. Taylor (Eds.), Seafoods -Quality, *Technology and Nutraceutical Applications* (pp. 1-5). Berlin: Springer.

Aubourg, S. P., Sotelo, C. G., & Pérez-Martín, R. (1998). Assessment of quality changes in frozen sardine (Sardina pilchardus) by fluorescence detection. *Journal of the American Oil Chemists' Society*, **75**(5), 575-580.

Bao, Y., & Ertbjerg, P. (2015). Relationship between oxygen concentration, shear force and protein oxidation in modified atmosphere packaged pork. *Meat science,* **110**, 174-179.

Bernárdez, M., Pastoriza, L., Sampedro, G., Herrera, J. J., & Cabo, M. L. (2005). Modified method for the analysis of free fatty acids in fish. *Journal of agricultural and food chemistry*, **53**(6), 1903-1906.

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.

Bono, G., & Badalucco, C. (2012). Combining ozone and modified atmosphere packaging (MAP) to maximize shelf-life and quality of striped red mullet (Mullus surmuletus). *LWT-Food Science and Technology*, **47**(2), 500-504.

Caglak, E., Cakli, S., & Kilinc, B. (2014). Comparison chemical, sensory, microbiological and textural changes of cuttlefish (Sepia officinalis) stored under different packaging. *Bulgarian Journal of Agricultural Science*, **20**, 1046-1053.

Chaijan, M., Benjakul, S., Visessanguan, W., & Faustman, C. (2006). Changes of lipids in sardine (Sardinella gibbosa) muscle during iced storage. *Food Chemistry*, **99**(1), 83-91.

Chapman, R. A., & Mackay, K. (1949). The estimation of peroxides in fats and oils by the ferric thiocyanate method. *Journal of the American Oil Chemists' Society,* **26**(7), 360-363.

Choubert, G., Brisbarre, F., Parfouru, D., & Baccaunaud, M. (2008). Argon modified atmosphere packaging for fillets of rainbow trout (Oncorhynchus mykiss) fed astaxanthin or canthaxanthin. *Journal of Aquatic Food Product Technology,* **17**(2), 117-136.

Dalgaard, P., Gram, L., & Huss, H. H. (1993). Spoilage and shelf-life of cod fillets packed in vacuum or modified atmospheres. *International Journal of Food Microbiology*, **19**(4), 283-294.

Devlieghere, F., Debevere, J., & Van Impe, J. (1998). Concentration of carbon dioxide in the water-phase as a parameter to model the effect of a modified atmosphere on microorganisms. *International Journal of Food Microbiology*, **43**(1-2), 105-113.

Erkan, N., Özden, Ö., Alakavuk, D. Ü., Yildirim, Ş. Y., & İnuğur, M. (2006). Spoilage and shelf life of sardines (Sardina pilchardus) packed in modified atmosphere. *European Food Research and Technology*, **222**(5-6), 667.

Fagan, J. D., Gormley, T. R., & Mhuircheartaigh, M. U. (2004). Effect of modified atmosphere packaging with freeze-chilling on some quality parameters of raw whiting, mackerel and salmon portions. *Innovative Food Science & Emerging Technologies*, **5**(2), 205-214.

Hsieh, R. & Kinsella, J. (1989). Oxidation of polyunsaturated fatty acids: mechanisms, products and inhibition with emphasis on fish. *Advances in Food and Nutrition Research,* **33**, 233–341.

Losada, V., Barros-Velázquez, J., Gallardo, J. M., & Aubourg, S. P. (2004). Effect of advanced chilling methods on lipid damage during sardine (Sardina pilchardus) storage. *European Journal of Lipid Science and Technology*, **106**(12), 844-850.

Lowry, R. R., & Tinsley, I. J. (1976). Rapid colorimetric determination of free fatty acids. *Journal of the American Oil Chemists' Society,* **53**(7), 470-472.

Méndez, L., Fidalgo, L. G., Pazos, M., Lavilla, M., Torres, J. A., Saraiva, J. A., ... & Aubourg, S. P. (2017). Lipid and protein changes related to quality loss in frozen sardine (Sardina pilchardus) previously processed under high-pressure conditions. *Food and Bioprocess Technology*, **10**(2), 296-306.

