DOI: https://doi.org/10.21323/2414-438X-2020-5-2-26-38

AND SAFETY OF MEAT PRODUCTS

Review paper APPLICATION OF HIGH HYDROSTATIC PRESSURE TECHNOLOGY TO IMPROVE CONSUMER CHARACTERISTICS

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Keywords: *high hydrostatic pressure, structure modification, functionality, myofibrillar protein, low salt meat products, low phosphate meat products, low fat meat products, additive-free foods, water retention, microbiological safety, quality, oxidation processes*

Abstract

Recently, there has been a growing demand for healthy processed foods, such as comminuted or gel-type meat and fish products with reduced content of salt (sodium chloride), phosphate (sodium phosphate) and/or fat, while maintaining their texture and quality characteristics. As know, a high intake of dietary sodium is associated with cardiovascular diseases and strokes. On the other hand, high phosphate intake has a potential health risk, especially with regard to bone metabolism, cardiovascular and kidney diseases. High hydrostatic pressure (HHP) technology has been recognized as a useful method for successfully reducing salt, phosphate and/or fat content in processed muscle products. The texture, yield and organoleptic properties of products are closely related to the structure and functionality of myofibrillar proteins (MP). Application of moderate high hydrostatic pressure at 100–200 MPa has been successfully used to increase the functionality of myofibrillar proteins by modifying the structure due to denaturation, solubilization, aggregation or gelation. The ability to reduce sodium content and achieve a high binding and water retention using this technology is an important task for the production of healthy food products.

Introduction

The consumer demand for healthy food products has been steadily growing worldwide. With the increasing understanding of the link between nutrition and health the growth in changes in lifestyle and nutrition structure of consumers, the extension of knowledge about food processing, many processed foods products containing the high level of sodium and/or fat have been criticized due to the problem of their health effects. Healthy nutrition is understood by consumers as foods with different origin and composition; with that, the requirements for reducing edible sodium, fat and other food additives continue to be in the first place. The demand for healthy meat and fish products with reduced content of salt (sodium chloride), inorganic phosphate (sodium polyphosphate) and/or fat with maintenance of texture and taste characteristics of finished products has been growing.

Refusal or restriction of excessive consumption of foods contained saturated fats is linked with risks of obesity, development of chronic metabolic disorders and diseases of circulatory system [1,2].

High dietary sodium intake is associated with cardiovascular diseases and stokes [3,4]. In 2010, the global mean sodium chloride intake was 10.06 g/day [5]. The limit of sodium chloride intake recommended by WHO is 5 g/day. Japan and Russia Federation belong to countries with sodium intake above the recommended level (Figure 1). The



Figure 1. Countries with high sodium chloride intake. Adapted from Powles et al. [5]



trend towards refusal of high sodium intake will allow a worldwide reduction in the risk of cardiovascular diseases, including strokes [3,4,6].

With that, humans have an increasingly active life, in which less time is available for food preparation at home. Processed foods became the main source of dietary sodium intake by modern humans — up to 77% of daily sodium intake comes with such products [7]. Moreover, processed meat products^{*} account for 20–30% of daily sodium intake [8,9].

In processed meat products, sodium is contained mainly as sodium chloride. Its total amount in a product is built up due to its addition (1% to 5.5% of raw meat material weight) and its natural content in meat (less than 1%).

Another source of sodium in meat products is different sodium food additives — glutamates, phosphates, citrates, lactates, acetates and others. Among food additives used in the meat industry, food phosphates, which are used in an amount of up to 0.5% of meat raw material weight, should be highlighted as a significant sodium source. It is less than the addition of sodium chloride, which contains 39.7% sodium. However, food phosphates contain sodium in a comparable amount. For example, sodium tripolyphosphate (E451) contains 31.3% sodium and sodium pyrophosphate (E450) has 34.6% sodium [9,10].

