Use of Nitrogen to Improve Stability of Virgin Olive Oil During Storage

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ABSTRACT: Experiments were carried out to study the possibility of improving the stability of extra virgin olive oil by using nitrogen as a conditioner gas during storage. With this aim, virgin olive oil samples, obtained from Leccino and Coratina cultivars, were stored in the dark, in closed bottles conditioned with air or nitrogen at 12–20 and 40°C. Results indicated that the FFA percentage increased over 1% only when oils were stored at 40°C. The PV and the K_{232} value (light absorbance at 232 nm) of oils increased over the limit value allowed by European Union law when the bottles were only partly filled and air was the conditioner gas. The use of nitrogen as conditioner gas helped to avoid this risk during 24 mon of storage at 12–20°C. The total phenolic content of both cultivars oils decreased during storage because their oxidation protected the oils from autoxidation. The content of total volatile compounds in oils decreased continuously during storage at 12–20°C, whereas it increased over 10 (Coratina cv.) and 15 (Leccino cv.) mon and then diminished when the storage temperature was 40°C. The same behavior, i.e., increase then decrease, was ascertained for *trans*-2-hexenal. The hexanal content of oils increased continuously during storage because this compound is formed by the decomposition of the 13-hydroperoxide of linoleic acid.

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KEY WORDS: Conditioner gas, olive oil stability, olive oil storage, virgin olive oil.

Fats and oils deteriorate during storage because of the autoxidation process, which is favored by a high content of PUFA such as linoleic and linolenic acids. In comparison with other vegetable oils, virgin olive oil is more stable during storage because of its high content of monounsaturated FA (oleic acid), its low content of PUFA, and its significant content of natural antioxidants, such as the phenolic aglycons containing tyrosol and hydroxytyrosol that originate from the phenolic glycosides present in olive fruit. Virgin olive oil stability, however, depends on storage conditions, particularly exposure to light, contact with the air, and ambient temperature.

Several investigations of the change of qualitative characteristics (1–5) and the content of some phenolic compounds (6,7) in virgin olive oil under different storage conditions have been undertaken. Other researchers have studied the influence of natural antioxidants and chemical composition on the stability of virgin olive oil (8–13). Finally, other investigators have identified the compounds formed from decomposition of FA hydroperoxides during storage of olive oil under different conditions (14–16).

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The current research was undertaken to ascertain the effects of different storage factors on the qualitative characteristics of virgin olive oil and to verify the possibility of improving its stability by using nitrogen as a conditioner gas.

MATERIALS AND METHODS

Two extra virgin olive oils were mechanically extracted, by using an industrial three-phase centrifugal decanter, from ripe olives of Leccino cultivar, grown in many regions of Italy, and medium-ripe olives of Corativa cultivar, grown in the Puglia region. The oils were stored in a closed, fully filled stainless steel vessel, in the dark at 12–15°C. The aqueous phase was removed by decantation. The separated oils, which were slightly turbid, were then bottled into green bottles. These were closed, kept in the dark, and stored under the following conditions: (i) temperature: 12–20°C (room temperature) or in a 40°C oven; (ii) volume of oil in the bottle: 98% (fully filled), 90% capacity (not fully filled), and 60% capacity (not fully filled); (iii) conditioner gas: air or nitrogen. In total, 96 samples were prepared, four for each treatment combination.

Oil samples were analyzed initially (0 mon) and after 5, 10, 15, and 24 mon from bottling to determine FA composition (at initial time only); FFA (%, as oleic acid); PV (meq O_2/kg); specific spectrophotometric absorptions at $\lambda = 232$ (K_{232}) and 270 nm (K_{270}); and organoleptic assessments (scores), according to the European standardized method (17), by a sensory panel of six trained tasters skilled in recognizing, identifying, and quantifying the sensory attributes of virgin olive oil. The different attributes of the oils were assessed, and their intensities were evaluated to produce a score between 1 and 9. Results obtained from six panelists were averaged. To be extra virgin, oil has to have an organoleptic score ≥6.5. Other parameters examined included total phenols content (mg/L, as gallic acid), by colorimetric method using the Folin–Ciocalteu's reagent (18); induction time (h), by Rancimat apparatus (19); chlorophyll pigments (mg/kg) (20); and composition of the volatile compounds in the headspace above the oils, by high-resolution GC following the dynamic method (21).

