High-Pressure Effects on Lipid Oxidation

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ABSTRACT: Rendered pork fat (0.44 water activity, A_w) was subjected to high-pressure treatment of 800 MPa for 20 min at 19°C prior to storage at 4, 25, and 50°C. In all cases, high pressure-treated samples oxidized more rapidly (had a shorter induction period) as shown by the peroxide value (PV), 2-thiobarbituric acid value, and ultraviolet absorbance. The effect was less marked at lower pressures. In contrast, at all water activities outside the range of 0.40–0.55, the PV of the high pressure-treated pork fat was lower than control samples stored similarly, and the PV of rancid fat decreased slightly on pressure treatment at 19°C, but not at higher temperatures. This effect may explain the observed inhibition of oxidation at most water activities. At 0.40–0.55 A_w, other factors, such as the liberation of transition metals, may override the destruction of peroxides.
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KEY WORDS: Conjugated diene, high pressure, lipid oxidation, peroxide value, rancidity, thiobarbituric acid value, water activity.

The application of high pressures (up to 800 MPa) to preserve and modify the properties of foods and food ingredients is receiving increasing attention because it can affect gel formation, enzyme activity, and the survival of microorganisms (1,2). There have been few studies, however, on the effects of high-pressure treatment on lipid changes in food, and, because lipid oxidation can limit product shelf life, such a study seemed desirable.

When cod muscles were exposed to 202, 404, and 608 MPa for 15 and 30 min, the peroxide value (PV) of the extracted oils increased with increasing pressure and processing time; even more pronounced effects were observed with mackerel muscle lipids (3). Tanaka *et al.* (4) studied sardine oils mixed with defatted sardine meats. When the samples were treated at 108 MPa, the PV and 2-thiobarbituric acid (TBA) number of samples stored at 5°C increased with processing time more rapidly than did the untreated sample. However, when the extracted lipids were treated similarly, but in the absence of defatted meat, oxidation was minimal. Thus, under most circumstances, the limited data reported suggest that high pressures catalyze lipid oxidation in fish tissues, although Tanaka *et al.* (4) found that, at a water activity (A_w) of 0.97, high pressure-treated sardine lipids in the presence of muscle tissue oxidized more slowly than untreated samples. These authors (4) used freeze-dried, defatted tissue to adjust A_w to various values and found that pressure was catalytic at lower water activities. The other groups (3) did not monitor A_w.

Wada (5) suggested that lipid oxidation after high-pressure treatment is due to a cooperative effect with the denatured protein in the meat. Tanaka *et al.* (4) believed that certain metal ions also may play an important role in promoting autoxidation of lipids in fish meats subjected to high pressures. To eliminate, as far as possible, the role of proteinaceous material, the present study was carried out on refined (rendered) pork fat. Because A_w is important in lipid oxidation (6), this parameter was varied.

MATERIALS AND METHODS

Preparation of sample. Rendered pork fat was prepared by heating fatty tissues from pig with water (40% w/w) at 120°C for 3–4 h. The upper layer of rendered fat was collected by decanting. The relative humidity of rendered pork fat, as determined by Protimeter Dewpoint Meter DP 383R (Marlow, Bucks, England), was 44% (0.44 A_w). Unless stated otherwise, freshly prepared fat or fat held in storage at –20°C for 2–3 wk was used.

High-pressure treatment. Rendered pork fat was sealed in polyethylene sachets (Stansted Fluid Power Ltd., Stansted, United Kingdom) and subjected to pressures up to 800 ± 10 MPa for 20 min as described previously (7). The pressure cell was held at the appropriate temperature by circulating water. The temperature in the cell was constantly monitored. During high-pressure application carried out over 20 min, in the first 2 min, the temperature increased by about 15°C but decreased to the initial temperature within a further 5 min. Untreated samples (control) also were held at the temperature at which the pressure was applied for 20 min.