Messina, C. M., Bono, G., Renda, G., La Barbera, L., & Santulli, A. (2015). Effect of natural antioxidants and modified atmosphere packaging in preventing lipid oxidation and increasing the shelf-life of common dolphinfish (Coryphaena hippurus) fillets. *LWT-Food Science and Technology*, **62**(1), 271-277.

Özogul, F., Polat, A., & Özogul, Y. (2004). The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (Sardina pilchardus). *Food Chemistry,* **85**(1), 49-57.

Pacetti, D., Lucci, P., Mozzon, M., Gagliardi, R., Fiorini, D., & Frega, N.G. (2015). Influence of deep-fat frying process on phospholipid molecular species composition of Sardina pilchardus fillet. *Food Control,* **48**, 155-162.

Pacheco-Aguilar, R., Lugo-Sánchez, M. E., & Robles-Burgueño, M. R. (2000). Postmortem biochemical and functional characteristic of Monterey sardine muscle stored at 0 C. *Journal of Food Science*, **65**(1), 40-47.

Pinheiro, A. C. D. A. S., Urbinati, E., Tappi, S., Picone, G., Patrignani, F., Lanciotti, R., ... & Rocculi, P. (2019). The impact of gas mixtures of Argon and Nitrous oxide (N2O) on quality parameters of sardine (Sardina pilchardus) fillets during refrigerated storage. *Food Research International*, **115**, 268-275.

Qadir, A., & Hashinaga, F. (2001). Inhibition of postharvest decay of fruits by nitrous oxide. *Postharvest biology and technology,* **22**(3), 279-283.

Randell, K., Hattula, T., & Ahvenainen, R. (1997). Effect of packaging method on the quality of rainbow trout and Baltic herring fillets. *LWT-Food Science and Technology,* **30**(1), 56-61.

Rocculi, P., Romani, S., & Dalla Rosa, M. (2005). Effect of MAP with argon and nitrous oxide on quality maintenance of minimally processed kiwifruit. *Postharvest Biology and Technology,* **35**(3), 319-328.

Sivertsvik, M., Jeksrud, W. K., & Rosnes, J. T. (2002). A review of modified atmosphere packaging of fish and fishery products–significance of microbial growth, activities and safety. *International Journal of Food Science & Technology,* **37**(2), 107-127.

Sivertsvik, M., Jeksrud, W. K., Vågane, Å., & Rosnes, J. T. (2004). Solubility and absorption rate of carbon dioxide into non-respiring foods: Part 1: Development and validation of experimental apparatus using a manometric method. *Journal of Food Engineering*, **61**(3), 449-458.

Spencer, K. C., & Humphreys, D. J. (2003). Argon packaging and processing preserves and enhances flavor, freshness, and shelf life of foods. *Freshness and shelf life of foods,* **836**, 270-291.

Spilimbergo, S., Matthews, M. A., & Cinquemani, C. (2011). Supercritical fluid pasteurization and Food safety. In Proctor, A. (Ed.), *Alternatives to Conventional Food Processing* (pp.145-174). Cambridge: Royal Society of Chemistry.

Stamatis, N., & Arkoudelos, J. S. (2007). Effect of modified atmosphere and vacuum packaging on microbial, chemical and sensory quality indicators of fresh, filleted Sardina pilchardus at 3 C. *Journal of the Science of Food and Agriculture*, **87**(6), 1164-1171.

Thippareddi, H., & Phebus, R. K. (2002). *Modified atmosphere packaging (MAP): Microbial control and quality.* FACTS National Pork Board, 4667, 1-8.

Verma, J. K., Srikar, L. N., Sudhakara, N. S., & Sarma, J. (1995). Effects of frozen storage on lipid freshness parameters and some functional properties of oil sardine (Sardinella longiceps) mince. *Food Research International, **28***(1), 87-90.

