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ScienceDirect
Food Chemistry 104 (2007) 1575–1579
Food Chemistry
www.elsevier.com/locate/foodchem

Effects of high pressure/thermal treatment on lipid oxidation in beef and chicken muscle

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Received 17 May 2006; received in revised form 29 November 2006; accepted 2 March 2007

Abstract

Lipid oxidation was studied in beef and chicken muscle after high pressure treatment (0.1–800 MPa) at different temperatures (20–70 °C) for 20 min, prior to storage at 4 °C for 7 days. Pressure treatment of beef samples at room temperature led to increases in TBARS values after 7 days storage at 4 °C; however, the increases were more marked after treatment at pressures ⩾400 MPa (at least fivefold) than after treatment at lower pressures (less than threefold). Similar results were found in those samples treated at 40 °C, but at 60 °C and 70 °C pressure had little additional effect on the oxidative stability of the muscle. Pressure treatments of 600 MPa and 800 MPa, at all temperatures, induced increased rates of lipid oxidation in chicken muscle, but, in general, chicken muscle was more stable than beef to pressure, and the catalytic effect of pressure was still seen at the higher temperatures of 50 °C, 60 °C and 70 °C. The addition of 1% Na_2EDTA decreased TBARS values of the beef muscle during storage and inhibited the increased rates of lipid oxidation induced by pressure. The inhibition by vitamin E (0.05% w/w) and BHT (0.02% w/w), either alone or in combination, were less marked than seen with Na_2EDTA, suggesting that transition metal ions released from insoluble complexes are of major importance in catalysing lipid oxidation in pressure-treated muscle foods.
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Keywords: High pressure; Thermal treatment; Beef muscle; Chicken muscle; Lipid oxidation; Antioxidants

1. Introduction

High pressure processed foods are a commercial reality in many parts of the world (Grant, Patterson, & Ledward, 2000) and interest in the technology is growing. Though the preservative effects of pressure processing on meat are well established (Ledward & Mackey, 2002), at sufficiently high pressure it makes the meat more susceptible to lipid oxidation (Angsupanich & Ledward, 1998; Cheah & Ledward, 1995, 1996; Kato & Hayashi, 1999; Ledward, 1998).

It is also well established that heat also markedly decreases the oxidative stability of muscle foods (Beltran, Pla, Yuste, & Mor-Mur, 2003; Keller & Kinsella, 1973; Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998; Tichivangana & Morrissey, 1985). Heat and pressure related damage to the cell membrane is thought to be at least partially responsible for these changes (Kato & Hayashi, 1999; Orlien, Hansen, & Skibsted, 2000), and several workers also believe that the release or activation of transition metal ions are involved (Apte & Morrissey, 1987; Shahidi & Hon, 1991), or that the haem pigments themselves are the key factors (Johns, Birkinshaw, & Ledward, 1989). Pressure treatment of beef liver at 600 MPa caused a significant increase in the total amount of soluble iron (Defaye & Ledward, 1999), although soluble iron in beef decreased as the haemoproteins denatured and became insoluble. In addition, metal chelators such as citrate and ethylenediaminetetraacetic acid are very effective inhibitors of pressure-induced lipid oxidation in pork (Cheah & Led-

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doi:10.1016/j.foodchem.2007.03.006

ward, 1997), cod (Angsupanich & Ledward, 1998) and chicken slurries (Beltran, Pla, Yuste, & Mor-Mur, 2004), strongly suggesting that metal ions are of major importance, since other antioxidants such as rosemary extract (Beltran et al., 2004) and BHA (Cheah & Ledward, 1997) were less effective.

Although all muscle foods so far studied are susceptible to pressure-induced lipid oxidation, it is not clear what the critical pressures are, as they appear to vary from meat to meat. Cheah and Ledward (1996) found that pressures of 300 MPa and above at ambient temperature caused increased rates of oxidation in pork, and Angsupanich and Ledward (1998) found increased rates of oxidation at 400 MPa and above at ambient temperature in cod muscle. In minced chicken breast muscle Beltran et al. (2003) found that treatments up to 500 MPa had no effects on the subsequent rates of oxidation at chill temperatures, and also claimed that chicken muscle was more stable than turkey muscle to pressure-induced changes in oxidative stability.

