Influence of Oxygen and Copper Concentration on Lipid Oxidation in Rapeseed Oil

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ABSTRACT: The influence of oxygen concentration and copper on lipid oxidation in rapeseed oil during storage at 40°C was investigated. The oil was stored in air, or with 1.1%, 0.17%, or 0.04% oxygen in the headspace, and 70 or 0.07 ppm copper was added. Volatile oxidation products and oxygen consumption were monitored. Addition of 70 ppm copper to the sample in air resulted in a 70-fold higher hexanal concentration after 35 d of storage, compared to the sample without added copper. The addition of 0.07 ppm copper to the sample stored in air gave a doubled hexanal concentration, compared to the sample without copper, after 35 d of storage. For the samples with 70 ppm copper at 0.17% and 0.04% oxygen, all oxygen was consumed after 7 d of storage. The results show the importance of minimizing the oxygen available for oxidation, especially when pro-oxidants are present. In the sample with 70 ppm added copper, in air, the hexanal increase was 65 times larger than for the same sample in 0.04% oxygen. A comparison of the effect of oxygen or copper on oxidation shows that the addition of 70 ppm copper to the 0.04% oxygen sample gave the same increase in hexanal content as an oxygen increase to 0.17%.

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Lipid oxidation is one of the major causes of quality losses in oils during storage (1). One important factor to influence the rate of oxidation is the concentration of metals in the oil. Copper and iron are the most active metals for inducing oxidation in oils (1–3); however, copper is reported most pro-oxidative (1,3). To ensure oxidative stability, it is recommended that the copper content be kept below 0.02 ppm in refined, bleached, and deodorized soybean oil (4). The amount and concentration of oxygen available for oxidation is another crucial factor that influences the rate of oxidation in food (5, 6). Even though much research has been done, there is still little known about the tolerance level of oxygen and oxygen consumption for different kinds of food (5).

Marcuse and Fredriksson (7) investigated the effect on lipid oxidation of the addition of various concentrations of copper salt to linoleic acid emulsions at various pH and oxygen concentrations. They found that, at pH 7 in air atmosphere, copper acted as a pro-oxidant, measured as oxygen consumption, and that the pro-oxidative effect increased with higher copper concentrations. However, with 1% oxygen in the atmosphere, copper was found to act as an antioxidant, instead of being a pro-oxidant, at certain concentrations and pH values.

Min and Wen (8) found that 0.1 ppm of added iron was sufficient to increase the rate of oxidation in soybean oil. By binding the trace metals (copper and iron) on grafted cellulose or a cationic resin, Benjelloun *et al.* (9) were able to decrease the rate of oxidation in rapeseed oil, thereby emphasizing the role of even small amounts of metal in the oxidation of oil. Karahadian and Lindsay (10) reported that 20 ppm of copper (II) ions had a strong pro-oxidative effect in fish oil. Evans *et al.* (1) found that addition of 0.003 ppm copper to soybean oil did not give a lower flavor score after 4 d of storage at 60°C, compared to samples without added copper. However, addition of 0.03 ppm had a measurable pro-oxidative effect after the same storage time. Min and Wen (11) found the level of dissolved oxygen to have a significant effect on the formation of volatile compounds in soybean oil. Even though the dissolved oxygen almost disappeared after 48 h of storage, the formation of volatiles continued after this point.

Investigations of the effects of trace metals and oxygen level on lipid oxidation in oil have previously been conducted separately (1,8–11). However, no investigations of the effect of copper in combination with various oxygen concentrations in pure oil have been reported. Also, the development of new packaging concepts with polymeric materials that are not absolute barriers to oxygen, instead of more traditional materials such as glass and metals, has focused interest on the influence of oxygen concentration on lipid oxidation. The lack of reports in the area of oxygen levels and oxidation is especially critical when it comes to oxidation at low oxygen concentrations and, even more so, if factors that have an accelerating effect on the lipid oxidation are also taken into account.

In this study, we investigated the lipid oxidation in rapeseed oil at four different oxygen headspace concentrations, without added copper as well as with added copper at two concentrations: 0.07 ppm, which is slightly above the level of 0.02 ppm recommended not to be exceeded in practice (4), and 1000 times higher. The oxygen concentrations ranged from 21% to 0.04%, with our main interest in the area of 1%

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oxygen and below, where changes of the oxygen concentration is considered to be particularly important.