Experiment 1: effects of high-pressure treatment at 800 MPa for 20 min and storage temperature at 0.44 A_w. After high-pressure treatment at 800 MPa for 20 min at 19°C, 3-g portions (±0.1 g) were placed into air-tight, screw-capped, amber glass bottles (120 mL) and stored at 4, 25, or 50°C. Control samples were stored in a similar manner. During storage, samples were taken out at suitable time intervals for the determination of PV (8), TBA value (9), and ultraviolet ab-

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sorption spectra (10). All chemicals used were of analysis grade.

Experiment 2: effects of the intensity of high pressure at 0.44 A_w. Rendered pork fat was subjected to 200, 400, and 600 MPa for 20 min at 19°C, divided into 3-g portions (±0.1 g), and stored at 50°C. Their PV and TBA values were determined after 3, 6, and 10 d. Control samples were stored in a similar manner.

Experiment 3: effects of water A_w. After high-pressure treatment at 800 MPa for 20 min at 19°C, 3-g portions (±0.1 g) were distributed thinly onto plastic dishes (5-cm diameter) and placed into air-tight bottles. Sulfuric acid of different concentrations was used to maintain the A_w in the bottles at predetermined values (11). Control samples were stored in a similar manner. During storage at 50°C, samples were taken out for the determination of PV and TBA at 4, 6, and 8 d.

Experiment 4: effects of high-pressure treatment at 0.76 A_w. The protocol was the same as in Experiment 3, except that rendered pork fat and untreated (control) were maintained at only one A_w, i.e., 0.76 A_w.

Experiment 5: effects of temperature during high-pressure (800 MPa) treatment at 0.76 A_w. The protocol was the same as in Experiments 3 and 4, except that rendered pork fat was subjected to high-pressure treatment at different temperatures (19, 25, and 45°C) prior to storage.

Experiment 6: Effects of temperature and pressure (800 MPa) on oxidized samples. Rancid samples (stored at 50°C for 4 d) were subjected to high-pressure treatment at 800 MPa for 20 min at different temperatures (19, 32, and 50°C) and analyzed immediately. Untreated rancid samples (control) were also held at the same temperatures for 20 min.

Statistical analysis. Differences between individual means were tested for significance by using the Student's *t*-test, and other data were subjected to analysis of variance (ANOVA) (12).

