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Stainless Steel Bottles for Extra Virgin Olive Oil Packaging: Effects on Shelf-Life

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SUMMARY

The quality changes in extra virgin olive oil afforded by the conservation in bottles of different materials were assessed in a 12-month shelf-life test. Transparent clear glass (TCG), green coloured glass (CG) and stainless steel (SS) 250-ml bottles were studied (in triplicates) alternating natural and fl uorescent light to simulate the 'in the drugstore' conditions. Every 2 months, the quality decay was assessed by monitoring some chemicals parameters and by sensory evaluation. Principal component analysis evidenced a clustering of the samples as a function of storage time and bottle type. The SS bottles showed the best storage performances, whereas only minor differences were detected in TCG and CG. A large variability was detected within the replicated glass bottles, probably as a consequence of some uncontrolled variations in the light exposure. This was confi rmed by the measure of light intensity over the storing surface, which showed a large variability (15%) around an average value of 380 lux. Under light exposure, a limited antioxidant effect of phenolic compounds was recorded. Only some specifi c phenols seem to play an important role in oil protection against oxidation.

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KEY WORDS: olive oil storage; oxidation; hydrophilic phenols

INTRODUCTION

Extra-virgin olive oil (EVOO) is generally considered the best among all different categories of olive oil and other edible vegetable oils, for either its organoleptic properties or chemical composition.1,2 The EVOO consumption is expanding all over the world because of the growing interest in the Mediterranean diet and consumers' choice to select least-processed and nutraceutically valuable foods.2,3 It is of course a major consumers' expectation that the high food quality be maintained during the period between purchase and consumption with minimal changes in sensory characteristics.4 As in any other fat-containing product, EVOO, it is susceptible to quality decay as consequences of two main natural degradation processes, i.e. lipolysis and oxidation. Lipolysis starts early when the oil is still in the fruit, whereas the oxidation, either photoxidation or autoxidation, begins at processing and proceeds during storage.4–6 In addition, EVOO quality either in terms of stability, or sensory and nutritional properties, is strictly related to the concentration of antioxidant minor components such as hydrophilic phenols. These are subject to degradation processes along with the oil fatty matrix during storage.7–9 Therefore, a proper packaging is a relevant factor for EVOO shelf-life for insuring a long life for distribution and marketing by adequately preventing the autoxidation processes leading to rancidity.

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Moreover, it is important for preserving antioxidant compounds from degradation, and maintaining the peculiar sensory and nutritional characteristics.10 The physic-chemical characteristics of the packaging material may signifi cantly affect the quality of EVOO. Migration and scalping phenomena may further affect the quality and safety aspects. Besides, an unwanted permeability to atmospheric oxygen and light may be relevant.10 The most common materials used for bottling and packaging of EVOO are tinplate, plastic and glass. The use of stainless steel (SS) is generally limited to tanks and oil tankers for transportation.10,11 As reviewed by Kanavouras et al. (2006) and Tsimis et al. (2002) 10,11 plastics materials and tinplate, although widely used for packing and bottling of vegetable oils, have some disadvantages deriving from chemical migration and the limited protection against oxygen. Glass offers advantages related to its impermeability to gases, but do not prevent photoxidation. In this contest, SS bottles appear as a new and promising solution. In fact, it combines the advantages related to chemicals migration, oxygen permeability and light exposure. However, the actual advantage of the use of steel packages in terms of preservation of EVOO quality was not still evaluated. Aim of this work was to compare the changes in EVOO quality between different types of containers, i.e. transparent clear glass (TCG), green-coloured glass (CG) and SS bottles, during a storage period of about 1 year. Quality changes of EVOO were monitored both on the base of some chemicals parameters and on sensory evaluation. Although the quality decay of EVOO during storage in different containers and/or under light exposure is well documented,12–17 the possible use of SS bottles for the oil packaging is a new important alternative. At the authors' knowledge, this solution has not been so far tested. Furthermore, from the work, come some new and unexpected results con- cerning the quality decay, which could contribute an improved knowledge on olive oil shelf-life.