Samples of each treatment combination were analyzed, and each determination was repeated twice using the same sample. Average values were reported in the tables and figures. The qualitative results were statistically treated by ANOVA, *F* test (22). Significance was set at 5%.

RESULTS AND DISCUSSION

Table 1 shows the FA compositions and the qualitative characteristics of two extra virgin olive oil samples stored at the

above mentioned conditions. Both oils had a high content of oleic acid, a low content of PUFA, and a favorable oleic acid/linoleic acid ratio, i.e., ≥10. Furthermore, the oils were similar in some qualitative characteristics, but they were different in the total phenols content, induction time, and chlorophyll pigments content.

The results of analyses carried out on Leccino and Coratina extra virgin olive oil samples during storage are reported in the Tables 2 and 3. The data indicate that the %FFA of oils from Leccino (Table 2) and Coratina (Table 3) cultivars did not vary significantly when the storage temperature was 12–20°C. When the storage temperature was 40°C, the %FFA of the two oils increased continuously to values greater than 1%, the limit value for extra virgin olive oil, after 24 mon of storage. High storage temperature (40°C) favors the enzymatic activity of lipase, if it is still present in the oil. The oxidation of compounds formed from the decomposition of hydroperoxides of FA, such as aldehydes, ketones, and alcohols, that can be partially oxidized to the corresponding acids will contribute to increased acid levels (FFA). At 40°C, %FFA levels were not affected by the volume of oil, the conditioner gas, and the cultivar.

The PV of oils, instead, showed differences according to the total phenol content, volume of air, and temperature. Oils of Coratina cultivar (Table 3), with a content of 254 mg/L (Table 1) of total phenol, showed lesser increases in the PV than those of oils from the Leccino cultivar (Table 2), which had a content of 97 mg/L (Table 1) of total phenols. When the bottles were fully filled (98%) with oil, the PV was lower than 20 meq O_2/kg (limit value of the European Union Standard) during 24 mon of storage at 12–20°C. When the bottles were partially filled (60%) and conditioned with air, the PV stayed within the limit value of the Standard for 10 (Leccino cultivar) and 15 (Coratina cultivar) mon of storage at 12–20°C.

The presence of nitrogen, as conditioner gas, helped to reduce the kinetics of peroxidation of oils. The PV of the oils with nitrogen were lower than 20 meq O_2/kg after 2 yr of storage at all temperatures tested. This important result shows that it is possible to improve the stability of virgin olive oil by replacing air with nitrogen during storage even when the containers are not fully filled.

ANOVA (*F* test) showed that the storage time and the different levels in the bottle to which oil was filled significantly ($P < 0.05$) affected the PV of oils of both olive cultivars when air was the conditioner gas. When nitrogen was the conditioner gas, the storage time affected only the PV significantly ($P < 0.05$). This confirms that the presence of nitrogen in the bottles protects extra virgin olive oil from the oxidation process, regardless of the volume of oil in the bottle.

PV showed a decrease with storage at 40°C because, at this temperature, the rate of decomposition of the hydroperoxides was higher than that of their production.

The K_{232} value, which mainly depends on the primary oxidation step and is correlated with the PV, increased during 24 mon of storage for oils from both olive varieties when air was the conditioner gas. When nitrogen was the conditioner gas, the K_{232} value of oils did not change and remained lower than the limit value of the law for extra virgin olive oil for 24 mon. ANOVA showed that K_{232} values were significantly ($P < 0.001$) affected by the different volumes of air in the bottles. When nitrogen was the conditioner gas, the variation of the K_{232} value was not significant. This result confirms the correlation between PV and the K_{232} value and demonstrates that it is possible to avoid or reduce the increase of this parameter by using nitrogen as conditioner gas for oil stored in incompletely filled containers. This will improve the stability and the shelf life of virgin olive oils in the market.

