



## REVIEW PAPER

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## The capacity of high pressure processing to preserving nutritional properties of the food products: A review

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### Abstract

High pressure processing (HPP) is a non-thermal technology used in the food industry because of its capacity to preserving most of the nutritional, sensory and functional properties of the processed food products and diminish the microbial load. HPP maintains the nutritional value and quality of food and therefore does not result in any undesirable changes associated with thermal processing. This thechnology is an emerging nonthermal technology that can ensure the same level of food safety as heat pasteurization and produces fresher-tasting, minimally processed foods. Processing aims to prolong the shelf life while the original sensory and nutritional properties are maintained as high as possible within the constraints put forward by microbial safety. There is a relationship between pressure induced changes on proteins, fats and selected quality parameters in different foods. The aim of this study was to understand the effect of HPP on the proteome and gain further insights into how this impacts on nutritional properties of food products.

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## Introduction

High-pressure (HP) processing is an interesting alternative to traditional food processing and preservation methods due to its limited effects on covalent bonds resulting in minimal modifications in nutritional and sensory quality (Oey *et al.*, 2008). Under HPP, natural flavors can be retained to provide food of superior quality and nutritional value. All food-processing techniques must conform to sanitation and safety-related specifications before they can be employed in food and commercially applied (Ghasemkhani *et al.*, 2014). The most appreciated characteristic of HPP is that it causes microbial death while virtually eliminating heat damage and the use of chemical preservatives/additives, thereby leading to improvements in the overall quality of foods. For example, HPP has a well-tested potential to pasteurize foods by inactivating the most pathogenic species, such as *Escherichia coli* O157:H7 or *Salmonella* spp., without changing the fresh-like characteristics of the product (Balasubramaniam *et al.*, 2008).

During HP processing (100-1000 MPa/-20 °C to 60 °C), (i) cell wall and membrane disruption (Van Buggenhout *et al.*, 2005); (ii) enzyme catalyzed conversion processes (Verlent *et al.*, 2005); (iii) chemical reactions (Nguyen *et al.*, 2006; Oey *et al.*, 2006) and (iv) modification of biopolymers including enzyme inactivation, protein denaturation and gel formation (Van der Plancken *et al.*, 2005) can occur at the same time. HPP is still an emerging processing technique, necessitating further investigation of its related scientific theories, parameter standards, and commercial applications (Huang *et al.*, 2014; Ghasemkhani *et al.*, 2014). HPP is a novel non-thermal food processing technique that can inactivate pathogenic microorganisms in food at room temperature, extend the shelf life of foods, and reduce damage to heat-sensitive food components caused by high temperatures. In HPP, the operating principle is simple. The food materials are sealed in packages and placed in an enclosed and insulated container. Using a liquid (typically water) as the transfer medium, an ultra-high pressure of 100- 600 MPa is generated in

commercial applications to process the Food (Hsiao *et al.*, 2014). Along with other thermal (microwave, radiofrequency, ohmic heating) and non-thermal (ultrasound, pulsed-electric fields) techniques, high hydrostatic pressure processing is one of the minimal processing food preservation technologies (Bermudez-Aguirre and Barbosa-Cánovas, 2011). In the last decades, the operating pressure levels increased from about 300–400 MPa to 800 MPa and above, reducing pressure holding time from 15–30 min to 5 min or below (Bermudez-Aguirre and Barbosa-Cánovas, 2011).

Among the many advantages of using this technology, there is the ability to enable food processing at ambient temperature or even lower temperatures, acting instantaneously and uniformly throughout the food matrix independently of size, shape and composition. In addition to food preservation, high-pressure treatment can result in food products acquiring novel structure and texture (meat texture and muscle protein gelatinization, among others), and hence can be used to develop new products or increase the functionality of certain ingredients.

If the food contains a significant amount of fat, such as butter or cream, the temperature rise is greater (8–9 °C/100MPa) (Rasanayagam *et al.*, 2003). The temperature distribution during the pressure-holding period can change depending on heat transfer across the walls of the pressure vessel, which must be held at the desired temperature to achieve truly isothermal conditions (Rastogi *et al.*, 2007). In the case of some proteins, a gel is formed when the rate of compression is slow, whereas a precipitate is formed when the rate is fast.

### *Effect of HPP on meat proteins*

The application of pressure on proteins leads to different degrees of protein structure modification. As a general mechanism, the application of pressure induces unfolding of the protein structure and subsequent folding after pressure release. This can lead depending on the specific protein and conditions applied to partial or total denaturation and tuning of

electrostatic interactions which are further explained later.