MATERIALS AND METHODS

Experimental procedure

A shelf-life test was performed on EVOO stored in three different type of bottles (250 ml), i.e. TCG, CG and SS bottles. Extra virgin olive oil produced from a blend of frantoio, moraiolo and leccino cultivars (crop season 2006–2007, drupes harvested at medium ripeness near Florence, Italy) in a centrifugal continuous extraction plant, was used for the experimental test. Three months after production, the EVOO was aliquoted in the bottles leaving a free headspace of about 3%. The fi lled bottles were hermetically sealed and stored on a table under natural diffused light and fl uorescent artificial light (8 h/day) so to simulate the typical market storage conditions. Temperature was recorded daily during the storage period and it results of about 22(±3)°C on the average. The total length of the storage period was 12 months. At sche-duled time, i.e. every 2 months, bottles samples (in triplicates) were randomly withdrawn from storage and analysed both for chemical and sensory analyses. The remaining bottles were periodically rearranged. Enough bottles, i.e. 18, were prepared for each trial so that no sample, once withdrawn from storage and analysed, had to be reused. The samples were chemically analysed for free acidity (FA), peroxide value (PV), UV specifi c extinction coefficients (K232, K270, ΔK), High Performance Liquid Chromatography (HPLC) analysis of hydrophilic phenols composition and subjected to sensory evaluation, i.e. triangle test. At time 0 also the fatty acid composi-tion was determined in addition to total hydrophilic phenols concentration and the organoleptic assessment in order to characterize the oil. The initial EVOO characteristi-cs are reported in Table 1.

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Chemicals analyses

Free acidity, PV, UV specifi c extinction coeffi cients and fatty acid composition were carried out according to the European Offi cial Method of Analysis (2003).18 The HPLC hydrophilic phenols profile was determined according to the method of the SSOG Technical Commission (2006).19 The method allows the extraction and quantifi cation of different phenolic compounds in olive oils, such as the natural and oxidized derivatives of oleuropein and ligstroside, lignans, fl avonoids and phenolic acids (Table 1). The HPLC equipment consisted of a Hewlett Packard 1200 diode-array detector

Table 1. Chemical composition and sensory profile of EVOO before storage. The column headed 'code' reports the variables coding used for data elaboration.

Standard quality parameters	Value	Code
Free acidity (%w/w oleic acid)	0.14	FA
Peroxide value (mEq O_2/kg)	4.20	PV
K232	1.64	K232
K270	0.10	K270
dK	0.00	dK
Fatty acid composition (%)	**Value**	**Code**
Myristic	0.00	–
Palmitic	12.92	–
Palmitoleic	0.83	–
Heptadecanoic	0.04	–
9-Heptadecenoic	0.07	–
Stearic	1.77	–
Oleic	76.07	–
Linoleic	6.86	–
Linolenic	0.61	–
Arachidic	0.32	–
11-Eicosenoic	0.28	–
Behenic	0.10	–
Lignoceric	0.04	–
E-oleic	0.01	–
E-inoleic + *E*-linolenic	0.10	–

Hydrophilic phenolic compounds (mg/kg)	Value	Code
Hydroxytyrosol	1.39	F1
Tyrosol	1.33	F2
Vanillic acid	0.88	F3
Vanillin acid	2.75	F4
Para-coumaric acid	1.74	F5
Ferulic acid	1.19	F6
Decarboxymethyl oleuropein aglycone, dialdehyde form	80.74	F7
Decarboxymethyl oleuropein aglycone, oxidized dialdehyde form	14.79	F8
Oleuropein	20.75	F9
Oleuropein aglycone, dialdehyde form	25.78	F10
Decarboxymethyl ligstroside aglycone, dialdehyde form	34.75	F11
Decarboxymethyl ligstroside aglycone, oxidized dialdehyde form	7.26	F12
Pinoresinol, 1 acetoxy-pinoresinol	42.08	F13
Cinnamic acid	9.84	F14
Ligstroside aglycone, dialdehyde form	5.99	F15
Oleuropein aglycone, oxidized aldehyde and hydroxylic form	91.89	F16
Luteolin	3.68	F17
Oleuropein aglycone, aldehyde and hydroxylic form	29.10	F18
Apigenin	11.56	F19
Ligstroside aglycone, oxidized aldehyde and hydroxylic form	20.85	F20
Methyl-luteolin	11.54	F21
Ligstroside aglycone, aldehyde and hydroxylic form	3.18	F22
Total	423.05	TOT
Total hydrophilic phenols (colorimetric method)	364.00	–