The K_{270} value, which is related to the presence of some secondary oxidation compounds and, in particular, to those containing a carbonyl functional group, did not vary significantly and always remained lower than the limit value of the law ($K_{270} \leq 0.20$) for the oils of both olive varieties stored at 12–20°C. At 40°C, the K_{270} value increased quickly over the limit value of the law when air was the conditioner gas, but it remained under this limit for 15 mon when nitrogen was the conditioner gas.

The results of ANOVA showed that the variation in the K_{270} values for oils from both olive varieties was significantly ($P < 0.05$) affected by the different room temperatures only. This confirms the negative effect of high temperature (≥40°C), which favors the oxidation process and thus the increase in the K_{270} value.

The results of the organoleptic assessments (scores) carried out by a panel of six trained tasters (Tables 2 and 3) show

TABLE 1
Initial Qualitative Characteristics and FA Composition of Extra Virgin Olive Oils Obtained from Leccino and Coratina Olive Cultivars

Determinations	Leccino	Coratina
Qualitative characteristics		
FFA (%)	0.31	0.30
PV (meq O_2/kg)	9.2	6.6
K_{232}[a]	1.75	1.60
K_{270}[b]	0.09	0.11
Organoleptic assessment (score)[c]	7.0	7.5
Total phenols (mg/L, as gallic acid)	97	254
Induction time (h)[d]	6.6	13.0
Chlorophyll pigments (mg/kg)	3.8	20.5
FA composition (%)		
$C_{16:0}$	12.6	11.4
$C_{16:1}$	1.0	0.6
$C_{17:0}$	0.1	0.1
$C_{17:1}$	0.1	0.1
$C_{18:0}$	2.1	2.4
$C_{18:1}$	76.3	76.2
$C_{18:2}$	6.5	7.6
$C_{18:3}$	0.5	0.6
$C_{20:0}$	0.3	0.4
$C_{20:1}$	0.3	0.4
$C_{22:0}$	0.1	0.1
$C_{22:1}$	0.1	0.1
$C_{18:1}/C_{18:2}$	11.7	10.0

[a]Specific spectrophotometric absorption at λ = 232 nm.
[b]Specific spectrophotometric absorption at λ = 270 nm.
[c]Score of organoleptic assessment; when score is ≥6.5, the oil is classified extra virgin.
[d]Induction time measured by the Rancimat apparatus at *T* = 120°C and air flow = 20 L/h.

TABLE 2
Variations in the Qualitative Characteristics of Extra Virgin Olive Oil (cv. Leccino) Stored for 24 mon in Closed Bottles, in the Dark, and Under Different Conditions of Temperature and Conditioner Gas[a]