EXPERIMENTAL PROCEDURES

Sample preparation. Rapeseed oil was prepared without added copper and with 0.07 and 70 ppm added copper, at four different oxygen concentrations: air, 1.1, 0.17, and 0.04%. $CuSO_4 \cdot 5H_2O$ (Fisher Scientific Company, NJ) was dissolved in methanol (E. Merck, Darmstadt, Germany) and added to the rapeseed oil (59% oleic acid, 21% linoleic acid, and 9.5% linolenic acid) (Zeta AB, Sweden). The original copper concentration of the oil was less than 0.1 ppm, according to the manufacturers. To eliminate the possible influence of methanol, the same amount of methanol was added to all samples in the study. The study was performed in two consecutive experiments. In the first experiment, oil without added copper and oil with 70 ppm added copper were studied, and in the second experiment, oil with 0.07 ppm added copper was studied together with a control, which consisted of oil without added copper with air in the headspace. Both samples, with and without added copper, were treated according to the following procedure. After the addition of the copper solutions and/or pure methanol, the oil was shaken for 2 min and then evaporated (Zymark Turbo Vap® LU Evaporator, Zymark, MA) for 10 min at 35°C to reduce the methanol content of the sample. The oil was stirred with a magnetic stirrer during further preparation. To maximize the surface area of the oil and thereby minimize the effect of oxygen diffusion, the oil was applied to dental cotton wool rolls. The rolls (100% cotton, Top Dent, Switzerland) were sliced with a scalpel to give an incision along the roll. Half a mL of oil was dropped into the incision of the roll with a pipette. Five oil-containing rolls were put into a 500-mL glass bottle, which was sealed with a stopper made of butyl rubber (Industrigummi, Solna, Sweden).

To obtain oxygen concentrations below 21%, the bottles were evacuated and filled with nitrogen by the method described by Andersson and Lingnert (12). The samples were stored in darkness at 40 ± 1°C and were analyzed after 0, 7, 13, 21, 28, and 35 d of storage. Samples from all of the prepared oil batches were also frozen and kept at −70°C.

Measurement of peroxide value (PV). PV were measured by the official ferric thiocyanate method of the International Dairy Federation (IDF) (13) as modified by Ueda *et al.* (14). *Iso*-hexane (HPLC grade, Fisons, Loughborough, England) was used instead of *n*-hexane to dissolve the rapeseed oil (Undeland, I., H. Lingnert, and M. Stading, unpublished data).

Analysis of α-tocopherol and γ-tocopherol. Tocopherols were determined by normal-phase HPLC, by the method of Piironen *et al.* (15) with minor modifications. The undiluted oil was injected onto the column (LiChrosorb 5 Silica, 250 · 2.10 mm, Phenomex, Torrance, CA), and the tocopherols were eluted with *iso*-hexane:2-propanol (99.8:0.2) (LiChrosolv, E. Merck, Darmstadt, Germany) at a flow rate of 0.5 mL/min. A fluorescence detector (Hewlett-Packard 1046A) was used with an excitation wavelength of 292 nm and an emission wavelength of 324 nm. Identification and quantitation were done by using standard solutions (>95%, E. Merck, Darmstadt, Germany). Peaks were integrated with a Softron PC program (version 1.0).

Oxygen analysis. The oxygen headspace concentrations in the bottles after packaging and storage were analyzed with a gas chromatograph by the method described by Andersson and Lingnert (16).

Analysis of volatile oxidation products. The volatile oxidation products were measured by a dynamic headspace sampling method described by Hall *et al.* (17). The sample was allowed to equilibrate at 40°C for 1 h, and 5 L of headspace gas was sampled. Cartridges that contained the adsorbent material were stored at −18°C until analysis. Analysis of the volatile oxidation products was done in triplicate in a gas chromatograph coupled to a flame ionization detector (FID) and a mass spectrometer as previously described by Andersson and Lingnert (12). A Hewlett-Packard 3550 laboratory data system was used to collect data and quantitate the amounts of volatiles.