The present study was designed to improve our understanding of the relative susceptibility of different muscles to pressure-induced oxidation and their stability to treatments combining both pressure (0–800 MPa) and temperature (20–70 °C). The effectiveness of different classes of antioxidants (metal chelators and free radical scavengers) was also undertaken to give further insight into the mechanism involved.

2. Materials and methods

2.1. Preparation of samples

Post-rigor beef *longissimus dorsi* of pH 5.4 was obtained directly from the University of Bristol (Ma & Ledward, 2004). The beef was trimmed of all visible fat and cut into approximately 3 × 3 × 6 cm pieces with the fibres parallel to the longest axis, and packed in Multivac bags (Bosley, International), which were subsequently stored at −18 °C until required. For each treatment samples were chosen at random and, prior to use, the frozen samples were left at 4 °C for 12 h to thaw.

For the experiments concerned with the role of antioxidants, the beef *longissimus dorsi* of pH 5.56 was obtained from a supermarket in Nanjing (China); the meat was from a 20–24 month old Luxi × Limousin crossbreed, and had been kept at 4 °C for 5 days following slaughter. It was trimmed of fat and connective tissue, minced and divided into four portions, and either 0.05% (w/w) vitamin E, 0.05% vitamin E with 0.02% BHT (butylated hydroxytoluene), or 1% Na_2EDTA (the sodium salt of ethylenediaminetetraacetic acid) were added to three of the four portions; the fourth portion served as control. The portions were mixed in a Waring blender (Stomacher 400, France) at 15,000 rpm for 30 s at 20 °C. Samples (about 30 g each) were sealed in plastic bags and stored at −18 °C prior to use. For each treatment, samples were chosen randomly and, prior to use, the frozen samples were left at 4 °C for 12 h to thaw.

Chicken breast was obtained from a local supermarket. All the visible fat was removed. The samples were cut into 3 × 3 × 6 cm pieces and sealed in Multivac bags (Bosley, International) and stored at 4 °C for 4 days before use. Though inevitably different muscles were used for the different pressure treatments, they were all from the same retail outlet and were at normal pH (Zamri, Ledward, & Frazier, 2006). At any given pressure the same muscle was used for all different temperatures.

2.2. High pressure and heat treatment

Intact beef muscle samples were pressurised at 200–800 MPa at room temperature, 40 °C, 60 °C and 70 °C for 20 min (Stansted Fluid Power Ltd., Stansted, UK), as described by Ma and Ledward (2004). The minced samples were treated at 200 MPa and 600 MPa at room temperature, 40 °C and 60 °C for 20 min in a high pressure rig (Kefa New Technology Food Machine Ltd., Baotou, China). Some samples were heated in water baths at 40 °C, 60 °C or 70 °C for 30 min.

Chicken muscle samples were pressurised at 200–800 MPa at room temperature, 40 °C, 50 °C, 60 °C and 70 °C for 20 min (Stansted Fluid Power Ltd.) as described by Zamri et al. (2006). Samples were also heated in water baths at 40 °C, 50 °C, 60 °C or 70 °C for 30 min.

2.3. Determination of TBARS

TBARS was determined according to the method of Pearson (1988). The TBARS number is expressed as milligrams of malonaldehyde (MA) per kilogram of sample, using a conversion factor of 7.8. In all cases 3 determinations were carried out on 3 samples.

2.4. Statistical analysis

Data were analysed by analysis of variance, using SPSS 12.0 for Windows. Levels for significant differences were set at $P < 0.05$.

3. Results and discussion

3.1. High pressure effects on lipid oxidation

After pressure treatment at room temperature (~20 °C) all beef samples showed a greater than threefold increase in TBARS values after 7 days storage in air at 4 °C (Table 1). However, the increases were more marked after treatment at pressures ⩾400 MPa. This pattern was replicated in samples treated at 40 °C, but at 60 °C or 70 °C there was little difference in the samples, irrespective of pressure treatment, suggesting that, at these elevated temperatures, pressure causes little additional susceptibility to oxidation. It is interesting to note that after treatment at 70 °C and

Table 1
The effect of heat and pressure processing on TBARS values (mg malonaldehyde/kg sample) of intact beef muscle immediately after processing (<4 h) and storage for 7 days at 4 °C