	Storage	12–20°C						40°C					
	Time	Conditioner gas: air			Conditioner gas: nitrogen			Conditioner gas: air			Conditioner gas: nitrogen		
Determinations	(mon)	Oil 98%[b]	Oil 90%[b]	Oil 60%[b]	Oil 98%	Oil 90%	Oil 60%	Oil 98%	Oil 90%	Oil 60%	Oil 98%	Oil 90%	Oil 60%
FFA	5	0.30	0.31	0.33	0.31	0.30	0.33	0.44	0.47	0.39	0.49	0.44	0.39
(%)	10	0.33	0.27	0.33	0.32	0.33	0.31	0.65	0.76	0.55	0.65	0.73	0.70
	15	0.38	0.38	0.33	0.34	0.33	0.33	0.78	0.80	0.61	0.68	0.81	0.84
	24	0.39	0.40	0.40	0.37	0.37	0.39	1.40[a]	1.48[a]	1.43[a]	1.16[a]	1.30[a]	1.36[a]
PV	5	8.7	10.5	14.0	8.6	8.8	10.6	9.3	11.4	15.3	8.1	9.6	8.1
(meq O_2/kg)	10	8.4	12.5	17.4	8.2	9.2	10.9	6.2	7.3	12.4	5.1	6.1	6.6
	15	11.9	20.7[c]	24.2[c]	12.3	14.3	13.9	8.1	18.9	13.7	5.5	5.8	4.9
	24	18.7	21.3[+,c]	36.2[+,c]	15.0	15.1	15.0	4.9[+]	5.3[+]	12.8[+]	5.9	5.0[+]	5.6[+]
K_{232}	5	1.75	1.85	2.38	1.75	1.75	1.75	1.79	1.86	2.55[b]	1.67	1.70	1.79
	10	1.72	1.93	2.50	1.72	1.78	1.75	1.70	1.77	2.43	1.60	1.61	1.71
	15	1.77	2.15	2.53[b]	1.74	1.78	1.78	1.72	1.79	2.49	1.73	1.60	1.62
	24	1.74	2.10	2.55[b]	1.70	1.79	1.73	1.69	1.69	2.28	1.51	1.60	1.90
K_{270}	5	0.09	0.09	0.09	0.08	0.09	0.09	0.12	0.12	0.14	0.12	0.12	0.12
	10	0.09	0.10	0.10	0.09	0.09	0.10	0.15	0.16	0.19	0.16	0.15	0.16
	15	0.10	0.10	0.11	0.10	0.10	0.11	0.23[a]	0.24[a]	0.26[c]	0.16	0.16	0.17
	24	0.09	0.12	0.12	0.10	0.10	0.10	0.24[a]	0.25[a]	0.29[c]	0.16	0.19	0.20
Organoleptic	5	7.0	6.5	6.5	7.0	7.0	7.0	6.5	6.5	6.5	7.0	6.8	6.5
assessment	10	7.0	6.5	6.3[a]	7.0	6.8	6.8	6.0[a]	6.0[a]	6.0[a]	6.5	6.5	6.5
(score)	15	6.5	6.0[a]	6.0[a]	7.0	6.5	6.5	6.0[a]	6.0[a]	6.0[a]	6.5	6.0[a]	6.0[a]
	24	6.3[a]	6.0[a]	6.0[a]	6.5	6.5	6.5	5.5[a]	5.5[a]	5.0[b]	6.3[a]	5.5[a]	5.5[a]
Total phenols	5	95	90	87	95	101	95	85	90	85	102	90	88
(mg/L)	10	91	87	92	90	90	99	90	86	82	95	91	83
	15	41	36	38	41	42	38	39	38	36	40	39	38
	24	39	38	34	39	38	37	38	36	34	38	37	37
Induction time	5	6.7	6.6	4.4	6.8	6.5	7.0	6.7	6.3	4.6	6.2	5.6	7.2
(h)	10	6.6	6.2	3.8	6.6	6.6	6.0	7.8	8.0	5.8	8.4	8.0	7.3
	15	6.7	6.6	3.9	6.8	6.7	6.5	8.5	7.1	5.4	8.4	8.1	8.7
	24	6.9	6.1	4.2	7.0	6.8	7.0	10.1	9.1	7.8	8.4	10.4	10.7

[a]Superscript roman letters indicate a change of the qualitative category of extra virgin olive oil to: a = virgin, b = ordinary, and c = lampante. +, Kreis test positive.
[b]The volume of oil in the bottle was 98, 90, or 60%.