Based on the principle of Le Chatelier and Braun, reactions with a decrease in volume are favored by pressure. Protein denaturation is one of the key mechanisms for microbial inactivation and irreversible changes in muscle proteins start at a comparative level to that required for the inactivation of microorganisms. Covalent bonds have a low compressibility and are much less sensitive to changes in pressure (Bajo *et al.*, 2012). HPP induces the breakdown of salt bonds, due to electrostriction and also parts of hydrophobic interactions. In contrast, hydrogen bonds appear to be slightly strengthened under pressure. Quaternary structure is mainly held by hydrophobic interactions and thus they are very sensitive to pressure. Major changes in the tertiary structure are observed beyond 200 MPa and changes in secondary structure will take place only at very high pressure above 700MPa (Rastogi *et al.*, 2007). Muscle proteins including myofibrillar proteins are unfolded up to a pressure of 300 MPa. Pressures above this level result in increased denaturation, gel formation and agglomeration of proteins. This fact can be employed in product development since enhanced gel structure and water binding capacity can be achieved by the use of certain HPP treatments (Sun and Holley, 2010). One of the remarkable effects of high pressure on meat is the modification of the actin–myosin complex. Pressure induces structural changes in the main constituent of muscle filaments, which are possibly caused by increased ATPase activity at 30 MPa as well as with an increase of soluble materials from the myofibrils enhanced by pressurization above 150 MPa (Nishiwaki *et al.*, 1996).

Begonya and Anne (2014) investigated the relationship between pressure induced changes on individual proteins and selected quality parameters in bovine longissimus thoracis et lumborum (LTL) muscle was studied. Pressures ranging from 200 to 600 MPa at 20 °C were used. High pressure processing (HPP) at pressures above 200 MPa

induced strong modifications of protein solubility, meat colour and water holding capacity (WHC). The protein profiles of non-treated and pressure Treated meat were observed using two dimensional electrophoresis. Proteins showing significant differences in abundance among treatments were identified by mass spectrometry. Pressure levels above 200 MPa strongly modified bovine LTL proteome with main effects being insolubilisation of sarcoplasmic proteins and solubilisation of myofibrillar proteins. Sarcoplasmic proteins were more susceptible to HPP effects than myofibrillar.

Begonya *et al.*, 2014 investigated the induced changes in beef muscle proteome by high pressure. High pressure can affect protein conformation and can lead to protein denaturation, aggregation or gelation (Picouet *et al.*, 2012). The outcome is dependent upon protein susceptibility, the applied pressure and temperature, and the duration of the pressure treatment (Sun and Holley, 2010).

The effects of HPP on proteins are mainly related to the rupture of non-covalent interactions within protein molecules (Galazka *et al.*, 1996). Covalent bonds and primary structures of the proteins are thought not to be affected by high pressure. Pressure induced denaturation of proteins is likely to occur because of the destabilisation of non-covalent interactions in the tertiary structure (Chapleau, Mangavel, Compoin, & Lamballerie- Anton, 2004). Even if pressurised proteins retain most of their secondary structure, a small degree of unfolding occurs, which exposes hydrophobic regions of the protein and can lead to protein aggregation (Sikes *et al.*, 2010).

Marcos and Maria (2014) studied the relationship between pressure induced changes on individual proteins and selected quality parameters in bovine longissimus thoracis et lumborum (LTL) muscle. Pressures ranging from 200 to 600 MPa at 20 °C were used. High pressure processing (HPP) at pressures above 200 MPa induced strong modifications of protein solubility, meat colour and

water holding capacity (WHC). The protein profiles of non-treated and pressure treated meat were observed using two dimensional electrophoresis. Proteins showing significant differences in abundance among treatments were identified by mass spectrometry. Pressure levels above 200 MPa strongly modified bovine LTL proteome with main effects being insolubilisation of sarcoplasmic proteins and solubilisation of myofibrillar proteins. Sarcoplasmic proteins were more susceptible to HPP effects than myofibrillar. Individual protein changes were significantly correlated with protein solubility, L\*, b\* and WHC, providing further insights into the mechanistic processes underlying HPP influence on quality and providing the basis for the future development of protein markers to assess the quality of processed meats.