Statistical evaluation. Student's *t*-test was performed to evaluate significant differences between samples.

RESULTS

Oxygen concentration. The changes in oxygen concentrations during storage are shown in Figures 1–4. The samples with 70 ppm added copper showed high oxygen consumption. After 7 d, the samples with initially 0.17% and 0.04% oxygen had consumed almost all oxygen. The samples with initially 1.1% oxygen concentration had consumed all oxygen after 13 d, and the sample packed in air had consumed all oxygen after 28 d of storage. The samples with 0.07 ppm added copper showed, as expected, much lower oxygen consumption than the samples with 70 ppm copper (see Figs. 1–4).

Volatile oxidation products. Hexanal and 2-hexenal were monitored as indicators of lipid oxidation. Hexanal is mainly formed from n-6 fatty acids, and 2-hexenal is produced from

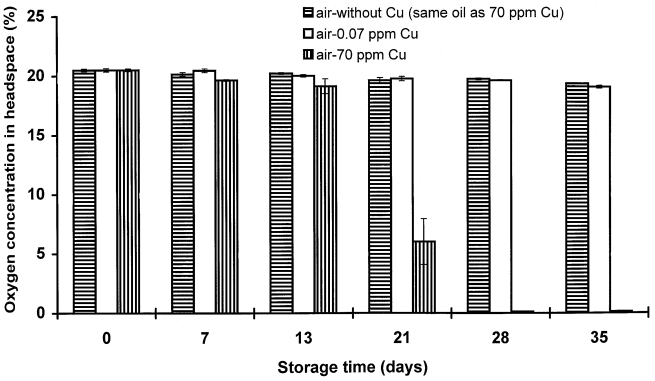

FIG. 1. Change in oxygen concentration in headspace (%) over time for the samples packed in air with no copper, 0.07 ppm copper, and 70 ppm copper. Each level is a mean value of three measurements.

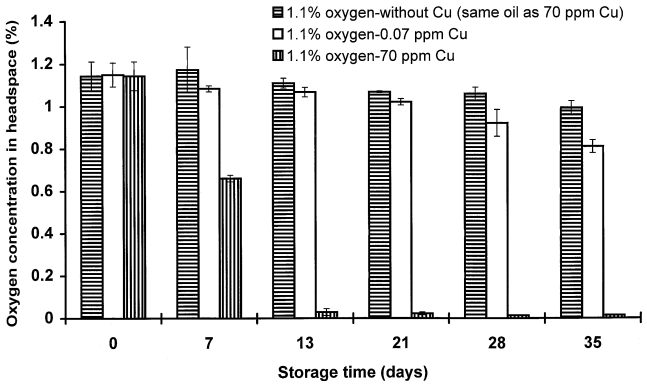

FIG. 2. Change in oxygen concentration (mean value of three measurements) in the headspace (%) over time for the samples with initially 1.1% oxygen in the headspace.

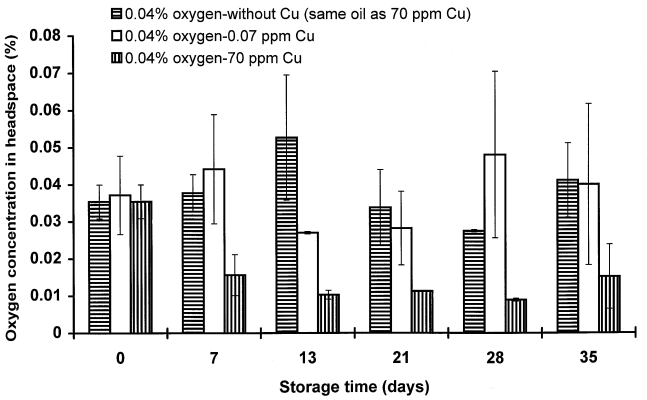

FIG. 4. Change in oxygen concentration in the headspace (%) for the samples with initially 0.04% oxygen in the headspace. Mean value of three measurements.

linolenic acid (18,19), which is the fatty acid most susceptible to oxidation in rapeseed oil.