TABLE 3
Variations in the Qualitative Characteristics of Extra Virgin Olive Oil (cv. Coratina) Stored for 24 mon in Closed Bottles, in the Dark, and Under Different Conditions of Temperature and Conditioner Gas[a]

	Storage Time	12–20°C						40°C					
		Air			Nitrogen			Air			Nitrogen		
Determinations	(mon)	Oil 98%[b]	Oil 90%[b]	Oil 60%[b]	Oil 98%	Oil 90%	Oil 60%	Oil 98%	Oil 90%	Oil 60%	Oil 98%	Oil 90%	Oil 60%
FFA	5	0.32	0.32	0.30	0.31	0.32	0.30	0.44	0.42	0.44	0.52	0.41	0.42
(%)	10	0.31	0.32	0.33	0.30	0.32	0.31	0.65	0.67	0.67	0.79	0.68	0.70
	15	0.30	0.32	0.33	0.28	0.27	0.28	0.71	0.78	0.79	0.77	0.69	0.67
	24	0.30	0.31	0.38	0.27	0.28	0.29	1.24[a]	1.30[a]	1.45[a]	1.21[a]	1.06[a]	1.28[a]
PV	5	5.8	6.5	12.9	5.8	6.2	8.7	8.2	10.3	12.3	7.0	8.1	6.3
(meq O_2/kg)	10	6.2	8.4	11.5	6.4	8.6	9.4	5.6	7.1	8.9	6.5	5.5	6.6
	15	9.9	13.7	18.0	9.7	10.8	11.1	6.2	6.4	9.0	7.0	4.4	6.1
	24	14.9	22.5[c]	29.4[c]	11.7	11.1	12.9	3.4	5.7	5.1[+]	2.7	2.8	2.9
K_{232}	5	1.62	1.70	1.84	1.60	1.60	1.62	1.64	1.72	2.20	1.56	1.57	1.55
	10	1.65	1.84	1.97	1.65	1.67	1.64	1.58	1.63	1.99	1.52	1.52	1.51
	15	1.67	1.89	1.97	1.70	1.75	1.66	1.53	1.51	2.02	1.52	1.54	1.51
	24	1.73	1.93	2.05	1.63	1.65	1.67	1.60	1.60	1.85	1.37	1.43	1.43
K_{270}	5	0.12	0.12	0.11	0.11	0.12	0.11	0.16	0.17	0.19	0.16	0.15	0.16
	10	0.13	0.13	0.13	0.13	0.13	0.13	0.21[a]	0.22[a]	0.27[c]	0.19	0.19	0.19
	15	0.13	0.14	0.13	0.14	0.13	0.13	0.43[c]	0.47[c]	0.45[c]	0.17	0.18	0.18
	24	0.13	0.13	0.14	0.13	0.13	0.13	0.51[c]	0.42[c]	0.50[c]	0.36[c]	0.39[c]	0.31[c]
Organoleptic	5	7.5	7.5	7.3	7.5	7.5	7.6	7.5	7.5	7.3	7.5	7.5	7.2
assessment	10	7.5	7.5	7.0	7.5	7.5	7.5	7.3	7.3	6.5	7.0	7.0	7.0
(score)	15	7.5	7.5	6.8	7.5	7.5	7.5	7.0	7.0	6.2[a]	7.0	6.7	7.0
	24	7.5	7.0	6.5	7.5	7.5	7.3	6.7	6.5	6.0[a]	7.0	6.5	6.5
Total phenols	5	254	232	201	240	248	240	236	214	198	225	230	215
(mg/L)	10	241	213	200	239	236	218	213	213	165	213	210	201
	15	136	116	128	130	122	128	110	123	102	109	116	110
	24	134	121	120	133	130	129	105	98	96	106	106	106
Induction time	5	13.0	12.1	8.1	13.0	12.6	11.6	11.6	9.6	9.0	11.6	12.0	13.0
(h)	10	12.3	10.8	9.8	13.4	13.1	10.0	15.0	14.8	12.8	16.7	16.8	15.9
	15	11.9	10.7	9.5	13.1	12.8	12.2	16.4	14.4	11.8	15.9	16.0	13.9
	24	13.3	11.3	10.5	13.3	13.2	13.4	17.7	17.9	18.2	18.6	16.5	17.2

[a,b]For footnotes see Table 2.

that the oils with a low content of total phenols (Leccino cultivar) developed the rancid defect (score lower than 6.5) after 10 mon of storage in partially filled bottles at room temperature (12–20°C) and with air as the conditioner gas. The oils conditioned with nitrogen remained without organoleptic defects (score ≥6.5) for 24 mon of storage under the same conditions. Higher storage temperatures (40°C) favored the oxidation of oils (Leccino cultivar), which became rancid after few months of storage, especially when air was the conditioner gas.