#### *Effects of HPP on lipid oxidation*

In recent years, there has been increasing focus on the response of lipid components to HPP, especially considering the deleterious outcomes that secondary products of oxidation have on the final product. Since 1990s HPP has been used as an alternative to thermal treatments to pasteurize and sterilize food products, such as meats and seafood. Many of these raw materials have a high content of lipids (among them triacylglycerols and cholesterol-derivative) that are susceptible to oxidation. During the last decade, there has been increasing interest on the response of lipid components to HPP, especially considering the deleterious outcomes that secondary oxidation-derivative molecules have on the final product. A better understanding of the underlying phenomena could lead to the development of predicting models which could be use in food industry.

#### *Effect of HPP on lipid oxidation of meat and meat products*

The first attempts made to determine the effect of HPP on lipid oxidation date back to the early 1990s, coinciding with the exploration of this technology as an alternative to thermal sterilization (Rivalain *et al.*, 2010). Clearly, researchers initially directed their attention to fish and meat products that present a

higher susceptibility to lipid degradation, due to the higher unsaturated fraction. Most of the studies have been performed using thiobarbituric acid (TBA or TBARS) or peroxide value (POV) tests to determine products derived from lipid oxidation; however, due to the broad spectrum of compounds that respond to both techniques, oxidized lipids determination is generally only quantitative. Pressure treatment had a little effect on lipid oxidation below 300 MPa, but increased linearly at pressures above this value. 300 to 400 MPa appears to be a critical pressure for inducing marked changes in meat (Ilce *et al.*, 2014). HPP of meat and meat products can trigger lipid oxidation. The effect of HPP on lipid oxidation on pork (Cheah and Ledward, 1997) and poultry (Bragagnolo *et al.*, 2007; Kruk *et al.*, 2011) has established that pressure levels between 300 and 600 MPa are critical for inducing lipid oxidation in fresh meat. Rendered pork fat (aW = 0.44) subjected to hydrostatic pressure of 800 MPa for 20 min shows a shorter induction time (3 days) with respect to lower pressures (4 days, at P b 800 MPa), estimated as the time necessary to reach an exponential increase of peroxides value and TBA (Cheah and Ledward, 1995). Washed muscle fibers and minced pork treated with HPP in a range of 300–800 MPa for 20 min showed a linear relationship between pressure and TBA number (Cheah and Ledward, 1996).

In cured meat products, Fuentes *et al.*, (2010) reported that pre-sliced dry-cured ham was more susceptible to oxidative reactions and lipid oxidation compared to the control (Fuentes *et al.*, 2010). While Clariana *et al.*, (2011) stated that the oxidative stability of dry-cured ham pressurized at 600 MPa was not affected. As for the color the effect on lipid oxidation by HPP is more pronounced for fresh meat than for cured meat products (Clariana *et al.*, 2011).

Addition of citric acid (0.02%) inhibited the increased rate of lipid oxidation pork meat, eliminating the catalytic effect of pressure treatment. It is possible that metal ions (Fe<sup>+2/+3</sup>, Cu<sup>+2/+3</sup>) were released during HPP, becoming available to generate free radicals (via Fenton's reaction) and addition of citric

acid effectively chelated the released ions. This argument is reinforced by the fact that the reduction of ferric iron (Fe<sup>+3</sup>) to ferrous ion (Fe<sup>+2</sup>) is promoted by pressure, since the reaction has a negative activation volume (Cheah and Ledward, 1997).

The mechanisms by which HPP induces lipid oxidation are not fully understood. Generally, it has been suggested that HPP triggers lipid oxidation by two mechanisms: increased accessibility for iron from hemoproteins and membrane disruption. The release of iron from hemoproteins can promote lipid oxidation. Several works have observed that the addition of ethylenediaminetetraacetic acid (EDTA), which can chelate metal ions, correlated with a reduction of the lipid oxidation in meat processed by HP, which indicates that transition metal ion catalysis is the major cause underlying the increased lipid oxidation (Bajo *et al.*, 2012). Membrane disruption facilitates contact between unsaturated lipids from the membrane and enzymes and heme and non-heme iron or other metal cations likely contributing to catalyze lipid oxidation.

Cava *et al.* (2009) showed that in dry-cured ham and loins TBA values significantly increase even at lower pressure (200 and 300 MPa), regardless the time of treatment. However, the positive effect of holding time and pressure in secondary product formation was amplified during storage at 4 °C for 60 in both products. In dry-cured ham, HPP at 600 MPa for 6 min at 12 °C enhanced the formation of lipid derived aldehydes, such as pentanal, hexanal, heptanal and nonanal, which are responsible for off-flavors in this kind of product (Fuentes *et al.*, 2010).