From the perspective of healthy nutrition, processed meat products are also criticized for the unbalanced phosphorus (P) and calcium (Ca) ratio. Food phosphate additives cause concerns due to the sharp shift of the Ca: P ratio from the natural ratio of these elements in muscle tissue (1:5), which is per se far from the ratio recommended by dieticians (1:1). Due to added phosphate, the phosphorus content in the processed meat products is nearly twice as high as in the initial meat raw materials [11]. The potential effect of phosphate additives on population health can be still underestimated, especially in the conditions of low consumption of dairy products rich in calcium.

High phosphate intake has a potential health risk, especially, regarding bone metabolism, development of cardiovascular and renal diseases. Phosphate additives in processed food products can be harmful for population health due to the damage of vessels, provoke ageing processes, lead to the development of hyperphosphatemia, which was identified during the last decade as a strong predictor of mortality in humans with chronic renal diseases [12].

In this connection, consumers who prefer foods with the so-called "clean-label" perceive phosphates as undesirable food additives.

Besides meat products, phosphates are widely used in significant amounts in the production of fish products, baked foods, cheeses, carbonated soft drinks and so on [12]. Since the 1990s, the use of phosphate food additives increased from 500 mg/day to 1000 mg/day, which indicates the topicality of the development of technologies that does not envisage their use [13].

Different countries of the world undertake legislative measures that guarantee the presence of healthy food products on the market for consumers. Similar measures are taken in Japan and the Russian Federation. For example, since 2018, the Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor) has been realizing the project of voluntary food product labeling "traffic light", which envisages color coding on product labels: green, yellow and red depending on the content of salt as well as sugar, fat, saturated fats, energy value (caloricity) with consideration for daily intake. The main task of this labeling is to give consumers visual information, which will allow them to make a correct choice when buying food, as well as facilitate adherence to the principles of healthy nutrition and reduction of risks for population health [14].

Since 2019, Rospotrebnadzor has been implementing measures aimed at propaganda of healthy nutrition principles and the creation of conditions in the Russian Federation that will facilitate the formation of healthy lifestyle in the framework of the Federal Project "Strengthening Public Health". The Federal Project presupposes the introduction of a system for monitoring nutrition of different population groups, including children, in regions, which will allow establishing a connection between the nutrition structure, food quality and population health state. The monitoring system will enable the accumulation of data that are necessary for assessing actual nutrition in different regions of the country and their use for developing targeted educational programs and updated recommendations on nutrition for different population groups [15].

This year, the Ministry of Health of the Russian Federation published the order No. 8 of January 15, 2020 "On approval of the strategy for the formation of a population healthy lifestyle, prevention and control of noncommunicable diseases for the period up to 2025". According to the strategy, one of the main measures of noncommunicable disease prevention is rational nutrition including daily consumption of fruit and vegetables (not less than 400 g/ day), reduction of free sugars (to a level of less than 10% total energy intake, which is equivalent to 50 g/day), consumption of fats in an amount of less than 30% of total energy intake, consumption of salt less than 5 g/day [16].

On the other hand, Japan has been implementing the targeted national 10-year program on improving health in the 21st century ("Health Japan 21"). The program was launched in 2013 and updated in 2018. According to the basic "Health Promotion Act", the program is aimed at the reduction of premature deaths, the extension of healthy life expectancy and improvement of life quality. Much attention is paid to the creation of environment for the prevention of lifestyle-related diseases and the assurance of food safety. In Japan, the Ministry of Agriculture, Forestry and Fisheries (MAFF), which is responsible for production

^{*} here and elsewhere this expression means meat and meat products that have not only raw meat materials in their composition.

and assurance of food quality, and the Ministry of Health, Labour and Welfare (MHLW), which regulates the stability of distribution and safety of foods, are together engaged in solving these tasks at all stages from production to consumers.

To stimulate an improvement in nutrition habits of the population, MHLW establishes and revises every five years Dietary Reference Intakes for Japanese. The basis of these recommendations is constant monitoring of the latest scientific achievements and international standards, as well as the analysis of the actual results of the annual National Health and Nutrition Survey in Japan. A consideration of the peculiarities of the traditional cuisine and lifestyle in developing recommendations results in several differences with the recommendations of the Codex Alimentarius Commission [17]. In particular, salt intake in Japan is significantly higher than the norm of 5 g recommended by WHO, although it has been constantly reduced. In recommendations of 2015, salt intake was established at a level of less than 8 g per day for males and 7 g per day for females. The last edition of 2020 recommends the intake of less than 7.5 g per day for males and 6.5 g for females. The daily intake of fats at a level of 25% of total energy intake is recommended [18,19].