Oils with a higher initial content of total phenols (Coratina cultivar) remained without organoleptic defects for a longer time because of the protection of these natural antioxidants. The protective effect of nitrogen as conditioner gas was evident at 40°C, for the oils stored in partially filled bottles (60%) remained without organoleptic defects for 24 mon, whereas oils stored with air became rancid after 15 mon of storage (Table 3).

The total phenolic content of oils of Leccino and Coratina olive cultivars diminished under the different test conditions. The total phenolic content of both oils changed significantly ($P < 0.001$) but only with respect to the storage time. Total phenols were not significantly affected by other parameters. Under the conditions used in this research, the total phenolic content diminished from 91 to 41 mg/L for oil of the Leccino cultivar, and from 241 to 136 mg/L for oil of the Coratina cultivar, during the period between 10 and 15 mon of storage; this parameter did not change significantly after 15 mon of storage (Tables 2 and 3). This observation was probably due to the consumption of phenolic substances with higher antioxidant capacity, such as *o*-diphenols, and to the residue of the phenolic compounds with a low antioxidant capacity, such as tyrosol and the phenolic aglycons containing tyrosol. The latter substances, although reducing the Folin–Ciocalteu's reagent utilized to determine the total phenol content of oils, have only a low protective power towards the oxidation of oil (9,13).

The values for induction times fell significantly ($P < 0.05$) for both oil samples (Leccino and Coratina olive cultivars) stored in partly filled bottles (60%), with air as conditioner gas and at room temperature (12–20°C). Under other conditions, induction time values did not change or else increased, especially at 40°C. This result allows us to affirm that at 40°C the positive correlation between the induction time value and the total phenols content of oils is not valid. This is probably due to the formation, during 40°C storage, conditions, of other compounds, such as polymeric TG, that could increase the resistance of oil to the forced oxidation that occurs with the Rancimat apparatus operating at 120°C and an air flow of 20 L/h.

The results of the chlorophyll pigments' determination, not reported in the Tables, indicate that their content did not change significantly during the storage of oils. The decrease in chlorophyll pigment content was more evident only for oils stored at 40°C in partially filled bottles and with air as conditioner gas.

The results obtained in the determination of volatile substances in the headspace above oils at room temperature (12–20°C), not reported in the Tables and Figures, indicate that, generally, the content of total volatile compounds, slightly decreased. In particular, the content of *trans*-2-hexenal, which is responsible, together with other corresponding alcohols, for the fresh-cut-grass aroma of oil, diminished continuously, especially when air was the conditioner gas. The presence of nitrogen helped to reduce the decrease in *trans*-2-hexenal. Conversely, the content of pentan-3-one and C_6 saturated and unsaturated alcohols, not reported in Table and Figures, increased for oils stored at room temperature and in partially filled bottles conditioned with air.

At 40°C, the total volatile compounds content increased up to 15 and 10 mon of storage for oils of Leccino and Coratina cultivars, respectively, as shown in the Figure 1. This is due to the increase in the content of hexanal (Fig. 2) and *trans*-2-hexenal (Fig. 3), which originate, respectively, from the decomposition of 13-L-hydroperoxides of linoleic and linolenic acids (23), favored by the higher storage temperature.

Figure 2 shows the variation in hexanal for Leccino and Coratina oils stored at 40°C. The content of this compound increased when air was the conditioner gas in partially filled bottles, causing the detection of rancid defect, as confirmed by the tasters and the scores of the organoleptic assessment. The presence of nitrogen, as conditioner gas, reduced the rate of formation of hexanal in all cases and delayed the formation of rancid off-flavor.