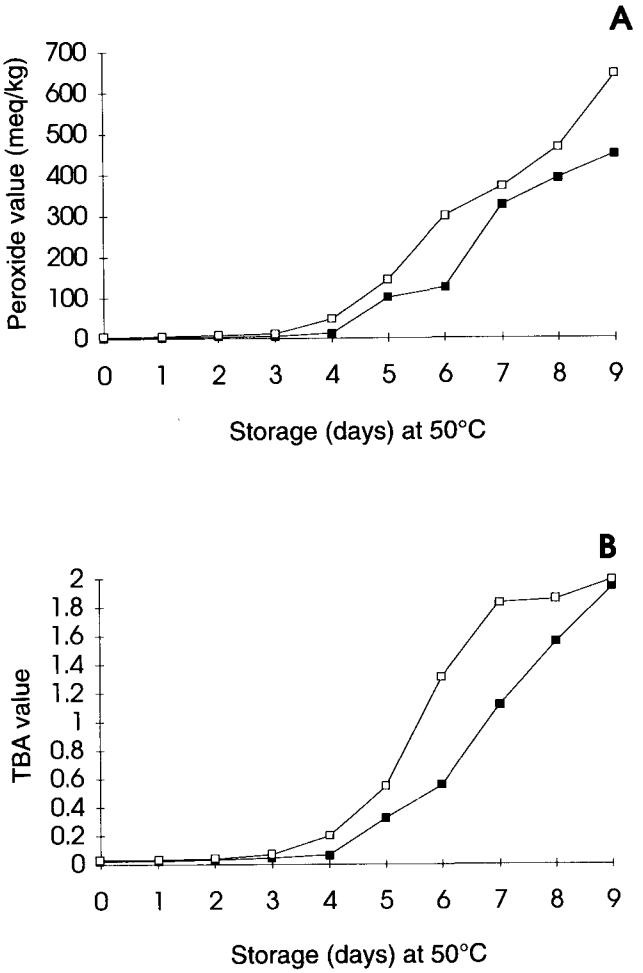

FIG. 1. Peroxide values (A) and 2-thiobarbituric acid (TBA) values (B) during storage at 50°C in the dark of rendered pork fat (■), and rendered pork fat treated at 800 MPa for 20 min at 19°C, prior to storage (□). Values are means of three determinations.

RESULTS AND DISCUSSION

Effects of high pressure at 800 MPa treatment for 20 min and storage temperature at 0.44 A_w. The "normal" A_w of the rendered pork fat was 0.44, and the changes in PV and TBA values during storage at 50°C are shown in Figure 1. The high pressure-treated samples had a shorter induction period (*ca.* 3 d) than the control sample (*ca.* 4 d). A further experiment confirmed these results. The specific absorbance ($E^{1\%}_{1cm}$) at λ_{max} (between 227 and 230 nm), caused by the formation of conjugated dienes, mirrored these results but were not convincing. At all storage times from 3 to 8 d, the high pressure-treated samples had significantly higher PV and TBA values ($P < 0.05$).

As expected, the induction periods were longer at 25°C than at 50°C. The results for PV are shown in Figure 2A, and the changes in TBA values (not shown) mirrored the PV results. High-pressure treatment reduced the induction period by about 15 d. The specific absorbance, caused by the formation of conjugated dienes at 25°C, also mirrored these results (Fig. 2B). At storage times from 70 to 90 d, all high pressure-treated samples had significantly higher PVs and $E^{1\%}_{1cm}$ ($P < 0.05$) than samples that were not pressure-treated. Because measurement of dienes is tedious and merely serves to confirm the PV and TBA results, this technique was not used in subsequent studies.

At 4°C, the PV of high pressure-treated samples after 8 mon of storage was higher (10.2 meq/kg) than that of the control samples (6.1 meq/kg) (data not shown). As the results at 50°C were similar to those observed at lower temperatures, all subsequent experiments were carried out at this temperature (50°C).

Effects of the intensity of high pressure at 0.44 A_w. Table 1 shows the PV of the control and high pressure-treated rendered pork fat (0.44 A_w) after 10 d of storage at 50°C. ANOVA showed that high-pressure treatment had a significant effect on the PV ($P < 0.05$). Table 1 shows that the effect increased with pressure, although only after treatment at 600 and 800 MPa, was the increase over the control significant.