Treatment	Day 0	Day 7
20 °C		
0.1 MPa	0.47 ± 0.06	0.48 ± 0.11
200 MPa	0.49 ± 0.04	1.23 ± 0.09
400 MPa	0.61 ± 0.02	6.71 ± 0.21
600 MPa	0.90 ± 0.04	5.25 ± 0.39
800 MPa	0.69 ± 0.03	4.08 ± 0.29
40 °C		
0.1 MPa	0.55 ± 0.01	0.58 ± 0.06
200 MPa	0.73 ± 0.12	2.88 ± 0.17
400 MPa	1.12 ± 0.27	2.97 ± 0.30
600 MPa	0.14 ± 0.04	3.99 ± 0.15
800 MPa	0.42 ± 0.11	4.15 ± 0.12
60 °C		
0.1 MPa	0.61 ± 0.13	3.79 ± 0.58
200 MPa	0.59 ± 0.07	3.70 ± 0.23
400 MPa	0.68 ± 0.08	5.02 ± 0.18
600 MPa	1.25 ± 0.09	4.20 ± 0.20
800 MPa	0.76 ± 0.08	4.13 ± 0.17
70 °C		
0.1 MPa	0.60 ± 0.09	2.57 ± 0.08
200 MPa	2.36 ± 0.91	5.95 ± 0.87
400 MPa	1.42 ± 0.17	5.07 ± 0.26
600 MPa	0.96 ± 0.08	5.63 ± 0.23
800 MPa	0.44 ± 0.10	1.83 ± 0.17

All TBARS values are the mean ± standard deviation of three replicates.

800 MPa lipid oxidation appeared to be reduced, which is contrary to expectation. However, values are the means of only three replicates and thus this result must be viewed with caution.

Chicken muscle was more stable than beef, although the muscles were treated and analysed relatively soon after slaughter, whilst the beef muscle had been held in frozen storage for several weeks. However, in chicken muscle the increased rate of lipid oxidation induced by pressure was readily observed in those samples treated at 600 MPa or 800 MPa at all temperatures (Table 2). Unlike the beef muscle, there did not appear to be a synergistic effect of temperature and pressure since application of pressures of 600 MPa or 800 MPa at elevated temperatures (50–70 °C) was still necessary to cause increased susceptibility to oxidation. As with the effects of different pressure/temperature regimes on texture (Zamri et al., 2006), it is noteworthy that higher pressures are apparently required to significantly modify the oxidative stability of chicken, compared with beef muscle (Ma & Ledward, 2004). Beltran et al. (2003) also found that the oxidative stability of minced chicken breast muscle was not affected by pressures up to 500 MPa and believed this was related to the integrity of the cell membrane.

The above results extend the results of previous studies (Angsupanich & Ledward, 1998; Cheah & Ledward, 1996) and show that elevated pressures at room temperature decrease the oxidative stability of fish, white and red meat.

Table 2
The effect of heat and pressure treatment on TBARS values (mg malonaldehyde/kg sample) of poultry muscles, immediately after processing (<4 h) and after storage at 4 °C for 7 days

Treatment	Day 0	Day 7
20 °C		
0.1 MPa	0.333 ± 0.002	0.254 ± 0.003
200 MPa	0.352 ± 0.003	0.339 ± 0.005
400 MPa	0.377 ± 0.006	0.385 ± 0.005
600 MPa	0.416 ± 0.007	0.579 ± 0.001
800 MPa	0.492 ± 0.004	1.190 ± 0.004
40 °C		
0.1 MPa	0.460 ± 0.005	0.440 ± 0.006
200 MPa	0.550 ± 0.008	0.490 ± 0.003
400 MPa	0.681 ± 0.012	0.490 ± 0.003
600 MPa	0.782 ± 0.021	2.07 ± 0.009
800 MPa	0.852 ± 0.007	5.13 ± 0.005
50 °C		
0.1 MPa	0.508 ± 0.004	0.362 ± 0.001
200 MPa	0.566 ± 0.009	0.508 ± 0.006
400 MPa	0.659 ± 0.007	0.715 ± 0.010
600 MPa	0.742 ± 0.014	2.85 ± 0.009
800 MPa	0.833 ± 0.008	3.39 ± 0.007
60 °C		
0.1 MPa	0.525 ± 0.006	0.411 ± 0.010
200 MPa	0.545 ± 0.006	0.560 ± 0.004
400 MPa	0.558 ± 0.006	0.608 ± 0.003
600 MPa	0.631 ± 0.006	1.89 ± 0.004
800 MPa	0.757 ± 0.010	4.45 ± 0.003
70 °C		
0.1 MPa	0.458 ± 0.005	0.417 ± 0.009
200 MPa	0.559 ± 0.004	0.497 ± 0.006
400 MPa	0.525 ± 0.010	1.57 ± 0.007
600 MPa	0.659 ± 0.007	2.08 ± 0.011
800 MPa	0.768 ± 0.004	2.78 ± 0.007

All values are the mean ± standard deviation of three replicates on one muscle.