Vernocchi, P., Maffei, M., Lanciotti R, Suzzi G, Gardini F (2007) Characterization of mediterranean mussels (Mytilus galloprovincialis) harvested in Adriatic Sea (Italy). *Food Control*, **18**(12), 1575-1583

Wang, T., Sveinsdóttir, K., Magnússon, H., & Martinsdóttir, E. (2008). Combined application of modified atmosphere packaging and superchilled storage to extend the shelf life of fresh cod (Gadus morhua) loins. *Journal of Food Science*, **73**(1), S11-S19

ANNOTATED REFERENCES

Chaijan, M., Benjakul, S., Visessanguan, W., & Faustman, C. (2006). Changes of lipids in sardine (Sardinella gibbosa) muscle during iced storage. *Food Chemistry*, **99**(1), 83-91.

This work evaluated different parameters related to fish oxidation in sardine fillets during storage. It was very important to discuss results observed in this research.

Choubert, G., Brisbarre, F., Parfouru, D., & Baccaunaud, M. (2008). Argon modified atmosphere packaging for fillets of rainbow trout (Oncorhynchus mykiss) fed astaxanthin or canthaxanthin. *Journal of Aquatic Food Product Technology,* **17**(2), 117-136.

This work is one of the very few researches that evaluated the sue of argon in th packaging of fish fillets. The comparison with our results was very important

Stamatis, N., & Arkoudelos, J. S. (2007). Effect of modified atmosphere and vacuum packaging on microbial, chemical and sensory quality indicators of fresh, filleted Sardina pilchardus at 3 C. *Journal of the Science of Food and Agriculture*, **87**(6), 1164-1171.

This research is focused on use of MAP for sardine fillets, which is a similar topic of the present study. The development of a sensorial analysis method for sardine fillets was based on this research that was also used for the discussion of results

Pinheiro, A. C. D. A. S., Urbinati, E., Tappi, S., Picone, G., Patrignani, F., Lanciotti, R., ... & Rocculi, P. (2019). The impact of gas mixtures of Argon and Nitrous oxide (N2O) on quality parameters of sardine (Sardina pilchardus) fillets during refrigerated storage. *Food Research International*, **115**, 268-275.

This work was carried out by our own research group and evaluated the use of novel gases for MAP of sardine fillets determining the effect on microbial shelf life. Results were exploited and used for explaining some of the results of the present study.

Figure captions

Fig. 1. Relationship of oxygen concentration on myoglobin chemical state (modified from Thippareddi and Phebus 2002).

Fig. 2. Images of fresh sample (a), and of samples air (b), N_2 (c), N_2O (d) and Ar (e) after 12 days of storage at 3 ± 1°C

Fig. 3. Peroxide values (A), Conjugated Dienes (B) and Conjugated Trienes (C) and Free Fatty acids (D) content measured in sardine fillets in MAP during storage at 3 °C. Different letters indicate significant differences among samples at the same storage time ($p<0.05$).

Table 1. Changes in the colour parameters of sardine fillets under different MAP conditions at 3°C.