Organoleptic assessment	Median	CVr[b](%)	Code
Defects	0	0	–
Fruity	2	15.43	–
Bitter	4	3.86	–
Pungent	4	3.86	–

[a] Data are scores median of the 8 assessors.
[b] Robust variation coefficient.
CVr, robust coefficient of variation; dK, delta K; TOT, total phenols.

system and a Hewlett Packard model 1100 autosampler: Agilent Technologies, Santa Clara, California, USA. Analytical conditions were: HPLC column: LiChrospher 100 endcapped RP-18, 5 μm, 250 × 4.6 mm ID; injection volume: 20 μL; solvent: pH 2.5 H2O/acetonitrile gradient as described in the method; wavelength: 280 nm. Syringic acid was used as the internal standard. Total hydrophilic phenols were extracted by liquid–liquid partition with an 80 : 20 methanol/water solution. The total phenol content of the extract was determined by the Folin-Ciocalteau spectrophotometric method at 765 nm, using gallic acid as the calibration standard.20

Sensory evaluation

Sensory evaluation of EVOO samples was performed by means of a panel group constituted by eight tasters, trained and prepared to distinguish between similar samples. A triangular sensory test (ISO 4120 : 2004, 2004)21 was applied to identify different sensory profi les among oils stored in different bottles. This forced-choice procedure allows determining whether a perceptible sensory difference or similarity exists between two samples. Three samples, two of which were identical, were presented simultaneously to the tasters. Each of these samples was prepared by blending the three replicates of each bottle type. Such simplifi cation was necessary because of the high number of comparisons, i.e. 27 different samples to be tasted at each panel session, which the complete analysis of all replicates would have required. Thus, by blending the three replicates of each bottle type, the number of samples was reduced to nine with no replicates. Each oil sample was analysed in different combinations for each session and the samples sets were randomly distributed among the tasters. Each taster was asked to identify the different sample and to give a preference along with the relative explanation in terms of positive or negative attributes. Further, at time 0, EVOO was sensory evaluated according to the 'Organoleptic Assessment of Virgin Olive Oil' IOOC procedure (IOOC 2007)22 Tasters randomly scored the fl avour descriptors of the sample on a normalized sheet (from 0 to 10). The median score was calculated for each sample. Sensory descriptors of olive oil can be classifi ed into 'positive attributes', such as fruity, bitter and pungent, and 'negative attributes', which describe defects of the oil. The latter includes fusty, musty, rancid, metallic, wine-vinegary and others. Olive oil is graded as 'extra virgin' when the median for the defects is 0 and the median for 'fruity' is above 0.