To prevent lifestyle-related diseases, the Guidelines for a balanced diet (from the Ministry of Agriculture, Forestry and Fisheries) recommend increasing consumption of vegetables up to 350 g and more, and fruit up to 200 g and more per day [20]. A strong emphasis is placed on the education of schoolchildren to increase familiarity of the nation with the issues of safety and balance of food products.

Since 2015, food labeling has been carried out according to the Food Labeling Act of 01.04.2015 with Consumer Affairs Agency (CAA) controlling its execution.

The Food Labeling Act applies to all food types (including food additives) and beverages (including alcoholic) with the exception of pharmaceutical drugs. The specific requirements for the label content significantly increased and are put down in the Food Labeling Standards. Labeling such components as the energy value, content of proteins, fats and carbohydrates, as well as salt equivalent (total sodium content) became mandatory. The mandatory requirements for labeling of the main allergenic foods and additives, the presence of L-phenylalanine compounds were introduced. From March 2022, marking countries of raw material origin on a label will become mandatory, even for the finished products finally packed or processed on the territory of Japan.

The inclusion of the content of saturated fats and dietary fibers into information for consumers that is placed on a label is voluntarily but recommended. Labeling of available carbohydrates, sugar, cholesterol, vitamins and minerals is completely voluntary [21].

Requirements for labeling food with health claims are different for each of the three categories. Products containing food components with proven effects on the physiological functions of the human body (Food for Specified Health Use; FOSHU) require mandatory control, registration in the state bodies — and have special labeling. Products with the declared food value (Food with Nutrient Function Claims: FNFC) have to account in labeling the recommended daily intake (for example, for vitamins and minerals) and are based on state standards. The most flexible category includes products with stated functional properties (Foods with Function Claims: FFC) and was introduced in 2015. The corresponding marking on a label is done according to available scientific research about the health benefits of an ingredient and is a responsibility of a producer. For any category, permission for placing information about one or another physiological effect from a product is given only after research confirming this effect and misleading consumers with false statements is punishable by law [21].

Therefore, food legislation in different countries set new tasks for the meat industry regarding further development of the functional product market and widespread reduction/exclusion of using undesirable components of meat products, first of all, salt, phosphates and/or fat. At the same time, sodium chloride and sodium phosphates are the main recipe components of meat products having an important function - extraction of myofibrillar proteins (solubilization) and formation of meat gel, which holds water, stabilizes fat in a product and form product consistency. Solubility, water-holding, gelation and emulsification of myofibrillar proteins depend on salt and phosphate addition and these functional and structural protein properties are important determining factors of consumer quality of meat products (appearance, color, texture, taste, aroma) as well as economic indices (yield and cost of finished products).

Processed meat products traditionally continue to be quite popular among consumers, but their assortment includes an insufficient quantity of products with low content of salts, phosphates or fat on markets in different countries. In other words, there is a growing demand for healthy, nutritional and economical muscle products with low content of food additives and maintenance of their textural and taste attributes. Nevertheless, reduction of the content of sodium salts or fat creates significant problems for manufactures of the meat industry and negatively influences such product quality characteristics as color, texture, organoleptic indices, as well as yield and primary cost of finished products. While a certain rise in product prices can be accepted by a consumer, maintenance of familiar sensory characteristics when excluding/reducing undesirable recipe components is the main problem of healthy nutrition products. Consumers will always want not only healthy but also tasty food. This makes scientists to search for new technical and technological solutions to create products of healthy nutrition with the maintenance of the required quality level. With that, in addition to meeting requirements for the composition and sensory characteristics, requirements for safety and shelf-life of meat products are progressively increasing.

