PRESSURE TREATMENT ON BEEF AND PORK MEAT: IMPACT ON THE LIPID AND PROTEIN OXIDATION

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Abstract – The aim of this study is to evaluate the impact of high pressure (HP) treatment (from 0.1 to 500 MPa) on flora inactivation and oxidation of pork and beef meat during storage.
A 500 MPa treatment is necessary to stop growth in pork and beef meat.
Oxidation indicators were followed in pork and beef minced meat just after HP treatment and after 7 and 14 days of storage. Lipid oxidation was evaluated by thiobarbituric acid reactive substances (TBARS) test and hexanal level. Protein oxidation was evaluated by 2,4-dinitrophenylhydrazine (DNPH) test.
The results confirm that HP processing above 400 MPa inactivates significantly aerobic flora in meat. Results related to lipid and protein oxidation depend on meat origin. Oxidation in beef seems less impacted by pressure than in pork. Contrary to beef, pressurization of pork meat promotes lipid oxidation but stabilizes protein oxidation.

Key Words – High pressure, Lipid and protein oxidation, Meat

I. INTRODUCTION

During the last years, HP processing appeared as a novel food preservation method, acting as a cold pasteurisation. In meat industry, HP is already applied to sliced or ready-to-eat meat products to reduce microbiological risk, but also led to a modification of quality parameters such as colour, texture and water holding capacity.
Meat is a raw material prone to spoilage and development of pathogens. The ability of HP to destroy spoilage and pathogenic microorganisms (bacteria, fungi or viruses) in meat is now well documented [1-3]. HP inactivates vegetative microbial cells mainly by damages caused to the cell membranes; this inactivation depends on the pressure level.
HP is therefore an efficient treatment to stabilize meat and meat products from a microbial point of view; however it could induce also various modifications of components, among which proteins and lipids.
Meat and meat products are rich in proteins and depending on the muscle type they contain variable quantities and proportions of lipids. Lipid oxidation in muscle foods was considered for a long period as the main reaction that impairs their qualities, especially the sensory ones. Since twenty years, proteins were also considered as a target of oxidation.
Thus, many authors were interested in the evaluation of oxidation in pressurized meat, in order to understand mechanisms inducing oxidation during pressure treatment. In particular, myoglobin fate upon HP treatment has been investigated.
Moreover, the extent of lipid oxidation depends on the treatment intensity and duration, the temperature applied during the HP treatment, and mainly on the type of meat or meat products [4]. Thus, beef samples seemed to be less oxidized than the other meat samples and contained for example five times less volatile compounds than chicken meat [5]. The interest of cross reaction between lipid and protein oxidation is quite recent especially as related to HP treatment.[6][7].
The aim of the study is to compare both lipid and protein oxidation damage in two low-fat meat model (pork and beef raw meat) after HP treatments (0.1 to 500 MPa) and during the subsequent storage.Lipid oxidation was assessed by measuring levels of carbonyl compounds by two ways: TBARS and hexanal. Protein oxidation was estimated by means of carbonyl compounds with DNPH.

II. MATERIALS AND METHODS

Chemicals and meat supply

All chemical substances were purchased by Sigma Aldrich.

Industrial minced raw beef at (6% fat) and fresh pork (2% fat) were purchased from a local market. Both minced meats were vacuum-packed in polyamide/polyethylene plastic bags.

Both beef and pork meat was then frozen and stored à -18°C. Before HP treatment, meats were thawed at 4°C during 72h.

HP treatment

HP treatment was carried out using a vertical 3 L high-pressure pilot unit (ACB, Nantes, France). The pressure-transmitting fluid was water; the vessel temperature was controlled at 20°C. The samples were inserted into the vessel and processed with a compression rate of 3 MPa/s until pressure target. Pressure from 0.1 (control) to 500 MPa was applied and held for 5 min; decompression was instantaneous (<2 s).The samples were then analyzed or stored at 4 °C until further analysis.

Total aerobic flora enumeration

On selected storage days (0, 7, 14, 21 and 28 days), 15 g of meat was placed in a sterile bag and diluted with 135 g of tryptone salt solution. Then, the bag content was homogenized using a stomacher blender during 2x30 seconds. Five serial dilutions were made and aerobic mesophilic counts were evaluated by plate technique: 1 mL of appropriate dilution was placed on plate count agar and incubated at 30 °C for 72 h. Plates were prepared in duplicate for each dilution.

Results were expressed as colony forming unit (CFU) per g of meat.

