Short Communication

TITLE: PRELIMINARY RESULTS ABOUT THE USE OF ARGON AND CARBON DIOXIDE IN THE EXTRA VIRGIN OLIVE OIL (EVOO) STORAGE TO EXTEND OIL SHELF LIFE: CHEMICAL AND SENSORIAL POINT OF VIEW.

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RUNNING TITLE: Ar and CO_2 in EVOO storage: preliminary results.

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Abbreviations: $ABTS^+$, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid); EVOO, extra virgin olive oil; LL EVOO, legal limit for extra virgin olive oil; FFA, free fatty acid; PV, peroxide value; TEAC, trolox equivalent antioxidant capacity.

Abstract

The aim of this work was to evaluate the effectiveness of different inert gases, other than nitrogen, to develop a preservation method that can maintain the high quality of EVOO increasing its shelf life. The EVOO was stored in the dark at 12 °C in glass containers for about 9 months, and Ar and CO_2 were selected as alternative head-space gases in comparison with air. While the acidity remained constant during the whole observation period, the oxidation-sensitive parameters showed significant differences over time as a function of the gas composition. After only two months of storage, the oil stored under air showed a marked decrease of quality, while the use of inert gases slowed oxidative degradation and Ar-treatment best preserved oil overall quality. Compared to samples stored under Ar, at the end of storage the air treatment reduced the phenolic content as well as the antioxidant capacity by 12.0 and 31.3%, respectively, and resulted in an increase in the peroxide value and K232 of 51.7 and 35.4%, respectively. As CO_2 determined a significant negative aftertaste development in oil, it is possible to conclude that this gas cannot be profitably used to optimize EVOO storage conditions.

Practical applications: the identification of an inert gas other than nitrogen able to slow down the oxidative degradation of EVOO both from sensory and chemical point of view, can contribute to identify some possible new strategy to be applied for EVOO long-term storage at industrial and market level.

According to the preliminary results described in this short communication, it should be interesting to better explore the suitability of the total or partial replacement of nitrogen with argon in EVOO storage and packaging.

In this context, as the extra-cost related to food-grade Ar utilization in comparison with N_2 appears close to 17%, the next step of our research will be further investigating also the economic feasibility of the possible combination packaging/storage head-space atmosphere adopted as a function of specific industrial goal.

1 Introduction

The quality of an olive oil depends on many factors such as cultivar, climate conditions, agronomic techniques, harvesting time and olive crushing method [1-3]. Furthermore, the storage and packaging conditions are decisive for the commercial life of several foods and beverages as well as olive oil [4-9]. As the stability of olive oil is primarily due to its fatty acid composition and the antioxidant activity of its polyphenols and tocopherols, when certain limits of lipid oxidation derivatives (hydroperoxides, conjugated dienes and trienes) are exceeded and/or rancid off-flavors occur, olive oil may lose the authorization to report the wording "extra virgin" or even "virgin" on the label [10].

The basic method to slow down the oxidative changes in fats is to limit the contact of oil with oxygen [11]. This goal can be reached using a packaging material characterized by low oxygen permeability and removing air in the headspace [12], either by fully filling the EVOO bottles or replacing air with inert conditioning gases, mostly nitrogen [13, 14].

Until now, many experimental studies have been carried out to verify that nitrogen can improve the stability and the shelf life of oil, slowing down its oxidative changes [13, 15-17].

The aim of this work was to evaluate the effectiveness of different inert gases, other than nitrogen, to develop a preservation method that can maintain the high quality of EVOO as long as possible, increasing its shelf life. As very few studies were focused on the use of Ar and CO_2 as alternative head-space gases for EVOO long-term storage, we focused our analysis choosing them as alternative gases. For what concerns Ar, it is an extremely stable chemical element, odorless and tasteless, available both in liquid and gaseous forms. Moreover, it has a specific weight higher than that of air and nitrogen, allowing the stratification of the gas at the bottom of an empty container or on the surface of a liquid mass contained therein. Carbon dioxide could have the advantage to be easily available, to be non-toxic, and not flammable, besides having a lower cost than Ar.