Over the last years, several approaches to the reduction of the sodium or fat content in processed meat products have been formed, among which are:

- use of salt replacers that exclude/reduce sodium intake, in particular, potassium chloride (KCl), in combination with substances masking off-taste;
- use of taste enhancers that increase the perception of product saltiness when used simultaneously with reduced salt amount;
- optimization of the physical form of added salt that allows enhancing and/or creating salty taste in a particular moment of product consumption (reduction of salt crystal sizes, formation of double emulsions);
- replacement of fat with hydrocolloids;
- use of special technologies for raw material processing (for example, processing with high hydrostatic pressure) [22,23,24,25,26,27,28,29,30,31,32,33,34,35].

Nowadays, in the Russian Federation, the main measures regarding sodium content reduction in processed meat products are directed towards decreasing doses of edible salt addition and the use of its replacers, which, in turn, cannot ensure clean-label [32].

There is an urgent need for searching alternative methods that will allow manufacturing products with low sodium and/or fat content and high functionality. Today, physical methods of processing have been finding increasingly wider application in the world and are regarded as promising technologies that enable a significant reduction of industry demand for food additives.

High hydrostatic pressure (HHP) pre-treatment at 100–200 MPa before heat treatment is one of the methods for successful reduction of the content of salt, phosphate and/ or fat in gel-type or minced meat and fish products.

It was shown that the use of high pressure impacts myofibrillar proteins (MP) similar to the effect of salts; consequently, both sodium chloride and sodium phosphates can be reduced by this method [36]. High pressure (HP) is used for increasing the functional properties of MP, causing structural modifications, leading to denaturation, aggregation and gelation. Improvement of functional properties by enhancing water-protein and protein-protein interactions changes the textural properties, improves water binding and increases stability of the meat system. These modifications depend on a product composition, introduced food additives, pressure parameters, pressure/temperature combinations and the sequence of application [37].

The above mentioned aspects determined the aim of this review: (1) to study the main details of structural and functional changes in myofibrillar proteins (MP), modified by high hydrostatic pressure (HHP); (2) to present an overview of achievements in the field of using high hydrostatic pressure (HHP) technologies to produce meat products of healthy nutrition with improved functionality and increased shelf-life.

Main part

Use of high pressure technology in the food industry

The first use of high pressure (HP) in the food industry was proposed by Hite in 1899 for the pasteurization of milk and fruit products [38]. However, at the end of the 1980s, it was Japan that became the first country using a hermetically sealed vessel specially designed for carrying out the HP procedure in the food industry to produce jam and fruit puree for commercial purposes. Since the first application in Japan, this technology was significantly developed with respect to technological aspects and assortment of pressurized food products on sale [39].

High pressure processing is a non-thermal technology in which a product is treated with a pressure of 100 MPa and higher using compression fluid [40]. There are three forms of such processing: a static process in a vessel at high hydrostatic pressure (HHP) during a certain time and at the certain temperature; the dynamic process in which fluid is pressed through a nozzle jet under high pressure (homogenization by high pressure); hydrodynamic processing with pressure or shocking waves which instantaneously development pressure waves up to 1 GPa [41].

The high pressure technology is based on two principles that determine the behavior of a product under pressure. An important principle underlying the effect on reaction equilibrium is known as the Le Chatelier's principle, according to which any phenomenon that is accompanied by volume reduction will be enhanced by a HP increase and vice versa. The use of HP leads to physical and chemical changes. However, chemical changes are minimal; HP does not affect covalent bonds, influencing only weaker ionic and hydrogen bonds; that is, hydrophilic and hydrophobic interactions. As HP does not impact covalent bonds, the majority of low molecular weight compounds such as vitamins and flavor substances do not change. Nevertheless, HP affects high molecular weight components of food and can cause protein denaturation and aggregation which leads to a change in raw material texture both with a positive and negative result of such the effect on final product quality [39]. HP significantly influences product microflora. Microbial inactivation that facilitates food preservation is a result of cell membrane damage, protein denaturation, changes in intracellular enzyme activities, ribosome damage, and other effects caused by HP [42].