#### *Changes in antioxidant activity*

Kwon *et al.* (2006) examined increasing antioxidative activities in garlic samples treated with high temperature and pressure (110-150 °C, 1- 5 h, 10 kgf/cm<sup>2</sup>) compared with control. They proposed that further research on antioxidative activities using non-thermal processing of HHP is necessary. Fengxia *et al.*, (2013) investigated the effects of high hydrostatic pressure and high temperature short time (HTST, 110

°C/8.6 s) on antioxidant activity, antioxidant compounds and color of mango nectars. Steam blanching was used prior to HHP and HTST to inactivate endogenous enzymes. Their results showed that antioxidant capacity (FRAP assay), L-ascorbic acid, sodium erythorbate, total phenols, total carotenoids, the redness (a\*), the yellowness (b\*) changed insignificant after HHP or HTST treatment. The lightness (L\*) exhibited a significant decrease in HTST-treated mango nectars, while no significant changes in HHP-treated samples. After 16 weeks storage at 4 and 25 °C, there were significant changes in antioxidant activity, antioxidant compounds and color, of mango nectars, whereas differences between HHP- and HTST-treated samples were not significant except for the decrease in L-ascorbic acid and sodium erythorbate, which was more pronounced in HHP-treated samples. As shown in Fig. 1, HHP and HTST treatments caused no significant change in antioxidant capacity of mango nectars using the FRAP assay, and a significant decrease using the DPPH assay was observed. Similar results have been well documented that in high pressure treated products antioxidant capacities were either unaffected or were reduced as compared to the fresh products (Fengxia *et al.*, 2013;Cao *et al.*, 2012). In contrast, Patras *et al.* (2009) reported higher antioxidant capacity of tomato and carrot puree after HHP treatment (400–600 MPa/15 min/20 °C). Fengxia *et al.*, (2013) reported after storage of 16 weeks, in HHP-treated mango nectars, the antioxidant capacity using ·DPPH assay decreased at 4 and 25 °C, and the antioxidant capacity using FRAP assay decreased. Their results were consistent with a study of Keenan *et al.*, 2010 that storage resulted in a significant reduction in total antioxidant activity by ·DPPH and FRAP assays for HHP (450 MPa/5 min) or thermally (P70≥10 min) treated fruit smoothies. It was found that there was a positive correlation between the antioxidant capacity and antioxidant.

Kyung *et al.*, 2014 investigated effect of high hydrostatic pressure treatment on flavor, physicochemical properties and biological functionalities of garlic. The antioxidative,

antimicrobial and antitumor activities of HHP treated garlic samples were decreased compared with control. A rapid decrease in antimicrobial and antioxidative activities was observed over 3 min HHP reaction time. No antitumor activities were observed after 3 min HHP reaction time. With different HHP reaction time and pH of solutions, they evaluated changes in antioxidative activities of HHP treated garlic samples (Fig. 2). The decrease in DPPH Free radical scavenging ability and total phenolic contents were observed in HHP treated garlic samples compared with control. As HHP reaction time increased, the DPPH Free radical scavenging ability of HHP treated garlic samples significantly decreased. It showed a rapid decrease after 56 s. After 3 min, statistical changes in the radical scavenging ability were affected by pH conditions and treatment time. It was reported that garlic samples treated with high temperature and pressure (120 °C, 20 min) decreased in antioxidative activities compared with control and the antioxidative activities in boiled, steamed and baked garlic were less affected (Jeon *et al.*, 2009).

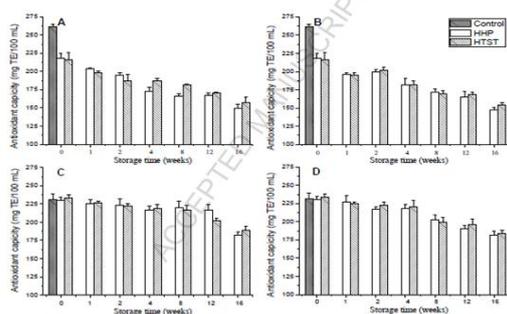
#### Changes in enzymatic activity

Kyung *et al.*, 2014 investigated the effect of high hydrostatic pressure (HHP) treatment on flavor, physicochemical properties and biological functionalities of garlic. They suggested that the alliinase activity depends on HHP reaction time rather than pH in solutions. It is generally considered that the optimal pH for alliinase activity is 6.0-6.5. In contrast, higher alliinase activity (40.3%) was observed in acidic condition of pH 1.8 solutions than