Samples	Storage time (days)						
	0	1	2	5	6	8	12
L*							
Air	46.56±1.5	45.52±0.5^{a}	43.71±2.8^{a}	43.68±2.6^{a}	45.07±2.7^{a}	43.94±2.6^{a}	45.55±2.4^{a}
N_2	-	45.47±1.8^{a}	43.98±3.1^{a}	43.15±1.74^{a}	43.43±2.2^{a}	42.03±2.6^{a}	44.33±1.6^{a}
N_2O	-	42.85±1.3^{b}	43.63±2.9^{a}	41.91±1.95^{a}	41.25±2.5^{a}	41.95±2.8^{a}	42.66±2.7^{b}
Argon	-	43.78±1.2^{ab}	45.30±2.0^{a}	42.61±1.92^{a}	43.50±2.1^{a}	42.66±2.4^{a}	43.70±1.6^{ab}
a*							
Air	1.22±0.9	1.96±2.1^{a}	3.07±1.8^{a}	2.35±1.1^{b}	4.41±1.8^{a}	4.36±2.7^{b}	3.89±2.5^{b}
N_2	-	0.99±1.5^{a}	3.52±1.6^{a}	5.05±2.4^{a}	5.71±1.3^{a}	6.53±2.3^{a}	5.94±2.6^{a}
N_2O	-	1.45±1.0^{a}	4.36±2.2^{a}	4.40±2.8^{a}	5.89±1.6^{a}	6.64±1.4^{a}	4.96±2.9^{ab}
Argon	-	2.05±1.4^{a}	2.94±2.3^{a}	4.55±2.6^{a}	5.89±1.3^{a}	6.24±1.5^{a}	6.26±2.6^{a}
H^0							
Air	80.21±11.2	79.02±19.7^{a}	70.29±13.4^{a}	75.68±12.3^{a}	62.91±11.2^{a}	65.88±13.8^{a}	54.45±13.3^{b}
N_2	-	83.89±11.6^{a}	66.59±13.6^{a}	59.84±11.6^{b}	54.12±5.0^{b}	51.48±9.1^{b}	56.20±11.1^{a}
N_2O	-	77.95±8.4^{a}	63.91±10.7^{a}	62.99±14.6^{b}	52.68±6.1^{b}	50.54±5.6^{b}	54.19±14.2^{a}
Argon	-	74.25±10.4^{a}	71.22±14.4^{a}	62.37±13.2^{b}	54.18±5.2^{b}	52.56±6.4^{b}	56.73±10.9^{a}

Different letters in the same column indicate significant differences ($P < 0.05$).

Table 2. Changes in the TBARS values (mg Malondialdehyde/kg) of sardine fillets under different MAP conditions at 4 °C.

Samples	Storage time (days)						
	0	1	2	5	6	8	12
Air	0.33±0.0	1.34±0.1^{a}	0.58 ±0.2^{a}	1.21 ±0.5^{a}	2.62 ±1.1^{a}	2.00 ±0.7^{a}	3.39 ±0.9^{a}
N_2	-	0.61±0.4^{b}	0.61 ±0.3^{a}	0.62 ±0.2^{a}	0.55 ±0.1^{b}	1.11 ±0.3^{a}	1.19 ±0.1^{b}
N_2O	-	0.59±0.1^{b}	0.58 ±0.3^{a}	0.73 ±0.1^{a}	0.63 ±0.1^{b}	1.61 ±0.7^{a}	1.01 ±0.1^{b}
Argon	-	0.44±0.2^{b}	0.45 ±0.0^{a}	1.08 ±0.3^{a}	0.71 ±0.0^{b}	1.34 ±0.1^{a}	1.09 ±0.4^{b}

Different letters in the same column indicate significant differences ($P < 0.05$).

Table 3. Release of FFAs (mg/kg) in *Sardina pilchardus* fillets during storage at 3°C in relation to the packaging atmosphere.