The hexanal (Figs. 5 and 6) and 2-hexenal (Figs. 7 and 8) concentrations increased with increasing storage time. After 35 d of storage, the hexanal concentrations for the air samples and the 1.1% oxygen samples with 70 ppm added copper reached values of 30,000 ng/L headspace (HS) and 2140 ng/L HS, respectively. Comparison of the samples without and with addition of 70 ppm copper (Figs. 5 and 7) shows that this addition, as expected, greatly increased oxidation. At the end of the storage period, the hexanal increase for the samples stored in air with copper was 70 times larger than that of the samples stored without copper. The difference between the samples with and without copper decreased with a decreasing amount of oxygen. With 0.04% oxygen in the headspace, almost the same hexanal increase was seen for the samples with and without copper. Without added copper, only small increases in volatiles for the samples with reduced oxygen concentration were seen. However, the increase in hexanal was almost seven times larger for the sample stored in air, without added copper, than for the sample stored in 0.04% oxygen, also without added copper.

In the experiment with 0.07 ppm copper (Figs. 6 and 8), the sample with added copper with air in the headspace showed larger hexanal and 2-hexenal increases than the air-packed sample without added copper (after 35 d of storage, approximately 2 and 1.7 times the amount, respectively). The samples with 1.1%, 0.17, and 0.04% oxygen in the headspace with 0.07 ppm copper, showed less hexanal production than the sample without added copper packed in air.

Oil analyses prior to storage. As stated earlier, the study was performed in two consecutive experiments. Comparison between the two experiments—for instance, the hexanal content of the air samples without added copper in Figure 5 and Figure 6, respectively—indicated differences between the oils used (two separate bottles from the same manufacturing batch). Therefore, oil from these two bottles was analyzed for PV and tocopherols. No significant differences in PV between the two oils were found. The PV was 0.35 ± 0.08 meq/kg oil. Neither were there any significant differences in α-tocopherol content (277 ± 9.2 μg/mL). However, the first oil had a

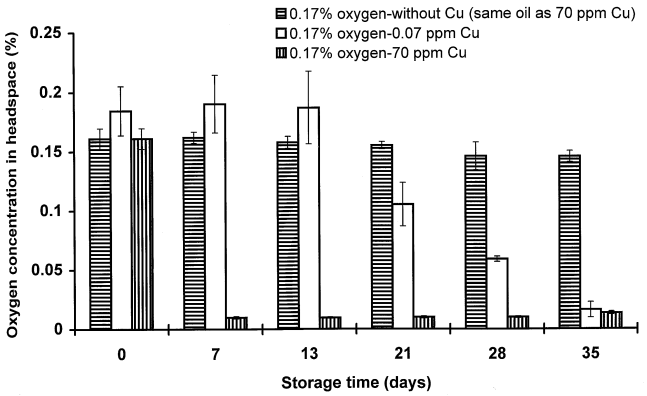

FIG. 3. Change in oxygen concentration in the headspace (%) for the samples with initially 0.17% oxygen in the headspace. Mean value of three measurements.

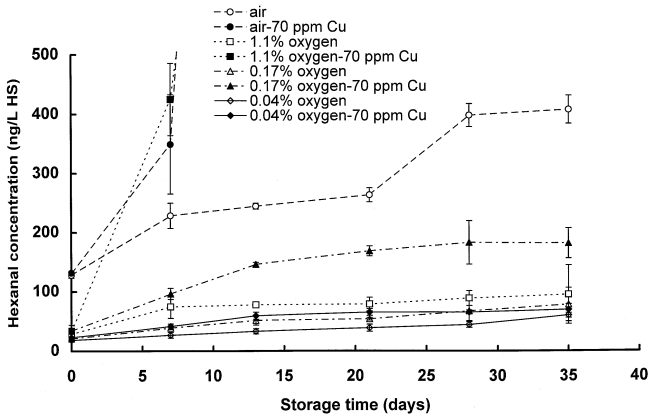

FIG. 5. Hexanal concentration [(ng/L) in the headspace (HS)] of the samples without and with 70 ppm copper in air, in 1.1% O_2, in 0.17% O_2, and in 0.04% O_2. Error bars indicate standard deviations from three measurements.