In this experimental work we followed the evolution of some chemical and sensory parameters of an EVOO stored in glass containers for about 9 months in a dark chamber at 12 °C with three different storage atmospheres (Ar, CO_2 and air). An innovative approach based on the overlapping between two series of information coming from both chemical and sensorial analysis was applied, and preliminary results are also discussed by an economical point of view.

2. Materials and Methods

2.1 Olives and oil sample

The EVOO used in this study was produced during the 2016/17 olive milling, and was a blend of two olive cvs of the Campania region: Ravece (70%) and Ogliarola campana (30%), both grown in Irpinia (Castelfranci, AV) at 400 m a.s.l. The olives were processed within 24 hours from harvest by an industrial plant (Alfa Laval, Tavernelle V.P. Florence, Italy) equipped with a hammer crusher, a traditional horizontal covered malaxer, a two-phase decanter extractor and a double vertical separator. The malaxation was carried out for 40 min at 27 °C. The EVOO obtained was immediately submitted to filtration and stored in stainless steel silos under nitrogen at constant temperature (12 °C ± 1 °C) until the experimental set up.

2.2 Experimental set up

After a chemical and sensorial characterization at the beginning of the storage period (reference time zero t0), the EVOO was divided into three aliquots and stored under three different gaseous atmospheres (Ar, CO_2 and air) in glass containers, in the dark and at controlled temperature (12 ± 1 °C). The sealing system consisted of a 500 ml coded tube, a gas burette, and a separatory funnel saturated with NaCl. Sampling was scheduled as follows: t0 = time at the beginning of the experiment; from t1 to t9 oil samples were collected every 15 days; t10 = 170 days of storage; t11 = 215 days of storage; t12 = 250 days of storage.

2.3 Legal quality parameters

The determinations of free fatty acids (FFA), of the peroxide value (PV) as well as the specific absorbance at 232 and 270 nm (K_{232} and K_{270}) of the EVOO were carried out in duplicate according to the Regulation (EU) No. 1348/2013 [18].

2.4 Preparation of phenolic extracts

Phenolics were extracted from the EVOO according to the method described by Montedoro *et al.*, 1992 [19] with some modifications reported in a previous work [20].

2.5 Total phenol determination

The total phenol concentration of the EVOO was determined colorimetrically at 765 nm, using the Folin–Ciocalteau reagent [21]; calculations were performed using a calibration curve constructed with gallic acid as standard.

2.6 Antioxidant capacity

The antioxidant assay of the methanolic extracts was carried out according to the method described by Sgherri *et al.*, 2016 [22]. The radical cation $ABTS^{\cdot+}$ was generated as described by Pellegrini *et al.*, 1999 [23]. The activities of the extracts were quantified by using a dose-response curve of Trolox in the 0.2-1.5 mM range, expressing them in terms of Trolox equivalent antioxidant capacity (TEAC) L^{-1} extract.

2.7 Sensorial characterization

The sensorial profiles of the olive oil as a function of the storage time were evaluated by a trained panel (10 assessors, 7 males and 3 females, aged between 30 and 65). All the panelists were included in the “expert panel” of the Department of Agriculture, Food and Environment of the University of Pisa (Italy) according to the internal procedure for assessor selection and training [24, 25].

The sensorial characterization followed the method described in the Regulation (EC) No. 640/2008 [26]. To better describe the organoleptic evolution of the stored oil, during the whole observation period the Panel was provided with a technical sheet specifically developed for this purpose. Thus, it was possible to obtain a sensory profile of the stored oil as a function of the storage conditions based on the first order descriptors of colour, flavouring and taste, and of the hedonic parameter related to the overall pleasantness.

As the perception of the oil evolution can be directly related to the whole oil evolutionary state, the panelist were asked to define also the “life time lapsed” of the oil: higher values of “evolutionary state” correspond to oils perceived with an advanced state of degradation.