	Raw material	Air - 8d	Air - 14d	N_2 - 8 d	N_2 - 14d	N_2O - 8d	N_2O - 14d	Ar - 8d	Ar - 14d
C13:0	-*	-	4.28±0.34	-	-	6.52±2.39	-	-	-
C14:0	-	3.24±0.13	3.25±0.48	3.47±0.05	3.53±0.40	3.96±0.52	4.49±0.78	1.56±2.21	4.10±0.40
C16:0	37.25 ±5.92	26.94±2.62	36.23±1.23	36.49±1.90	30.90±2.38	35.58±5.33	49.26±18.76	38.78±5.63	38.16±1.35
C17:0 iso	-	9.99±4.13	-	-	-	-	-	-	-
C18:0	29.81 ± 2.25	8.74±0.18	12.09±1.2	13.88±0.72	9.98±0.88	9.07±1.25	10.79±1.10	16.39±3.25	12.37±1.14
C22:0	-	-	-	-	30.15±2.63	-	-	-	
Σ saturated	***67.06***	***48.91***	***55.85***	***53.84***	***74.56***	***55.13***	***64.54***	***56.73***	***54.63***
% saturated	91.93	49.70	51.77	52.73	60.84	51.28	53.37	50.39	51.45
C14:1n9	5.89 ±1.67	-	-	-	-	-	-	3.63±0.87	-
C16:1n9	-	5.00±0.26	4.63±0.1	4.97±0.57	5.45±1.30	5.56±0.25	9.33±1.74	4.94±1.00	6.54±0.17
C18:1n9c	-	3.07±0.82	-	3.14±1.00	2.55±0.37	2.55±0.05	-	5.14±0.10	2.80±0.76
C18:1n9t	-	2.02±0.01	-	2.23±0.39	-	2.53±0.37	-	-	2.89±0.42
Σ monounsaturated	***5.89***	***10.09***	***4.63***	***10.34***	***8.00***	***10.64***	***9.33***	***13.71***	***12.23***
% monounsaturated	8.07	10.25	4.29	10.13	6.53	9.90	7.72	12.18	11.52
C18:2n6c	-	1.82±0.16	3.62±1.10	1.34±0.60	2.64±0.22	1.39±0.64	6.55±0.89	-	-
C18:3n3	-	-	2.59±0.2	2.26±0.40	2.12±0.37	-	2.86±0.98	-	-
C20:4n6	-	1.70±0.16	-	1.27±0.13	-	1.39±0.64	-	-	-
C20:5n3	-	14.05±1.63	13.68±2.14	12.53±0.90	13.91±4.33	14.71±0.04	20.50±9.00	13.67±1.40	16.10±1.59
22:6 (n-3)	-	21.85±7.77	27.51±5.61	20.53±1.75	21.43±5.53	24.24±2.84	17.14±2.56	28.47±0.48	23.22±7.90
Σ polyunsaturated		***39.42***	***47.4***	***37.93***	***40.1***	***41.73***	***47.05***	***42.14***	***39.32***
% polyunsaturated	0.00	40.05	43.94	37.15	32.72	38.82	38.91	37.43	37.03
Total	**72.95**	**98.42**	**107.88**	**102.11**	**122.56**	**107.5**	**120.92**	**112.58**	**106.18**

* *not detected*

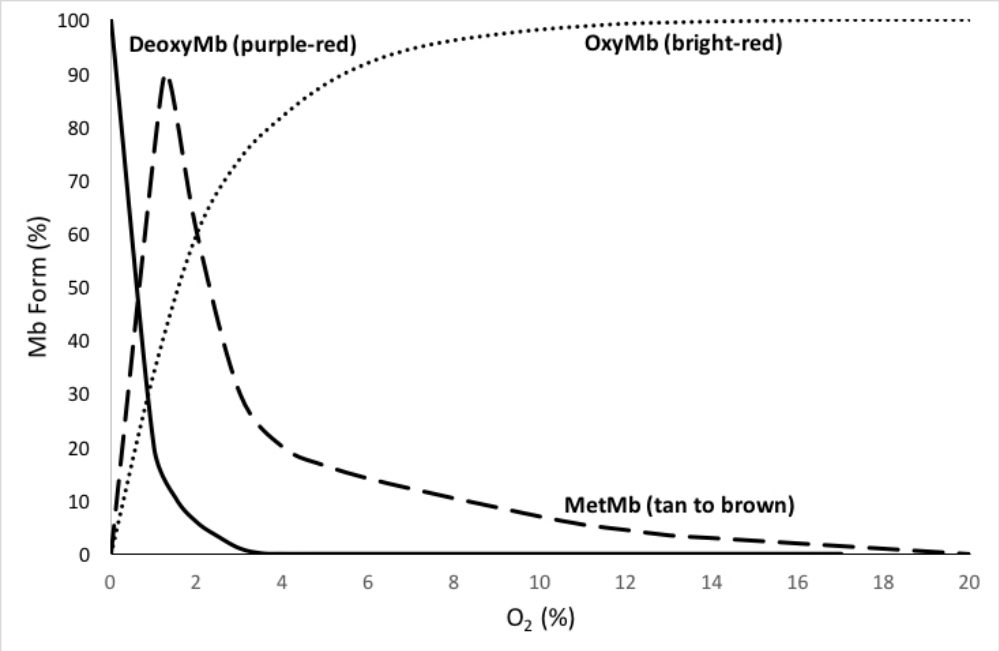

Fig. 1. Relationship of oxygen concentration on myoglobin chemical state (modified by Thippareddi and Phebus 2002).