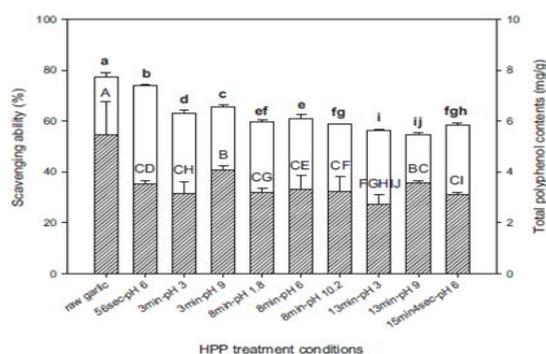
the others (pH 6 & pH 10.2) with 8 min HHP reaction time. The upward pH adjustment (pH 6 and pH 10.2) was not effective to enhance the alliinase activity as the activity showed 20.5% and 21.8%, respectively in HHP treated garlic samples. Up to 56 s HHP reaction time, the alliinase activity was not changed significantly but it was dramatically decreased at a longer HHP reaction time compared with control, showing higher stability in acidic condition than alkaline condition. They said the alliinase activity of HHP treated garlic samples with 56 s HHP reaction time was similar with that of control as shown at Table 1. The highest value of enzymatic activity was obtained in their samples among other HHP treated garlic samples while no enzymatic activities were obtained in HHP treated garlic samples with 13 min (pH 9) and 15 min (pH 6) HHP reaction time. High enzymatic activities were found in HHP treated garlic samples with 3 min HHP reaction time (pH 3, 6) compared with others. The acidic condition in samples maintained more enzymatic activities than samples in alkaline condition. They said similar effects were found in HHP treated garlic samples with 8 min and 13 min HHP reaction time. The decline in enzymatic activity (less than 50% compared with control) was caused due to longer HHP reaction time (over 8 min). Similarly, Sohn *et al.* (1996) reported that a significant decrease (around 90%) in alliinase activity of garlic soaked in distilled water with HHP (500 MPa) over 10 min HHP reaction time was observed, and a full inactivation of alliinase with 20 min HHP reaction time was reported.

**Table 1.** Changes in alliinase activity of HHP treated garlic samples with different HHP reaction time and pH (Kyung *et al.*, 2014).

	Activity of alliinase (nmol/min, mg protein)
Raw garlic	17.821 ± 0.595 <sup>a</sup>
56 s – pH 6	17.294 ± 0.0834 <sup>ab</sup>
3 min – pH 3	15.625 ± 0.369 <sup>c</sup>
3 min – pH 9	13.801 ± 1.013 <sup>d</sup>
8 min – pH 1.8	7.173 ± 1.38 <sup>e</sup>
8 min – pH 6	3.661 ± 0.0526 <sup>fg</sup>
8 min – pH 10.2	3.877 ± 0.474 <sup>f</sup>
13 min – pH 3	3.15 ± 0.213 <sup>fh</sup>
13 min – pH 9	0 ± 0.254 <sup>i</sup>
15 min 4 s – pH 6	0 ± 0.163 <sup>ij</sup>



**Fig. 1.** Changes in antioxidant capacity in mango nectars after high hydrostatic pressure and high temperature short time treatments and during 16 weeks storage at 4 and 25 °C (A, DPPH assay at 4 °C; B, DPPH assay at 25 °C; C, FRAP assay at 4 °C; D, FRAP assay at 25 °C) (Fengxia *et al.*, 2013).



**Fig. 2.** Changes in antioxidative activities and total polyphenolic contents of HHP treated garlic samples according to different HHP reaction times and pH. The different letters on the bars were significantly different ( $P < 0.05$ ) (Kyung *et al.*, 2014).

## Conclusion

Chemical modifications due to innovative technologies are important and relevant topics in the field of Food Science and Engineering. The thermo-labile nature of protein meat systems and the described effects of pressure on meat and meat proteins allow the development of novel meat based products and the usage of HPP as a time and energy saving processing step for the meat industry. Lipid degradation in HPP must be contextualized within the broad transformation of biological structures of foods. For example, the liberation of radicals or precursors of radicals due to the breakdown of cell membranes induced by pressure is one of the most

important causes of increase in lipid degradation. Furthermore, the contribution of pro-oxidative enzymes in lipid degradation seems to be relevant, considering the effects that high pressures have on proteins. HHP and HTST treatments can be used as alternative methods for the preservation of mango nectars with a microbiological stability. The antioxidative activity of HHP treated garlic samples decreased along with increasing HHP reaction time. No enzyme activity was observed in HHP treated garlic samples.

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