RESULTS AND DISCUSSION

The EVOO analysed before the storage not only widely conformed to the limits imposed by EEC/2568/91 Regulation, deserving to be labelled as ''extra-virgin', but resulted also of excellent quality for its high phenolic concentration and the good sensory profi le. During the storage period, a general quality decay occurred on all the oil samples as indicated by the increased oxidative indexes and decreased concentration for almost all the identifi ed phenolic compounds. However, marked differences were recorded in quality decay among samples stored in different bottles, which after 12 months of storage showed large differences in several parameters (Table 2). The oils stored in SS bottles showed a better qualitative level as indicated by signifi cant higher levels of phenols and lower values of the oxidative indices (both primary and secondary oxidation by-products). On the contrary, the EVOO from glass bottles had PV exceeding the legal limit and large quality decay confi rmed by the sensory evaluation. As showed in Table 3, a marked distinction was evidenced by the tasters for SS as compared with TGC and CG at 12 months. Indeed, they were able to identify the SS samples just after 4 and 6 months of storage (in comparison with TGC and CG bottles, respectively), whereas TGC and CG samples were not distinguished until 10 months. However, after 12 months TCG and CG showed quite the same degradation level with differences not statistically signifi cant, albeit the CG had PV apparently intermediate with respect to the other two oils series. This result probably depended on the large variability among the replicated glass bottles, for both TCG and CG, as stated by the large PV ranges reported in Table 2. This occurrence will be discussed later. The analytical and sensory data were analysed by means of multivariate statistical analysis for an overall understanding EVOO quality evolution during shelf-life. A matrix with 54 rows (oil samples at different storage time in different bottles) and 26 columns (chemicals parameters) was built and

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Table 2. Chemical composition of EVOO stored in different bottles after 12 months.

	SS		TCG		CG	
Parameter[a]	Median[b]	Range	Median[b]	Range	Median[b]	Range
FA (%)	0.18a	0.01	0.19b	0.01	0.19ab	0.01
PV (mEq O_2/kg)	10.00a	2.60	74.75b	83.75	54.85b	56.00
K232	2.12a	0.22	2.31a	0.64	2.23a	0.81
K270	0.14a	0.01	0.19c	0.03	0.15b	0.01
dK	0.00a	0.00	0.01b	0.00	0.00a,b	0.01
F1 (mg/kg)	7.12a	0.17	11.81b	3.49	9.97b	0.67
F2 (mg/kg)	3.01a	0.47	4.90b	1.37	4.44b	1.06
F3 (mg/kg)	0.73a	0.03	0.70a	0.03	0.72a	0.02
F4 (mg/kg)	2.80a	0.09	4.13b	1.27	4.14b	1.18
F5 (mg/kg)	0.51b	0.06	0.39a	0.04	0.47b	0.05
F6 (mg/kg)	2.84c	0.97	0.48a	0.13	1.11b	0.61
F7 (mg/kg)	50.93b	5.58	21.22a	23.74	24.68a	14.64
F8 (mg/kg)	12.55a	0.69	14.39a	5.92	13.18a	0.77
F9 (mg/kg)	13.91b	1.02	3.77a	1.86	4.33a	2.62
F10 (mg/kg)	18.66b	2.46	4.90a	1.48	8.36a	3.87
F11 (mg/kg)	35.20b	3.49	17.25a	16.46	18.15a	7.69
F12 (mg/kg)	10.02b	1.11	8.79a	1.67	9.88b	2.13
F13 (mg/kg)	45.52a	2.11	44.17a	0.59	43.06a	2.09
F14 (mg/kg)	4.28b	0.88	2.86a	0.50	3.50a	0.89
F15 (mg/kg)	10.97b	1.53	5.93a	2.36	6.44a	2.94
F16 (mg/kg)	32.05a	0.62	26.19a	5.84	28.50a	7.36
F17 (mg/kg)	6.08a	0.62	14.38b	3.21	11.37b	0.67
F18 (mg/kg)	33.36a	1.96	29.28a	53.48	28.71a	19.78
F19 (mg/kg)	6.30b	2.41	4.60a	2.27	4.19a	0.97
F20 (mg/kg)	9.93b	0.28	7.26a	2.22	7.19a	1.37
F21 (mg/kg)	2.14a	1.70	1.54a	3.55	2.13a	1.36
F22 (mg/kg)	7.83a	0.18	6.01a	14.46	7.39a	3.30
TOT (mg/kg)	318.35a	12.62	237.60ab	105.76	241.80b	64.25

[a] Data are median of three independent replicates.
[b] Within each row, median with different letters are significantly different (p at 0.05) according to Kruskal–Wallis non-parametric one-way analysis of variance and Mann–Whitney U post-hoc test.