258x168mm (72 x 72 DPI)

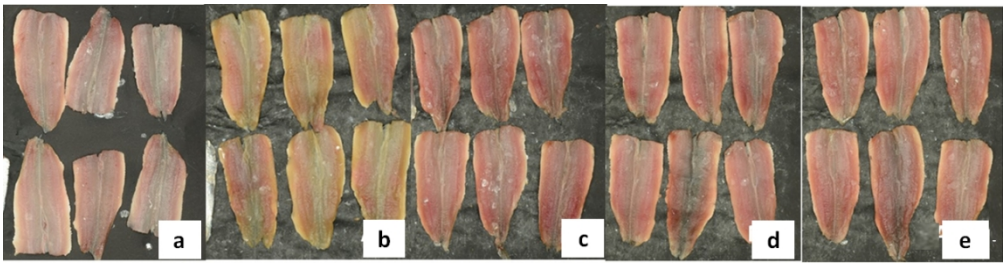

Fig. 2. Images of fresh sample (a), and of samples air (b), N2 (c), N2O (d) and Ar (e) after 12 days of storage at 3 ± 1°C

334x87mm (96 x 96 DPI)

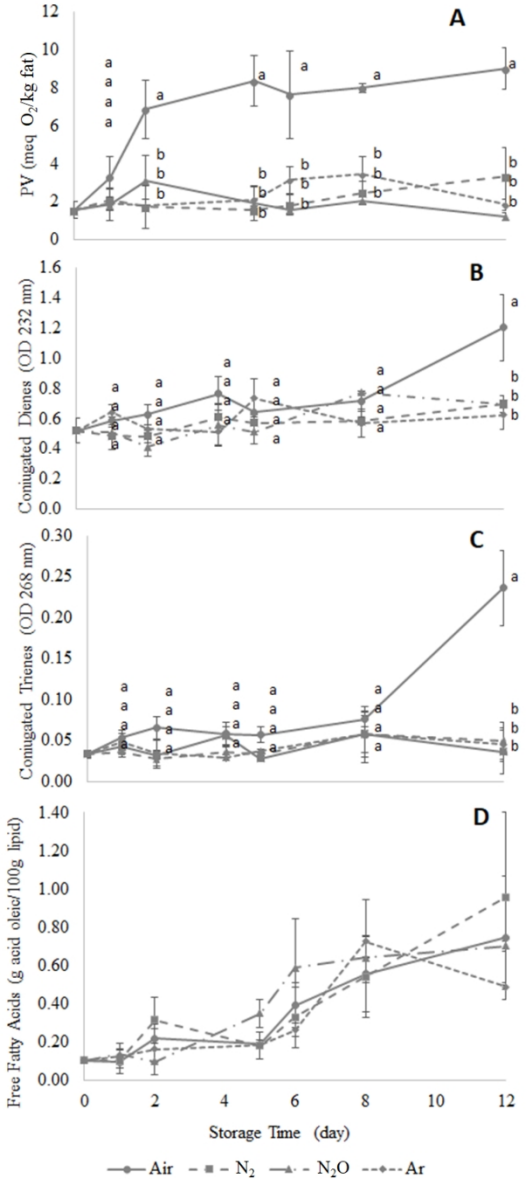

Fig. 3. Peroxide values (A), Conjugated Dienes (B) and Conjugated Trienes (C) and Free Fatty acids (D) content measured in sardine fillets in MAP during storage at 3 °C. Different letters indicate significant differences among samples at the same storage time ($p<0.05$).

184x411mm (96 x 96 DPI)

Table S1. Sensory acceptance* during sardine storage under different MAP conditions at 3 °C.

Sample	Storage time (days)					
	1	2	5	6	8	12
Air	E	B	C	C	C	C
N2	E	A	A	B	C	C
N2O	E	A	A	B	C	C
Ar	E	A	A	B	B	B

* Freshness categories: E (excellent), A (good), B (fair) and C (unacceptable). Raw fish was category E.