All assessments were repeated in duplicate in two different days by the same group of panelists.

2.8 Statistical analysis

The results are the means ± SD values of three independent experiments. The significance of differences among means was determined by one-way ANOVA (CoStat, Cohort 6 software). Comparisons among means were performed by the Bartlett’s X2 corrected test ($p < 0.05$).

Pearson’s correlation coefficient test was also carried out to measure the strength of the correlation among chemical and sensorial results [27].

3 Results and discussion

3.1 Effect of storage atmosphere on EVOO chemical parameters

The chemical parameters of the olive oil at the beginning of the experiment (t0) were within the limits established by the Regulation (EU) No. 1348/2013 for EVOO [18].

While the acidity remained constant during the whole observation period (Table 1), the oxidation-sensitive parameters showed remarkable differences over time among the different treatments (see Table 1 and Figure 1).

As showed in Figure 1, the PV increased over time in all samples, the EVOO samples stored under air showing the worst values. Statistically significant differences among EVOO samples stored under different atmospheres was firstly evidenced after 45 days (t3) from the beginning of the experiment. While the olive oil stored under air exceeded the legal limit by 13% (22,6 ± 0.01) after 215 days of storage (t11), both samples stored in the inert atmospheres failed out of EVOO parameters for PV after an extra-time of almost 1 month (t12).

A trend similar to the PV was shown by the absorbance at 232 nm, which is related to the presence of conjugated dienes. Storage under air differed from the other two treatments after 60 days of storage (t4), and it was the only sample to overcome the legal limit after 170 day from the splitting of the oil (t10).

At the end of the observation period, the lowest concentration of phenols (Table 1) and the highest peroxide number (Figure 1) were detected in the EVOO stored under air. The same trend was also confirmed for the antioxidant capacity, with the Ar-stored sample showing the highest value of this parameter after 250 days of storage, while the air-stored oil showed the lowest one.

3.2 Effect of storage atmosphere on EVOO sensorial parameters

The sensorial characterization at t0 classified the olive oil as extra virgin, according to the Regulation (EC) No. 640/2008 [26]. At starting time, the peculiar aromatic notes were tomato, artichoke and almond, while visually a high clearness and a prevalence of green shadows could be detected.

As showed in Figure 2, a loss of aromatic profile was observed over time for all the storage atmospheres in comparison with the starting sample. The most negatively affected parameters were “apple” and “almond” that were completely absent at the end of the experiment; “artichoke” decreased more than twice in intensity for all the treatments and “tomato” decreased of one, two and three points of intensity under air, Ar and CO_2, respectively.

Although no difference was observed for green fruity, a descriptor mainly attributed to C6 and C5 aromatic volatile compounds [28], the evolutionary state and whole pleasantness showed remarkable differences among the different samples. The olive oil perceived with an evolutionary state more similar to the starting value was the Ar-stored oil, and this result is in agreement with the chemical data, in particular the health-promoting compounds like phenolics as well as the antioxidant capacity (Table 1). The decrease of green shadows, smell, bitter and pungent descriptors underlines a remarkable loss of oil sensory qualities, even if this loss was not sufficient to declass the air- and Ar-treated EVOO to lower categories.

This was not true for the oil stored under CO_2. In fact, the organoleptic impact of CO_2 determined not only a higher perception of the evolutionary state of the product, but also a considerable lower whole pleasantness, linked to the appearance of a highly negative aftertaste that made the product unacceptable for the panelists. At best of our knowledges no data are available in literature about the influence of this gas on the sensorial expression of stored oil. Anyway, a possible explanation could be charged to the high solubility in oil of the non-polar CO_2 molecule [29], so that the sensorial profile of the EVOO stored under CO_2 was adversely modified. In contrast, the EVOO stored under Ar, although showing a less intense and complex flavor in comparison with the starting sample, maintained organoleptic characteristics preferable than the others.