Table 3. Results of triangle sensory test on EVOO stored in different bottles.

Bottles	Months	Proof assayed	Minimum successes	Successes	Significance
TCG vs CG	2	8	6	2	ns
	4	8	6	2	ns
	6	8	6	5	ns
	8	8	6	2	ns
	10	8	6	5	ns
	12	8	6	8	0.001
CG vs SS	2	8	6	5	ns
	4	8	6	5	ns
	6	8	6	6	0.05
	8	8	6	6	0.05
	10	8	6	7	0.01
	12	8	6	6	0.05
TCG vs SS	2	8	6	5	ns
	4	8	6	7	0.01
	6	8	6	6	0.05
	8	8	6	7	0.01
	10	8	6	7	0.01
	12	8	6	8	0.001

ns, not significant.

analysed by means of principal component analysis (PCA) to recognize pattern and structure in the analysed data.23 In our case the first two principal components (PC1, PC2) explained over 70% of the total variance of the data after rotation (varimax method). The score plot defined by the two principal components (Figure 1a) shows a separated cluster, represented by SS bottles with positives high scores on PC1. Both PC1 and PC2 almost equally contribute to explain the change of EVOO with time and showed a general trend to assume negative values with the longest storage time. The loading plot (Figure 1b), i.e. the scatter plot of correlations among variables and components, provides indications upon the parameters influencing the samples separation. On PC1, the relevant parameters were many phenolic compounds (high positive loadings), and on the opposite side some oxidative indexes such as PV with high negative loading. Such pattern was in agreement with the opposite trend of these two groups of parameters as a function of the storage time. In Figure 1a, the glass bottles appear more scattered over the plot area and it is quite difficult to distinguish between the two kinds of glass. This behaviour agrees with the above-mentioned variability which occurs within the glass bottles. In particular, CG samples tend to move from the first plot quadrant to the third quadrant by a triplet samples grouping as a function of the storage time. Unexpectedly, one of the triplet samples at 8 and 10 months of storage lied in the fourth instead of the third quadrant, distant from the other samples. A similar behaviour can be observed for TGC, i.e. in correspondence of 10 and 12 months of storage one of the triplet samples was found in the fourth quadrant. Such variability was not detected for SS bottles and this let hypothesize that some uncontrolled different light exposures of the bottles on the storing table may have occurred. On the other hand, when the experiment was set up, such condition was not predictable considering the limited area extension of the storing surface (2 m2), where a homogeneous light exposition was reasonably assumed. This evidence seems to suggest that small variations in the light exposure were able to determine large variations in the oil quality decay. To confirm this hypothesis, the light intensity over the storing surface was randomly measured (20 measurements) a posteriori, by a portable light meter (model HD 2302.0 Lightmeter, DeltaHOM srl, Padova, Italy), under the same light exposure conditions applied during the experiment. The overall average light intensity resulted of about 380 lux with a coefficient variation of about 15%. This observation confirms that certain differences in the light exposition occurred during the experiment and supports the hypothesis that such differences were probably important for EVOO stability as stated by the large variability within the glass bottles which were transparent to light. Furthermore, as only slight differences were recorded between clear and coloured glass bottles for almost all the chemicals parameters, the green color had only minor effect in oil protection against