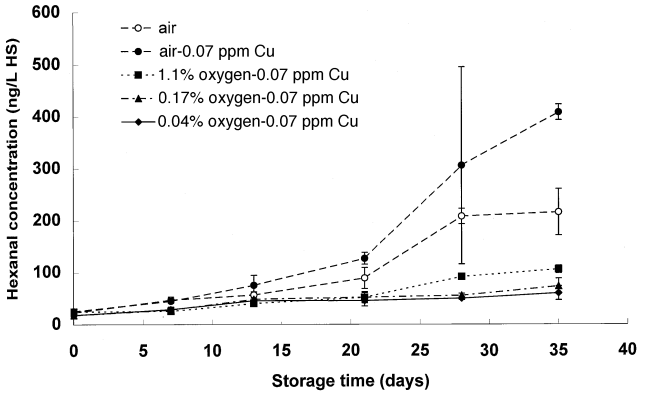

FIG. 6. Hexanal concentration (ng/L HS) of the samples without copper in air and with 0.07 ppm copper in air, in 1.1% O_2, in 0.17% O_2, and in 0.04% O_2. Error bars indicate standard deviations from three measurements. See Figure 5 for abbreviation.

γ-tocopherol content that was significantly ($P = 0.0002$) lower than in the samples from the second experiment, 403 ± 7.5 μg/mL compared to 430 ± 9.5 μg/mL.

DISCUSSION

Even though the samples in the two experiments were not directly comparable because of initial differences between the oils used, it is obvious that the effect of copper increases at higher concentrations. The differences in γ-tocopherol content, together with differences in initial content of volatiles (compare the air samples in Figs. 5 and 6), indicated differences in oxidative status at the start of the experiments. This difference could not be detected by PV measurements or in the α-tocopherol content. However, it has previously been reported that γ-tocopherol was oxidized before α-tocopherol in rapeseed oil (20).

The addition of 70 ppm copper to the oil gave a 70-fold increase in the hexanal content and an almost 200-fold increase in the 2-hexenal content after 35 d of storage for the sample packed in air. If the addition was only 0.07 ppm, there was a 1.5-fold increase in hexanal content and a 2-fold increase in 2-hexenal content during the same storage time in air. This confirms the importance of keeping the copper content as low as possible. The oxygen concentration also had a strong influence. After 35 d of storage, the increase of 2-hexenal in the sample without added copper in air atmosphere was 5 times larger than in the sample with only 0.04% oxygen. For the sample with the highest amount of added copper, the 2-hexenal increase in air was 280 times that at 0.04% oxygen. This result shows the need for keeping the amount of oxygen available for oxidation as small as possible, especially in samples susceptible to oxidation.

After 7 d, the samples with 70 ppm added copper and initially 0.17% and 0.04% oxygen in the headspace had already used almost all of their oxygen and were not expected to oxidize further. The sample stored in air still had oxygen in the headspace after 21 d of storage. The increase in volatiles seen after all oxygen had been consumed was in accordance with the results of Min and Wen (11), who found that the production of volatiles continued after the oxygen available for oxidation had been consumed. A difference in initial hexanal and 2-hexenal concentrations was seen between the samples with different oxygen concentrations (Figs. 5–8). This was due to the evacuation process, which, in addition to removing oxygen, removed part of the volatile oxidation products already present in the sample. Therefore, throughout this study, comparisons were always made regarding increase of volatiles.

No tendencies for copper to act as an antioxidant, as found by Marcuse and Fredriksson (7), were seen in this study. In their experiments, a linoleic acid emulsion was used as a model system, and oxidation was measured by monitoring oxygen consumption. They found that an emulsion with a 10^{-3} M copper concentration oxidized slower at pH 6 and pH 5 at low oxygen concentration (1%) than did an emulsion without added copper. However, our system was differ-