The pressures required to initiate these changes seem to be lower for beef (200 MPa), compared to pork (300 MPa), cod (400 MPa) and chicken (600 MPa), although the post-slaughter history of the samples varied. It has been claimed that this phenomenon is due to the release of ‘free’ iron from the iron complexes present in meat, as the concentration of ‘free’ iron increased in liver samples after pressure treatment (Defaye & Ledward, 1999), and chelating agents, such as EDTA, effectively prevented the increased rates of oxidation seen in pressure-treated cod (Angsupanich & Ledward, 1998) and pork (Cheah & Ledward, 1997). It is also possible that the effects of pressure may relate to changes in the integrity of the cell membrane (Beltran et al., 2003). It is worth noting that the chicken samples were from birds only a few weeks old, while the pork would be from animals 6–8 months old and the beef animals were the oldest, at about 24 months of age. All the samples were of normal pH being about 5.5 in beef (Ma & Ledward, 2004), 5.7–5.8 in pork (Cheah & Ledward, 1996), 6.6–6.7 in cod (Angsupanich & Ledward, 1998) and 5.8–6.0 in chicken (Zamri et al., 2006). It is difficult to envisage how

the effects of pressure could result in major changes in cell integrity and it is more likely that they reflect differences in the concentration and pressure sensitivity of potential pro-oxidants.

3.2. Role of antioxidants

Three well-established antioxidants were studied – two chain breakers or free radical scavengers (vitamin E and butylated hydroxytoluene (BHT)) and one metal chelator (the sodium salt of ethylenediaminetetraacetic acid) – on the oxidative stability of minced beef, pressure treated at 20 °C, 40 °C and 60 °C. At 20 °C, Na_2EDTA was very effective in all of the samples but especially at 200 MPa and 600 MPa (Table 3). Vitamin E and vitamin E with BHT minimised the effects of pressure but were far less effective than Na_2EDTA. Very similar effects were seen in those samples treated at 40 °C, with Na_2EDTA being by far the most effective antioxidant. These results agree with the work of previous researchers on a range of muscle foods (Angsupanich & Ledward, 1998; Beltran et al., 2004; Cheah & Ledward, 1997).

The beef used in the antioxidant experiments was apparently more stable than that used in the whole muscle work (cf. Table 1 with Tables 3–5), even though the experiments were carried out by the same researcher, albeit in two different countries, namely China and England. However, it is seen that a pressure of 200 MPa causes some loss of stability, agreeing with the greater pressure sensitivity of beef (Table 1), compared with cod and pork, where effects were only seen at 300–400 MPa (Angsupanich & Ledward, 1998; Cheah & Ledward, 1996) and chicken, where pressures above 400 MPa were necessary (Table 2). At 60 °C the effectiveness of Na_2EDTA was further confirmed, although, as seen in Table 1, pressure *per se* had little or no effect on the oxidative stability.

Table 3
Effect of antioxidants and Na_2EDTA on TBARS values (mg malonaldehyde/kg sample) in minced beef, following pressure treatment at room temperature and storage for 7 days at 4 °C

Treatment	Day 0	Day 7
0.1 MPa		
Control	0.261 ± 0.003^a	0.270 ± 0.003^a
Vitamin E	0.259 ± 0.003^a	0.260 ± 0.002^a
Vitamin E + BHT	0.236 ± 0.010^a	0.246 ± 0.001^a
Na_2EDTA	0.030 ± 0.002^b	0.048 ± 0.003^b
200 MPa		
Control	0.291 ± 0.008^a	0.405 ± 0.015^a
Vitamin E	0.263 ± 0.005^a	0.316 ± 0.020^b
Vitamin E + BHT	0.253 ± 0.004^b	0.268 ± 0.020^b
Na_2EDTA	0.043 ± 0.015^c	0.156 ± 0.021^c
600 MPa		
Control	0.357 ± 0.012^a	0.625 ± 0.018^a
Vitamin E	0.330 ± 0.030^b	0.383 ± 0.025^b
Vitamin E + BHT	0.270 ± 0.016^c	0.325 ± 0.011^c
Na_2EDTA	0.045 ± 0.016^d	0.123 ± 0.032^d

All TBARS values are the mean ± standard deviation of three replicates. TBARS with different superscripts in the same column at equal pressure were significantly ($P < 0.05$) different.