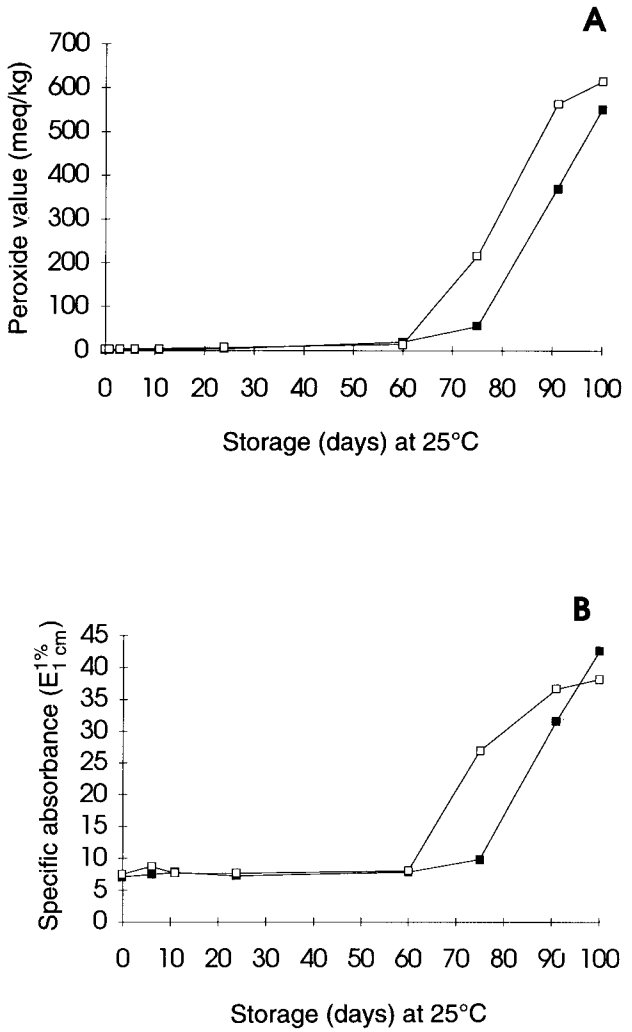

FIG. 2. Peroxide values (A) and specific absorbance (B) during storage at 25°C in the dark of rendered pork fat (■), and rendered pork fat treated at 800 MPa for 20 min at 19°C, prior to storage (□). Values are means of three determinations.

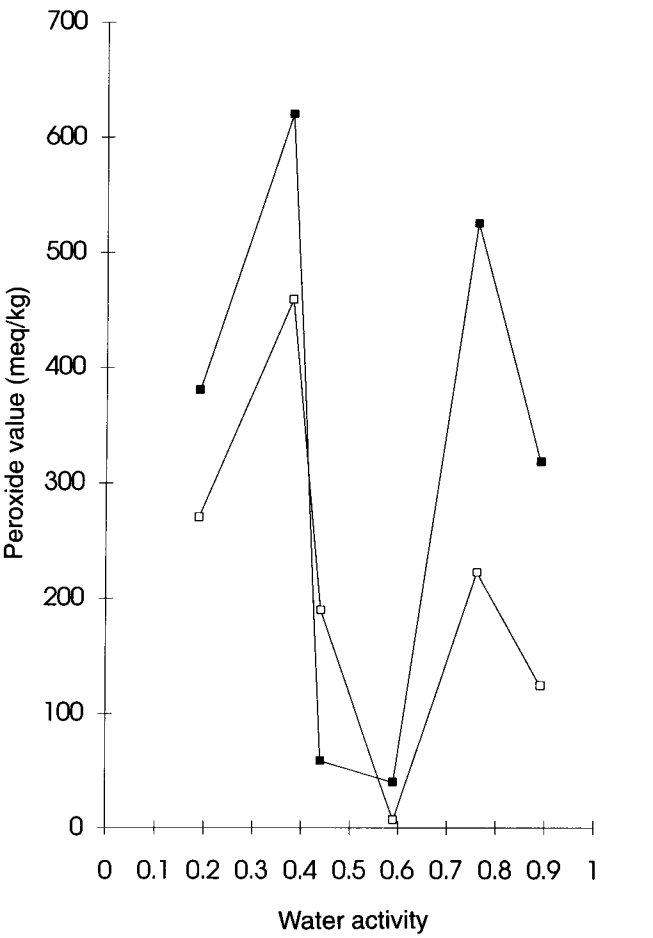

FIG. 3. Effects of water activity on peroxide values after storage for 8 d at 50°C in the dark of rendered pork fat (■), and rendered pork fat treated at 800 MPa for 20 min at 19°C (□). Values are means of eight determinations.

Effects of A_w. Figure 3 shows the effects of water activity on the PV of both the control and treated (800 MPa for 20 min) samples after storage in the dark at 50°C for 8 d. The differences between the treated and the control pork fat were significant ($P < 0.05$) at all water activities. Similar results were observed with the TBA values. Similar trends were observed after 4 and 6 d storage (data not shown). Both relationships are typical for the dependence of lipid oxidation on A_w (6). What is interesting is that, at all water activities except 0.44, high-pressure treatment inhibited lipid oxidation. Because most foods are found in the intermediate moisture range and above, this fact increases the appeal of high-pressure processing. In view of the positive effect of high-pressure treatment in the intermediate moisture range, subsequent work was carried out at 0.76 A_w.