TBARS

On selected storage days (0, 7 and 14 days), 1 g of crushed meat was homogenized by Ultra Turrax (125 IKA, 1000 rpm) for 3x15 seconds with 5 mL of buffer (5 µM propyl gallate,5 µM EDTA, 0.2 M trichloracetic acid) in ice bath. Homogenate was centrifuged (4000g for 10 min at 4°C) and the supernatant was filtered through filter n°4. Filtrate was incubated (v/v) with 0.3% (w/v) 2-thiobarbituric acid in a boiling bath for 30 min; reaction was stopped by cooling in ice bath. Absorbance was measured between 400 and 600 nm against buffer. Analysis was realized in triplicate for each sample.

TBARS concentrations were calculated using malondialdehyde (MDA) as standard. Results were expressed as mg of MDA equivalent per kg of meat, according to formula (a: slope of MDA curve):

$$TBARS = \frac{A_{532nm} \times M_{TBA} \times V_{extract}}{a \times M_{MDA} \times m_{meat}}$$

Hexanal

On selected storage days(0, 7 and 14 days), 5 g of crushed meat was inserted in a Head Space vial with 5 mL of water and 10 µL of 1 g/L 4-heptanone as internal standard. Volatile compounds were extracted 30 min at 60 °C in a head space analyzer (Turbo Matrix, Perkin Elmer) then injected (1 min, split less, 280 °C) in a gas chromatography (Clarus 500, Perkin Elmer), separated on 75 meter DB-624 column with helium at 40 kPa (1 min-40 °C-10 °C/min-180 °C-13 min-10 °C/min-200 °C-2 min) and detected by flame ionization (280°C). Analysis was realized in triplicate for each sample.

Results were expressed as mg of 4-heptanone equivalent per kg of meat.

DNPH

On selected storage days(0, 7 and 14 days), 1 g of crushed meat was homogenized by Ultra Turrax (125 IKA, 1000 rpm) for 3x20 seconds with 10mLof 0.6 M KCl. Homogenate was then filtered through gauze. 100 µL of the filtrate was centrifuged (10000g for 5 min at 4°C) with 10% (w/v) trichloracetic acid (TCA), and the supernatant was discarded. Pellet of protein was treated with 0.8 mL of 0.2% (w/v) DNPH. Mixture was agitated in Disruptor at 2000 rpm during 1 hour at room temperature in the dark. Proteins were precipitated by addition of 0.7 mL of 10% TCA and subsequently centrifuged (10000 g for 5 min at 4 °C). Supernatant was discarded and pellet was washed three times with 1 mL of ethanol/ethyl acetate (1:1, v/v) and centrifuged (10000 g for 5 min at 4 °C). Pellet was then dried during 1 hour at room temperature.

1 mL of 6 M guanidine was added to dissolve the pellet and the mixture was agitated in Disruptor at 2000 rpm during ½ hour at room temperature in the dark. After centrifugation (10000g for 10 min at 4°C), absorbance was measured between 250 and 500 nm against guanidine blank. Analysis was realized in triplicate for each sample.

DNPH concentrations were expressed as micromoles of carbonyl compounds per g of proteins, according to formula ($\varepsilon_{carbonyls\ at\ 280nm}$ = 0.021 µmol/L/cm):

$$DNPH = \frac{A_{370nm} / (\varepsilon_{carbonyls\ at\ 280nm})}{A_{280nm} / A_{280nm\ of\ BSA\ at\ 1g/L}}$$

III. RESULTS AND DISCUSSION

High pressure inactivation of mesophilic flora

Results were similar in both pork (data not shown) and beef (figure 1) meat. Initial populations were around3 log CFU/g. Just after treatment (day 0), there is no significant difference in populations for meats treated between 0,1 and 300 MPa, but a significant decrease of initial population was observed for meats treated at 400 and 500 MPa with a population below30 CFU/g. After 28 days at 4°C, a significant increase (5 log) was observed for meats treated between 0.1 and 400 MPa. No growth was observed on meat treated at 500 MPa after 28 days of storage.

This result showed that 500 MPa treatment is necessary to stop growth in pork and beef raw meat.

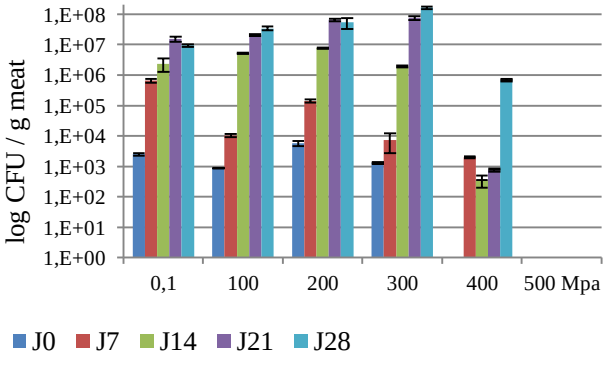

Figure 1. Influence of pressure on inactivation of mesophilic flora of beef meat during storage (n=5)

High pressure impact on oxidation indicators

Results are showed in figure 2 and were quite different between pork and beef meat.