3.3 Correlations among sensorial and chemical results

To evidence whether the storage conditions could influence the oxidation rate of EVOO, the main chemical parameters (e.g. the phenolic concentration) involved in the delay of the deteriorative processes occurring during EVOO storage were correlated for all storage conditions with the sensory attributes generally related to oil oxidation. Among them, only the parameters that showed some significant differences during storage time were selected. The Pearson's correlation coefficients are reported in Table 2.

As previously reported by Morales *et al.*, 2000 [30], phenolics, in particular secoiridoid compounds, showed a strong positive correlation with bitter and pungent tastes. The same relation could be also highlighted in the present experiment since the pungent character decreased in all the treatments, although maintaining the highest value in the oil stored under Ar at the end of the experiment.

The K232 index resulted positively correlated with the evolutionary state, being it a parameter sensitive to oxidative processes and representing the quality of the olive oil as a function of time. Therefore, this parameter was negatively correlated with the phenolic concentration. Some olfactory sensations such as artichoke and golden apple were also positively correlated with the phenolic concentration. On the contrary, the same sensorial attributes were negatively correlated with quality parameters, indicating the degradation of the lipid matrix, in particular with PV and the spectrophotometric parameter K232 (Table 2). Because of oxidation processes, the taste parameters (bitter and pungent) resulted negatively correlated with PV and K232 index.

4 Conclusions

In conclusion, storage conditions affected EVOO chemical quality as a function of time, particularly as regards the PV, the phenolic concentration and the spectrophotometric parameters, whereas FFA concentration did not change significantly. The oil stored under air showed a marked decrease of quality after only two months of storage, while the use of inert gases in the headspace of the container during storage can reduce the presence of oxygen and preserve, as much as possible, the compositional, nutritional and organoleptic qualities of the oil. Despite EVOOs stored under CO_2 and Ar showed only slight differences as regards their chemical parameters, they appeared deeply modified for what concerns the sensorial characterization. In particular, CO_2 determined a negative organoleptic interference that would not support its use for EVOO long-term storage.

Therefore, Ar treatment appears as the best solution alternative to nitrogen to preserve the quality of the EVOO over time.

As the extra-cost related to food-grade Ar utilization in comparison with N_2 appears close to 17%, next step of our research will be to investigate the suitability as well as the economic feasibility of the replacement of nitrogen with argon in extended EVOO storage at industrial and market level.

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Tables

Table 1. Phenolic content (PC, mg gallic acid/kg oil), antioxidant capacity (□mol TEAC/ml) and free fatty acids (g oleic acid/kg oil) of EVOO at time zero (t0) and of samples stored in air, Ar and CO2 at 12 °C for 250 days (t12) after experimental splitting. Data are reported as mean ± SD (n = 3). For each column different letters indicate significant differences among treatments at $p < 0.05$.

	PC	TEAC	FREE FATTY ACIDS
t0	340 ± 10 a	0.18 ± 0.01 a	0.14 ± 0.01 a
Air	233.85 ± 4.5 c	0.11 ± 0.01 b	014 ± 0.01 a
Ar	265.63 ± 3.7 b	0.16 ± 0.01 a	0.14 ± 0.01 a
CO_2	242.67 ± 7.5 bc	0.12 ± 0.01 b	0.14 ± 0.01 a

Table 2. Pearson's correlation coefficients obtained correlating the sensorial analysis with chemical results. Strong correlations are highlighted in bold. PC: phenolic content; PV: peroxide value.

	Evolutionary state	Artichoke	Golden Apple	Bitter	Pungent
PV	0.5	-0.6	**-0.7**	-0.6	-0.6
K232	0.6	-0.4	-0.5	-0.6	**-0.7**
K270	0.4	-0.1	**-0.7**	**-0.7**	-0.6
PC	-0.6	**0.7**	**0.8**	0.6	**0.7**

Figure captions

Figure 1. Time evolution of peroxide value (A) and in spectrophotometric indices (K232, B) in EVOO samples stored under different gaseous atmosphere. At each sampling time, different letters indicate significant differences among treatments at $p < 0.05$. Continuous line represents the legal limit for extra virgin olive oil (LL EVOO) regulation (EU) No 1348/2013.