TABLE 1
Peroxide Values (PV) of Rendered Pork Fat Subjected to Different High-Pressure Treatments for 20 min (0.44 A_w) After 10 d of Storage at 50°C

Pressure treatment for 20 min	PV (meq/kg)[a]
0 MPa (control)	369x
200 MPa	462xz
400 MPa	467xz
600 MPa	546yz
800 MPa[b]	709

[a]Results are means of eight determinations. Values with the same letter are not significantly different ($P < 0.05$).
[b]Value obtained by extrapolation from Figure 1.

Effects of high-pressure treatment at 0.76 A_w. The changes in PV of rendered pork fat (0.76 A_w) during storage at 50°C are shown in Figure 4. Student's *t*-test revealed that the PV of the control samples were significantly higher than the high pressure-treated samples throughout storage ($P < 0.05$). As expected, this was the reverse of what was observed with rendered pork fat stored at 0.44 A_w (Figs. 1 and 2). A comparison of Figures 3 and 4 shows that, although the effects were consistent and reproducible within an experiment, sample-to-sample variations made interexperimental comparisons difficult.

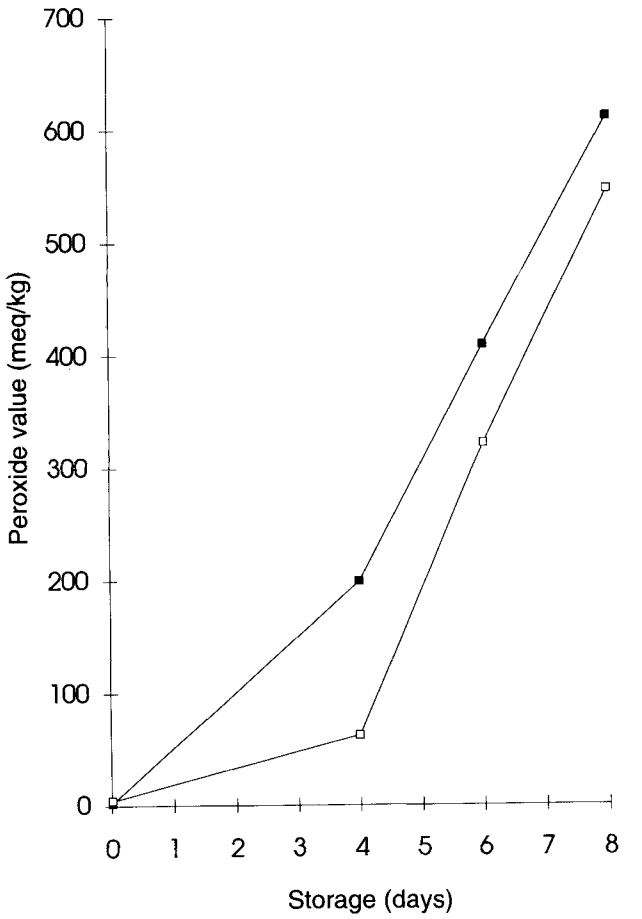

FIG. 4. Peroxide values after storage at 50°C in the dark of rendered pork fat (■), and rendered pork fat treated at 800 MPa for 20 min at ambient temperature (□), equilibrated to 0.76 water activity. Values are means of eight determinations.