Figure 3 shows that the *trans*-2-hexenal content of oils stored at 40°C increased in the first 5 or 10 mon. High temperature favored the formation and decomposition of the 13-L-hydroperoxide of linolenic acid, from which *trans*-2-hexenal originates (23). After 10 and 15 mon of storage, the content of this compound diminished, in agreement with the results reported in other papers (3,4).

The results from this research support the view that virgin olive oil keeps its quality characteristics for many months if it is stored in fully filled bottles, in the dark, and at room temperature (12–20°C). The quality of the oil deteriorates in only a few months if it is stored in partially filled bottles conditioned

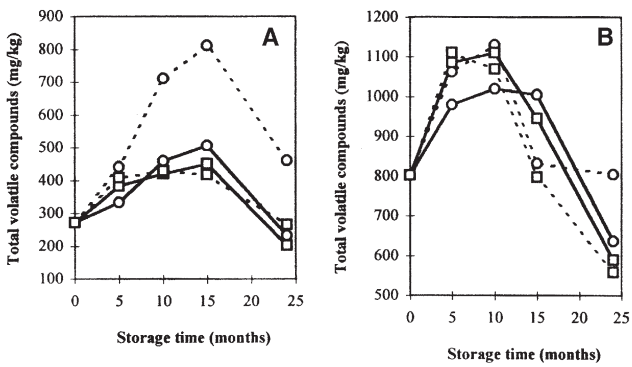

FIG. 1. Variation in total volatile compounds content of Leccino (A) and Coratina (B) oils stored at 40°C in fully (98%) filled (–○–, –□–) and incompletely (60%) filled (--○--, --□--) bottles conditioned by air (--○--, –○–) or nitrogen (--□--, –□–) gas.

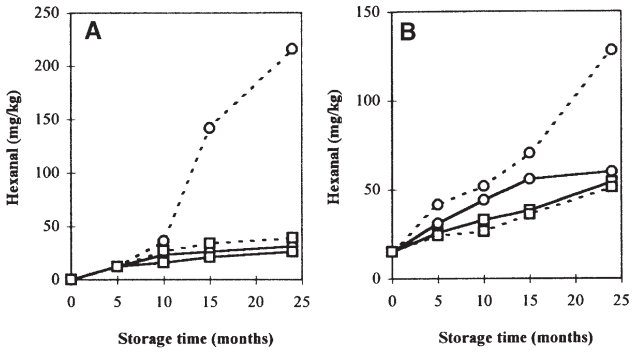

FIG. 2. Variation of hexanal content of Leccino (A) and Coratina (B) oils stored at 40°C. For key see Figure 1.

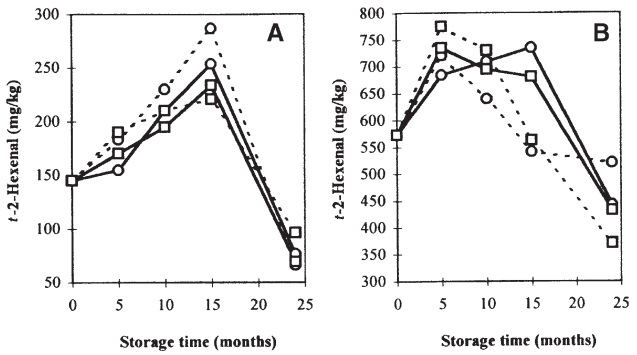

FIG. 3. Variation of *trans*-2-hexenal content of Leccino (A) and Coratina (B) oils stored at 40°C. For key see Figure 1.

with air. The presence of nitrogen, as conditioner gas of oil stored in partially filled bottles, improves the stability and the shelf-life of the oil and slows the oxidation process. These results allow us to predict that nitrogen can be used effectively in storing virgin olive oil in high-capacity stainless steel tanks before its bottling. During this step, the oil is exposed to the oxidation process, and it is important to protect it by storing it under a conditioned atmosphere with nitrogen. The temperature of 40°C is too high for the storage of virgin olive oil, because this condition favors the increase of the %FFA and the K_{270} value, with the development of the rancid defect and, consequently, a low score for the organoleptic assessment.

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