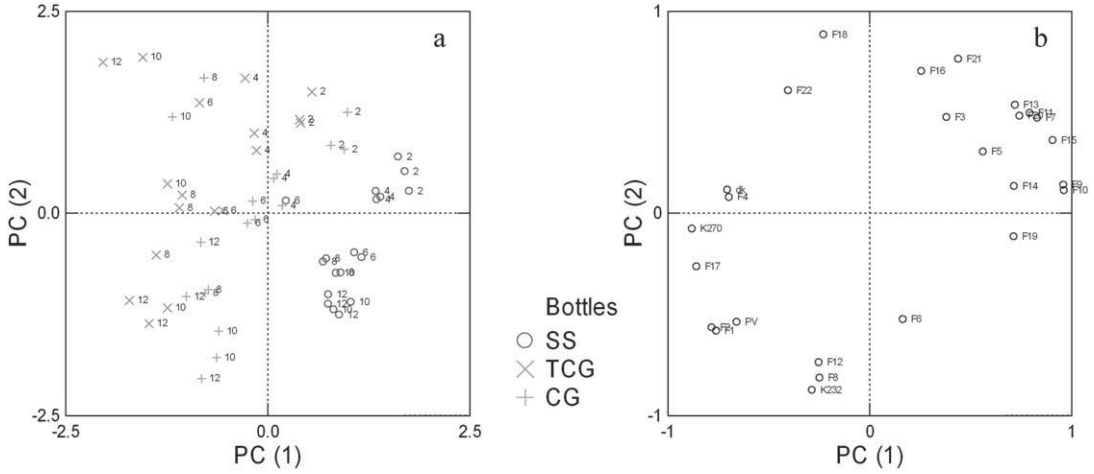

Figure 1. (a) PCA score plot defined by the two first principal components based on chemicals variables. PC (1) 55% explained variance; PC (2) 15% explained variance. SS: stainless steel bottles; TCG: transparent clear glass bottles; CG: colored green glass bottles; (b) PCA loading plot based on chemicals variables. See Table 1 for variables code.

photoxidation. In fact, no signifi cant differences were recorded for total Hydrophilic Phenols (HP) concentration, and the average decrement with respect to time 0 was quite the same for the two glass bottles. The decrease of hydrophilic phenols concentration of EVOO in glass bottles was lesser than expected (about of 60% of the initial value) on the basis of the huge increment of peroxides value (average fi nal value about 60 mEq O2/kg). This evidence may suggests that polar phenols, which act as chain-breaking antioxidants, play a rather limited role during exposure to light and that photoxidation rather than autoxidation is the main degradation mechanism occurring in the glass bottles. This could explain also the relative small increment of K232 recorded for the glass bottles at 12 months (Table 2). In fact, according to Kanavouras et al. (2004)12 only conjugated dienes (responsible for absorbance at 232 nm) are formed in free-radical autoxidation, whereas non-conjugated dienes can be found in photoxidation reactions. The small variations of K270 (related to the presence of secondary breakdown products) indicate that essentially all the oxidation products are represented by primary hydroperoxides (as also indicated by the high PV), and that an effective decomposition to secondary products was not begun.
Finally, some considerations can be drawn on the kinetic of phenolic compounds breakdown during the storage time. Figure 1b shows that different phenolic compounds were located in different positions of the area defi ned by the fi rst two principal components in relation to the storage conditions. For instance, hydroxytyrosol and tyrosol were close to the oxidative indices, with high negative load either on PC1 and PC2, whereas other compounds, e.g. dialdehydic aglyconic forms of oleuropeina and ligstroside, showed high positive loading on the two components. This pattern was probably the result of different degradation kinetics that involve the specifi c phenolic compounds and that can be better evidenced by the linear regression coeffi cients among storage time and singles phenols concentration (Table 4). The simple phenolic compounds, i.e. phenolic alcohols hydroxytyrosol and tyrosol, showed positive regression coeffi cients on storage time along with the oxidized oleuropein and ligstroside derivatives forms. These fi ndings could be explained in terms of degradation with the storage time by hydrolysis of larger phenolic molecules for the former and oxidation of the correspondents' precursors for the latter.24 On the opposite side, several compounds showed negative values of the regression coeffi cient indicating a marked degradation due to oxidation, with the dialdehydic form of the decarboxymethyl oleuropein aglycon which has shown the faster degradation kinetic. Further, as a general trend an increment of the regression coeffi cients moving from SS bottles to CG and TGC bottles was observed for almost the phenolic compounds, probably as results of the increasing oxidative conditions.6