Effects of temperature during high-pressure (800 MPa) treatment at 0.76 A_w. The PV of rendered pork fat (0.76 A_w) and fat subjected to high-pressure treatment at different temperatures is shown in Table 2. ANOVA showed that there were no significant differences among the control samples processed at different temperatures. However, the temperature at which the pressure treatment was carried out had a significant effect on the extent of oxidation—the extent increased with the temperature of the treatment (Table 2). For this reason, no significant difference was observed between the control and the treated samples processed at 45°C. It would seem that pressure treatment at higher temperatures diminishes the inhibiting/protective effect of pressure on lipid oxidation.

TABLE 2
Peroxide Values (PV) of Rendered Pork Fat (control) and Rendered Pork Fat Subjected to High-Pressure Treatment (800 MPa for 20 min) at Different Temperatures After 6 d of Storage at 50°C and 0.76 A_w

Sample	Processing temperature (°C)	PV (meq/kg)[a]
Control	19	438x
Pressure-treated	19[b]	315y
Control	25	431x
Pressure-treated	25[b]	365y
Control	45	431x
Pressure-treated	45[b]	412x

[a]Results are means of eight determinations. Values with the same letter are not significantly different (Student's *t*-test, $P < 0.05$).
[b]This shows the initial temperature. It increases by about 15°C during pressurization from 0 to 800 MPa; at 800 MPa it decreases to the initial temperature in 5 min.

TABLE 3
Peroxide Values (PV) of Oxidized Rendered Pork Fat (control) and Oxidized Rendered Pork Fat After High-Pressure Treatment (800 MPa) at Different Temperatures for 20 min

Temperature (°C) (profiles as per Table 2)	PV (meq/kg)[a]	
	Control	Treated
19	97.1 ± 2.6x	92.1 ± 4.2y
32	97.2 ± 3.6x	98.8 ± 4.4x
50	95.2 ± 4.3x	100 ± 6.4z

[a]Results are means SD of eight determinations. Values among both columns with the same letter are not significantly different ($P < 0.05$).

Effects of temperature and pressure (800 MPa) on oxidized samples. When oxidizing samples were subjected to 800 MPa at 19°C for 20 min, pressure caused a significant decrease in the PV, but at 50°C there was a significant increase (Table 3). At 32°C, the difference was not significant. Not unexpectedly, all controls were similar to each other because they were only held in a water bath at the respective temperatures and normal pressure for 20 min.

DISCUSSION

The application of pressure for only a short time (20 min) has significant effects on the stability of pork lipids during subsequent storage, indicating that the application of pressure leads to irreversible changes in the fat. Because most storage experiments were carried out at 50°C, where the fat is melted, differences in crystal structure and solid/liquid ratio are unlikely to be the cause. Peroxides were labile to pressure at 19°C because the PV of rancid fat decreased on pressure treatment (Table 3). Because fresh fats contained about 1–2 meq/kg of peroxides (Figs. 1 and 2), this destruction of peroxides could explain the inhibitory effects observed at water activities above 0.55 A_w (Fig. 3). That the effect of pressure is temperature-dependent (Table 3) suggests that the peroxides are more vulnerable to pressure in the solid phase, or that they are created at higher temperatures. Unfortunately, we do not have any information on how the solid/liquid ratio itself is affected by pressure.

As high-pressure treatment in the experiments reported in Figure 3 was carried out at 19°C, peroxides in rendered pork fat would have been destroyed (Table 3). However, at water activities in the range of 0.40–0.55, pressure is seen to be catalytic to the oxidation (Fig. 3). Thus, another factor must be involved. Pork fat may contain up to 1.5 ppm iron and 0.4 ppm copper (13), and, under pressure, transition metals may be freed from complexes because this should lead to a de-

crease in volume; such liberated ions will be powerful prooxidants (14). At higher A_w, free ions will hydrate because water is available; however, at lower water activities, such hydration may not be complete and increases the catalytic effect of the ion. Thus, in the range of 0.40–0.55 A_w, this effect may override the inhibiting effect of peroxide destruction. At low A_w, mobility considerations may further complicate the situation, leading to pressure being inhibitory. Further work is necessary to test these hypotheses.

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