The second important principle that explains the effect of HP is isostatic. According to this principle, the pressure is instantaneously and uniformly transmitted throughout a product volume without a gradient. Therefore, the HP is characterized as an isostatic quantity. Pressure does not depend on a product size or geometry, and pressure transmission to the product center does not depend on its mass and process duration. After pressure treatment, a product returns to its initial shape [39].

Programs on processing foods, beverages and other products (Figure 2) began to be performed from the 1990s, and one of the first works was a shelf-life extension of



Figure 2. Practical uses of HP pasteurization [43]

avocado-based products. This processing method leads to an effective reduction of the microbial contamination with minimum impact on the sensory characteristics and nutritional value. Very soon, the HP technology became known as a post-packaged preservation technology for ready-to-eat products: whole or sliced meat products, seafood, fresh-cut fruit and juices, as well as salads, sauces, dressings, soups and so on [36,43].

Today, more than 160 industrial units (vessels) are used worldwide [39]. A large part of the equipment (44%) is intended for processing products based on fruit and vegetables (Figure 3). It is explained by the fact that avocado processing was one of the main drivers of this innovative technology [44,45]. In addition to microbial inactivation at low temperatures, this technology can be used to develop new products without thermal impact or for manufacturing similar products with minimum effect on taste, color and nutritional value [40].

High hydrostatic pressure (HHP) processing had received wide recognition in the meat sector. It is, possibly, linked with the fact that meat products have a high commercial value which allows investing and covering expenses on such processing. At present, 25-30% of the whole volume of HP equipment is used in the meat industry [36]. Over the last decades, the technology has been widely used in meat product manufacture for inactivation of pathogenic microorganisms and, therefore, for shelf-life extension [46]. However, the HP technology is not regarded any more as a simple alternative to conventional pasteurization. Today, this technology is increasingly often used as a processing method that causes structural modifications to create innovative meat products with increased functionality, products with reduced content of food additives, products with a modified structure for certain population groups [37]. Therefore, HP continues to be an alternative to heat treatment to ensure microbiological safety and is used to change a product structure with maintenance of its nutritional value. This ensures the high potential for modification of the assortment of minced meat products, creation of innovative products by influencing the structural



Figure 3. Distribution of HP equipment in food industry [45]

and functional properties of myofibrillar proteins (MP). This can facilitate a reduction in the use of food additives with E-numbers and an increase in manufacturing products with clean-label [37].

Researchers [29,47,48,49,50,51] showed that the use of HHP in meat processing to improve functional properties of myofibrillar proteins opens wide possibilities for production of meat products with reduced content of salt, phosphate, fat and calories. HHP can be used to change the structure of MP modifying protein by disrupting noncovalent interactions (electrostatic and hydrophobic) in tertiary and secondary structures within protein molecules and the subsequent formation of intra/intermolecular bonds. The control of pressure-induced structural and functional changes in MP in meat systems is an important question for meat product manufacturers [37].

The result of structural modification of MP depends on inherent characteristics of a product (species, type, pH, composition, presence of one or another ingredient) and conditions of HHP application (pressure intensity, pressure gradient, duration, temperature, pressure/temperature ratio, sequence of stages of technological processing and pressure application) (Figure 4). The corresponding choice of HHP parameters can change meat quality, functional characteristics of muscle proteins and, partially, compensate a decrease in doses of salt and food additive introduction and in the fat content, thereby, increasing the meat product value [37,52,53].

Effect of high pressure on the meat structure

As known, a muscle contains three types of proteins classified by their solubility and location in muscle tissue — sarcoplasmic, myofibrillar and stroma proteins. Myofibrillar proteins (MP) or salt-soluble proteins, which are soluble only in solutions with relatively high ionic strength (>0.3 M NaCl or KCl) account for about 50 to 56% of the total quantity of muscle proteins and are linked with the meat structure. MP extracted from a muscle fiber, mainly consist of myosin and actin with myosin as the most abundant protein. Myosin is the major MP which is



Figure 4. Schematic presentation of processes of technological processing of meat products using HHP and formation of meat product characteristics. Adapted from Chen et al. [37]

Note: HHP — high hydrostatic pressure

responsible for the total solubility, emulsification, gelation, water- holding. These functional properties are important determining factors for yield, texture and organoleptic indices of meat products.