CONCLUSION

This work confi rms that EVOO storage under light irradiation results in large oxidative changes as compared with packing in SS bottles that showed the best storage performances. The large variability detected within glass bottles, both clear and green coloured, let suppose that even small light variations are able to affect the extent of the oil quality decay that, by contrast, seems not affected by glass color. Hydrophilic phenols dynamics did not show a univocal pattern during storage time. Only some phenol showed a substantial decrement, whereas other compounds seem to be independent with time. This result probably indicates that only some specifi c phenolic components play an important role in oil protection against oxidation. Furthermore, under light exposure, the antioxidant effect of phenolics appears rather limited as compared with fatty matrix autoxidation. The use of stainless bottles represents a new packaging solution which allows extending EVOO shelf-life slowing down the product quality decay. Recently, the EVOO marketing involves the diffusion of bottles with small dimensions, i.e. between 5 and 100 ml. In the case of glass bottles, the oils could be subjected to more oxidative stress because of the higher ratio between the light exposed oil surface and the oil mass volume into the bottle. The SS bottles overcome this problem as they protect the oil from the light thus preventing photoxidation regardless of bottle dimensions.

Table 4. Regression coefficients of specific phenols concentration vs storage time.

	SS		TCG		CG	
	Regression coefficient[Δ]	[a]R^2	Regression coefficient[Δ]	[a]R^2	Regression coefficient[Δ]	[a]R^2
F1	0.519**	0.974	0.825**	0.918	0.743**	0.962
F2	0.142**	0.933	0.290**	0.909	0.275**	0.916
F3	–0.012**	0.530	–0.010**	0.446	–0.014**	0.563
F4	–0.037*	0.207	0.100**	0.331	0.080[ns]	0.196
F5	–0.043*	0.319	–0.052**	0.405	–0.055**	0.505
F6	0.224**	0.650	–0.002[ns]	0.000	0.083[ns]	0.189
F7	–2.748**	0.913	–3.484**	0.650	–3.776**	0.688
F8	0.151[ns]	0.157	0.266[ns]	0.177	0.178[ns]	0.164
F9	–0.871**	0.788	–1.092**	0.857	–1.254**	0.854
F10	–0.995**	0.754	–1.338**	0.853	–1.486**	0.856
F11	–0.852**	0.525	–1.371**	0.445	–1.538**	0.575
F12	0.272**	0.731	0.170**	0.422	0.296**	0.724
F13	–0.412*	0.247	–0.398*	0.282	–0.536**	0.375
F14	–0.184*	0.260	–0.218*	0.264	–0.211*	0.261
F15	–0.220[ns]	0.103	–0.456**	0.406	–0.491**	0.414
F16	–1.499[ns]	0.168	–2.340**	0.378	–1.976*	0.269
F17	0.134*	0.321	0.869**	0.910	0.634**	0.863
F18	–0.379[ns]	0.055	–0.283[ns]	0.004	–0.709[ns]	0.039
F19	–0.135[ns]	0.088	–0.242*	0.229	–0.262*	0.301
F20	–0.519**	0.637	–0.519**	0.492	–0.580**	0.561
F21	–0.448**	0.561	–0.448**	0.477	–0.455**	0.545
F22	0.226*	0.280	0.403[ns]	0.110	0.206[ns]	0.059

[a] Determination coefficient.
[b] Significance of the regression coefficient was tested by means of *t*-test.
* $p < 0.05$; ** $p < 0.01$, ns, no significant.