Table 4
Effect of antioxidants and Na_2EDTA on TBARS values (mg malonaldehyde/kg sample) in minced beef, following pressure treatment at 40 °C and storage for 7 days at 4 °C

Treatment	Day 0	Day 7
0.1 MPa		
Control	0.379 ± 0.027^a	0.420 ± 0.039^a
Vitamin E	0.258 ± 0.018^b	0.443 ± 0.041^a
Vitamin E + BHT	0.214 ± 0.009^c	0.351 ± 0.036^b
Na_2EDTA	0.097 ± 0.031^d	0.279 ± 0.012^c
200 MPa		
Control	0.389 ± 0.026^a	0.625 ± 0.039^a
Vitamin E	0.265 ± 0.019^b	0.651 ± 0.016^a
Vitamin E + BHT	0.216 ± 0.017^c	0.464 ± 0.040^b
Na_2EDTA	0.086 ± 0.005^d	0.269 ± 0.022^c
600 MPa		
Control	0.545 ± 0.031^a	0.744 ± 0.049^a
Vitamin E	0.335 ± 0.015^b	0.436 ± 0.018^b
Vitamin E + BHT	0.289 ± 0.020^b	0.353 ± 0.031^b
Na_2EDTA	0.184 ± 0.017^c	0.289 ± 0.023^b

All TBARS values are the mean ± standard deviation of three replicates. TBARS with different superscripts in the same column at equal pressure were significantly ($P < 0.05$) different.

Table 5
Effect of antioxidants and Na_2EDTA on TBARS values (mg malonaldehyde/kg sample) in minced beef, following pressure treatment at 60 °C and storage for 7 days at 4 °C

Treatment	Day 0	Day 7
0.1 MPa		
Control	0.524 ± 0.025^a	0.944 ± 0.035^a
Vitamin E	0.314 ± 0.019^b	0.791 ± 0.034^a
Vitamin E + BHT	0.315 ± 0.022^b	0.525 ± 0.017^b
Na_2EDTA	0.193 ± 0.015^c	0.376 ± 0.007^c
200 MPa		
Control	0.348 ± 0.022^a	0.747 ± 0.008^a
Vitamin E	0.345 ± 0.005^a	0.702 ± 0.028^a
Vitamin E + BHT	0.316 ± 0.021^b	0.583 ± 0.008^b
Na_2EDTA	0.183 ± 0.013^c	0.299 ± 0.011^c
600 MPa		
Control	0.622 ± 0.068^a	0.854 ± 0.010^a
Vitamin E	0.398 ± 0.025^b	0.514 ± 0.025^b
Vitamin E + BHT	0.306 ± 0.031^c	0.428 ± 0.016^c
Na_2EDTA	0.145 ± 0.003^d	0.191 ± 0.018^d

All TBARS are the mean ± standard deviation of three replicates. TBARS with different superscripts in the same column at equal pressure were significantly ($P < 0.05$) different.

4. Conclusions

High pressure treatment at 20–40 °C caused decreases in oxidative stability of a range of muscle foods, including beef, pork, chicken and cod, but the different meats apparently become significantly more unstable at different pressures; beef is less stable than pork and cod, and chicken is the most stable of those so far studied. When subjected

to pressure at higher temperatures the catalytic effects of pressure were still seen in chicken (at 400 MPa and above, Table 2), but, in beef, all of the samples, irrespective of pressure treatment, were of similar stability.

Of the antioxidants studied, Na_2EDTA, a metal chelator, was the most efficient, supporting the contention that transition metal ions (iron) released from insoluble complexes (Cheah & Ledward, 1997; Defaye & Ledward, 1999) are of major importance in catalysing lipid oxidation in pressure-treated muscle foods.

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