One of the main effects of HP impact on meat proteins is depolymerization. It was confirmed that depolymerization of F-actin, extracted myosin and actomyosin occurs at the pressure intensity of 100 to 300 MPa. It is assumed that depolymerization facilitates an increase in solubility of MP [36,37,54].

Pressure-induced structural changes in myofibrillar proteins were also well studied. MP began unfolding under the pressure of 50 to 300 MPa, and higher pressure levels lead to increased denaturation, agglomeration and gelation. These structural modifications of meat proteins under pressure can be used in developing products to affect structure formation and/or water retention. Also, pressure-induced structural changes in myosin head (myosin subfragment-1, S-1) were described at pressure from 150 MPa [36,37,55]. Therefore, pressure can cause partial or full unfolding, denaturation, reversible or irreversible aggregation of MP.

One of the significant effects of HP on meat proteins is the modification of actin-myosin complex. Suzuki et al. (1990) studied the effect of HP (100–300 MPa) on beef post-rigor muscle and noticed that maximum fragmentation was achieved at 300 MPa within 5 min. In addition, the Z-line in myofibrils was not apparent in pressurized muscles [56]. Iwasaki et al. (2006) also described the destruction of the M-line and Z-line, and dissociation of thick and thin filaments within myofibrils under HP. This arrangement of the thick and thin filaments within myofibrils is mainly responsible for the ability to retain water in muscle cells. Therefore, any spatial rearrangement or disruption between filaments affects the ability to retain water [48].

It was noticed that HP pre-treatment changed the conformation and functional properties of MP which subsequently can influence the gelling property of protein upon heat treatment. Heat-induced gelation of myosin is a key factor in the production of minced meat and fish products (pastes and so on). The HP effect on denaturation and gelation of meat proteins significantly depends on many factors such as a protein system, ionic strength, presence of additional ingredients, and parameters of HP application. Treatment of MP with pressure higher than 100 MPa at low temperature causes protein solubilization and unfolding which are necessary for initiation of protein gelation. However, both the conformation condition of myosin and salt content can influence the gelation of myofibrils. HP-induced gelation of myosin chains occurs as a result of their interaction with each other similar to heat-induced gelation; however, tail to tail interaction of the protein structures is not observed when HP is applied [57,58,59,60]. Therefore, HP-induced meat gels are often weaker than heat-induced gels. Nevertheless, the use of HP pre-treatment at a low temperature before heat treatment can enhance the thermal gelation of myosin (Figure 5). Thus, myosin pre-treated with HP retains its ability to form aggregated structured gel as a result of heat treatment (after heating and cooling) [59].

For example, Macfarlane et al. (1984) showed that HP processing at 150 MPa for 10 min at 0 °C improved heatinduced (70 °C, 10 min) gelation of myosin from sheep muscle at low ionic strength [61]. They also reported that HP pre-treatment (150 MPa, 20 min, 0-3 °C) increased structural-mechanical characteristics (tensile strength) and water-holding capacity of cooked beef patties contained 1% NaCl.

Iwasaki et al. (2006) also suggested that depolymerization of thin filaments was a cause of high apparent elasticity of heat-induced myofibrillar gels when using HP pre-treatment at 200 MPa. Pressure processing at 200 MPa (10–20 min, 20–25 °C) before heat treatment at 70 °C increased the elasticity of pork patty contained 1% NaCl (at pH 6) and chicken myofibrillar gel by 2–3 times, respectively [48]. They suggested that HP processing at 300 MPa causes shortening of myosin filaments, which can facilitate a decrease in elasticity of heat-induced myofibrillar gels compared to pressurized gels.



Filament (0.1 M KCI)

Figure 5. Proposed mechanism of the HHP-induced aggregation and gelation of myosin in high ionic strength (0.5 M) or low ionic strength (0.1 M) solution at pH 6.0 based on the findings of Iwasaki et al. (2005); Yamamoto et al. (1990); Yamamoto et al. (2002) and Yamamoto et al. (1993) [57,58,59,60].