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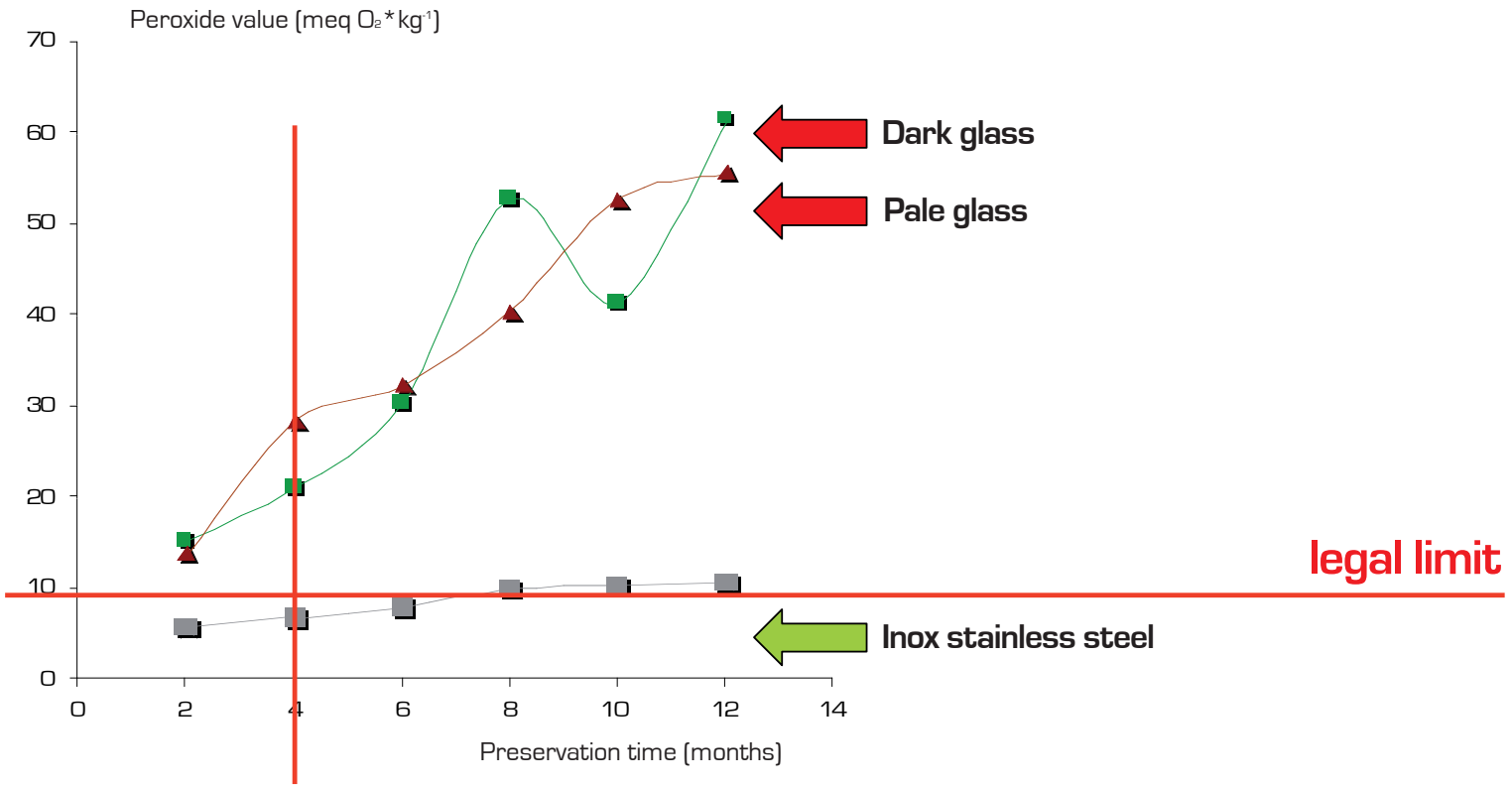
Qualitative effects:



"The conservation in stainless steel bottle keeps longer the quality of olive oil in terms of lower oxidation state, reduced loss in antioxidant compounds, the preservation of the sensory characteristics "

Prof. Alessandro Parenti - University of Florence, DEISTAF

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