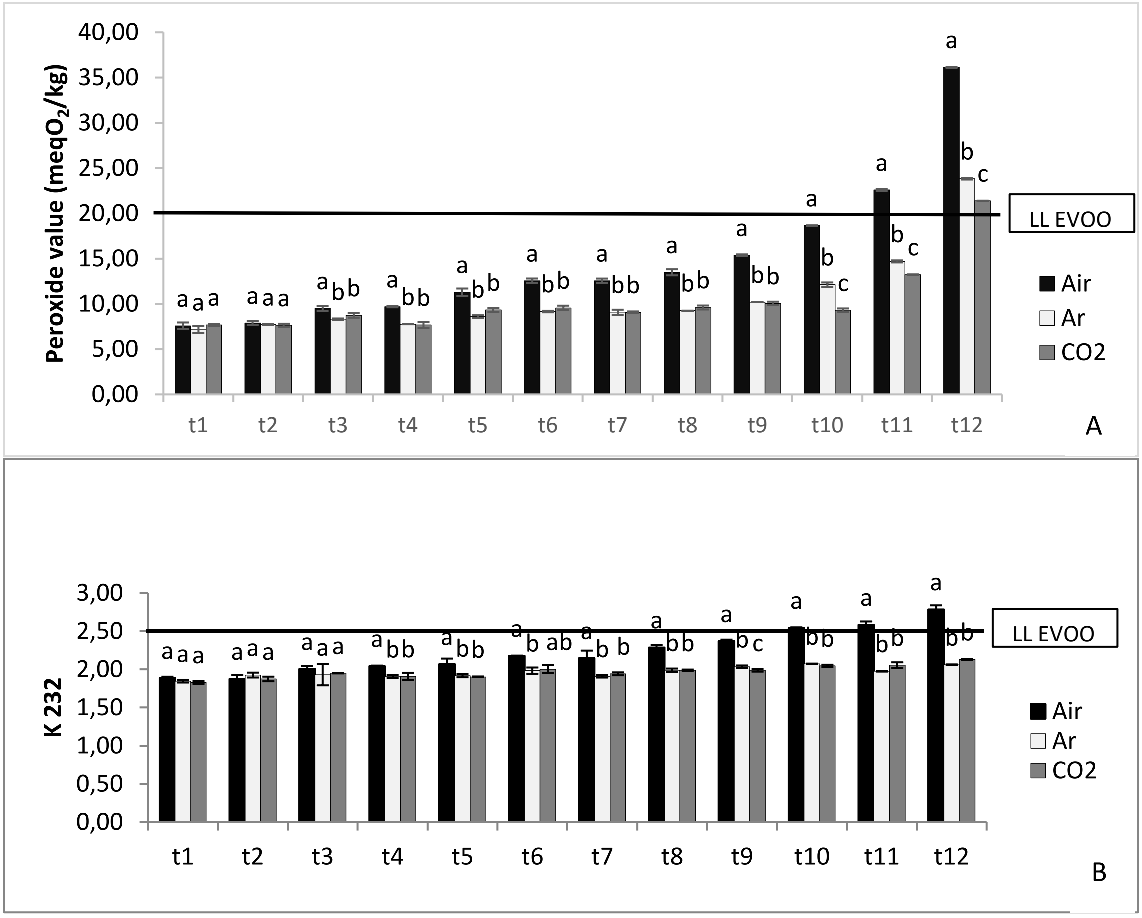

Figure 2. Sensory characterization of the oil sample as a function of storage conditions. A: overall descriptors; B: smell parameters; C: taste parameters. At each sampling time, means accompanied by different letters indicate significant differences among treatments at $p < 0.05$.