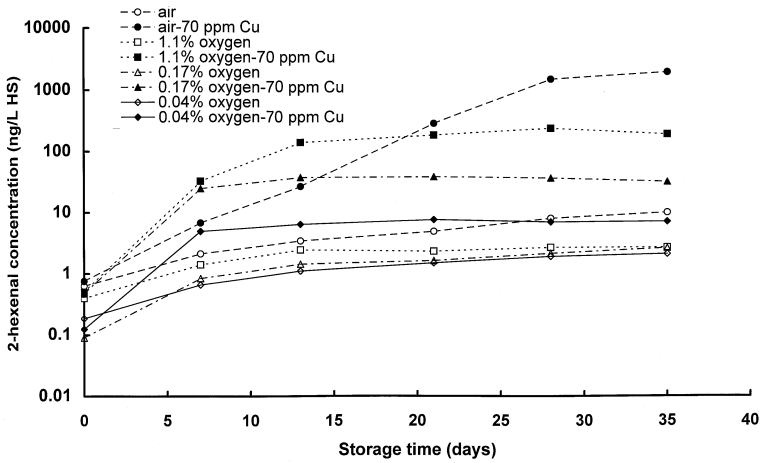

FIG. 7. 2-Hexenal concentration (ng/L HS) of the samples without and with 70 ppm copper at four oxygen levels as in Figure 5. Error bars indicate standard deviations from three measurements. See Figure 5 for abbreviation.

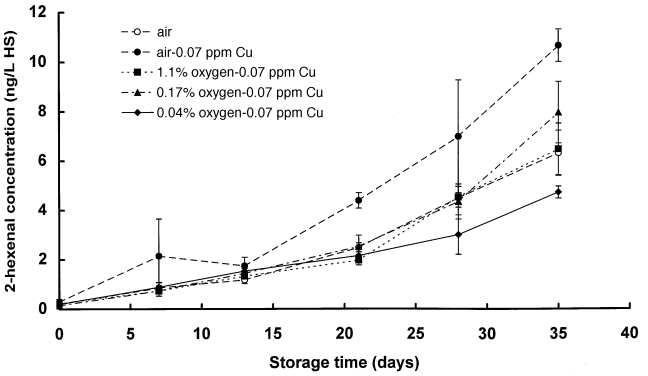

FIG. 8. 2-Hexenal concentration (ng/L HS) of the samples without copper in air and with 0.07 ppm copper at four oxygen levels as in Figure 6. Error bars indicate standard deviations from three measurements. See Figure 5 for abbreviation.

ent because we used a pure oil and not linoleic acid in an emulsion.

The relative importance of oxygen and copper in promoting lipid oxidation has to be determined before the change in oxygen concentration is too large—i.e., in this experiment, during the first 7 d of storage. Addition of 70 ppm copper to the oil approximately doubled the hexanal increase at 0.04% oxygen. The same increase was seen when the oxygen concentration was raised from 0.04% to 0.17%when no copper was added. However, at 1.1% oxygen, a more than 8 times larger hexanal increase was seen when 70 ppm copper was added, while the increase of oxygen to 21% only doubled the hexanal increase. An explanation of the smaller difference in hexanal concentration at the higher oxygen concentration (even though the increase in oxygen was much larger) could be that lipid oxidation is more influenced by changes in oxygen concentration at low oxygen levels (21, 22). The difference could also be due to consumption of all oxygen for the 0.04% sample with addition of copper. In the second experiment, both the sample with and that without copper in air showed the same increase in hexanal during the first seven days of storage, indicating that addition of 0.07 ppm copper is not so important during the first period of storage, even though this small addition seems to increase oxidation during longer storage.

A difference in order between the samples for hexanal and 2-hexenal production is seen (Figs. 5–8). The addition of copper seems to be more important for 2-hexenal production. The 2-hexenal production was larger for the 0.17% samples with copper (Figs. 7 and 8) than for the samples without copper in air, while the air samples without copper have higher hexanal production (Figs. 5 and 6) than the 0.17% samples with copper. In the oil where the addition of 0.07 ppm Cu was studied, the order between the 1.1% O_2 sample with added copper and the air sample without copper in hexanal and 2-hexenal formation was also reversed. One explanation for these order reversals could be that the oxidation reaction of linolenic acid is more influenced by copper concentration than the oxidation reactions of linoleic acid. This could, for example, be true if copper mainly influences the breakdown of hydroperoxides and this is the rate-determining step for the production of 2-hexenal, while the formation of hydroperoxides is rate-limiting for the production of hexanal. Addition of copper to the oil would then have a greater effect on the production of volatiles from linolenic acid.

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