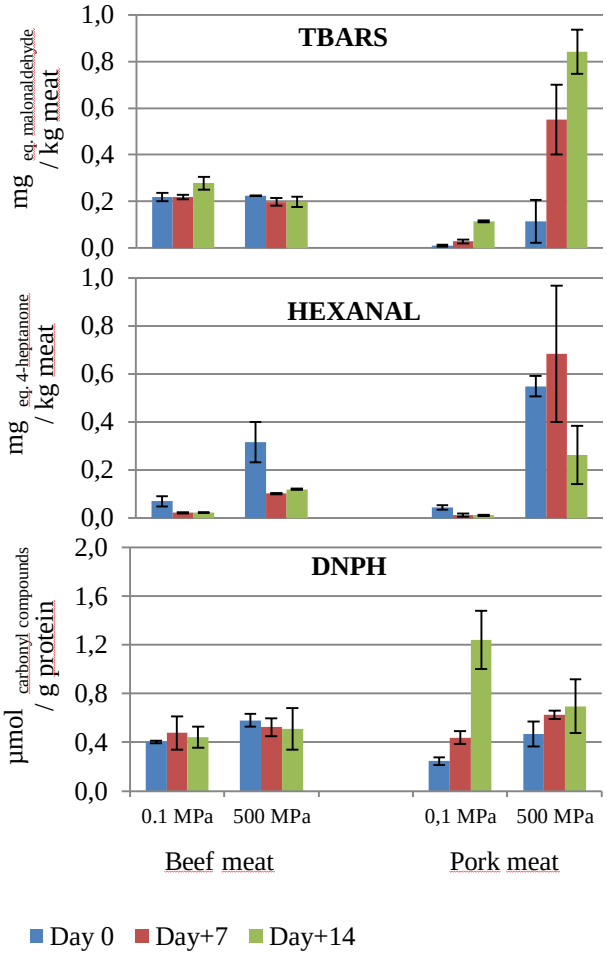

Figure 2. Influence of pressure on oxidation indicators of beef and pork meat during storage (n=3)

At 0.1 MPa, at the beginning of the storage (Day 0), the three oxidation indicators were higher in beef than pork. However, during the 4 °C storage, TBARS and DNPH were quite stable in beef meat contrary to pork meat where a significant increase of TBARS and DNPH level was noticed. In both meats, level of hexanal decreased during storage probably due to a lower releasing. This difference on level and ability of oxidation of pork and beef meat can be related to difference of fat content and composition of fatty acids. Thus, pork meat contains 2% of fat with 16 % of polyunsaturated fatty acids against 6% of

fat with less than 5% of polyunsaturated fatty acids for beef meat.

At 500 MPa, hexanal level was higher than control in beef meat but no significant differences on TBARS and DNPH level were observed during the 14 storage-days at 4°C.
On pork meat, the three oxidation indicator levels increased significantly just after 500 MPa treatment (day 0). During storage, TBARS level increased significantly contrary to DNPH.

Then, impact of HP on oxidation seems to depend of meat origin, notably of the polyunsaturated fatty acids proportion. Moreover, lipid oxidation results show a correlation between hexanal and TBARS in pork contrary to beef. Campus [8] shows no correlation between TBARS and volatile compounds in pork dry cured meat after pressurization.
Contrary to lipid oxidation, protein oxidation seems to be stabilized by pressure in both meats. As well, Cava [6] shows a noticed increase of lipid oxidation (TBARS) after HP treatment and during storage whereas no significant modification of the extent of protein oxidation (DNPH) occurred in pork loin pressurized and stored 90 days at 4°C.

IV. CONCLUSION

HP treatment is an efficient treatment that can slow down microorganism development up 400 MPa and inactivated microorganism from 500 MPa to stabilize pork and beef raw meat.

Our results do not evidence that HP treatment consistently induce oxidation of meat: it depends of the type of meat. Indeed HP treatment seems to increase lipid oxidation in polyunsaturated rich meat.
Moreover, protein oxidation tended to be lowered after HP treatment, especially in pork meat. Thus, this technology could be used to protect meat against natural protein denaturation during storage.

Future work will focus on the interaction of protein with lipid oxidation to understand these phenomena.

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