Note: HHP — high hydrostatic pressure

Ikeuchi et al. (1992) suggested that improved gelation of actomyosin induced by HP pre-treatment is explained by denaturation of actin in actomyosin and increased content of sulfhydryl, as well as increased surface hydrophobicity. As shown in Figure 6, the high quantity of F-actin negatively affects thermal gelation of actomyosin at low and high KCl concentrations according to the theory proposed by Yasui et al. (1980) [62]. HP pre-treatment at 100–150 MPa will effectively reduce a negative effect of excessive quantity of F-actin in actomyosin on gel strength of myosin via depolymerization of actin. As a result, HP pre-treatment at 150 MPa increases the heat-induced gel strength of actomyosin [63].

The initial effect of HP impact on MP consists in dissociation of the quaternary structure causing protein unfolding and solubilization, and exposing functional groups of the protein surface. As shown in Figure 7, treatment at moderate pressure levels (100–200 MPa) increases the solubility of MP due to complex dissociation and depolymerization of the protein structure. MP denature and moderately stretch causing destabilization of their protein structures and exposure of hydrophobic and sulfhydryl groups. During the following heating, the helical tail portion of myosin unfolds and multiple cross-links of the tail-tail type are formed for water holding. This leads to the formation of the regular, homogeneous, filamentous, three-dimensional network with increased water-holding capacity (WHC) and gel strength.

On the contrary, treatment with pressure higher than 200 MPa causes strong protein-protein interaction with



Figure 6. Proposed mechanisms on the effects of HHP on the thermal gelation of actomyosin in 0.6 M or 0.2 M NaCl/KCl at pH 6.0 based on the findings of Wang et al. (2017); Cao et al. (2012); Iwasaki et al. (2006); Ikeuchi et al. (1992) and Yasui et al. (1980) [48,62,63,64,65,66]. Note: HHP — high hydrostatic pressure; SH: sufhydryl groups



Figure 7. Proposed mechanism of HHP impact on denaturation, solubilization, aggregation and thermal gelation of MP in 0.6 M NaCl/KCl at pH 6–7 [64]

Note: HHP — high hydrostatic pressure, WHC — water-holding capacity

the participation of hydrophobic radicals, hydrogen and disulfide bonds forming large insoluble aggregates. When heating, the α -helical structure of the tail portion of myosin unfolds to a lesser extent. Water-protein interactions during association of myosin tails are suppressed and protein-protein interactions will advance. As a result of processing with pressure higher than 200 MPa, the globular aggregated network will be formed, which is characterized by cavities of a larger size between protein chains. Such segmentary structure will be weakened and cannot hold water effectively.

Therefore, when it is necessary to increase the WHC of protein gel after heat treatment, pressure of not more than 200 MPa is to be applied.

Nevertheless, salt and phosphate concentrations should be regarded as key factors influencing gelation under HP. It is assumed that the presence of 1% salt can increase the sensitivity of meat proteins to an impact of HP. Similar to the effect of meat protein denaturation, it depends on the intensity of pressure application and on the presence or absence of salt and phosphate in meat systems [37].

Use of high pressure technology to develop healthy, functionally improved gel-type or minced muscle products

Recently, the HHP technology was tested in several studies on different meat and fish products to satisfy consumer requirements regarding the reduction of salt, phosphate and fat content (Table 1).

Studies on the application of HHP pre-treatment of beef, pork and fish proved the applicability of this tech-

nology for the production of meat products with reduced sodium content. Sikes et al. [47] treated the beef batters contained 0%, 0.5%, 1%, and 2% salt at different pressure parameters (0.1–400 MPa) at 10 °C, for 2 min. They observed that beef sausages treated at 200 MPa and contained 1% salt had texture similar to sausages with 2% salt. With that, the samples of sausages had the same cooking losses. Thus, it was shown that HHP use leads to an increase in yield and improvement in finished product texture. The researchers suggested that improved WHC of low salt batters was determined by increased the solubility and gelling property of protein as a result of its partial unfolding under an effect of HHP treatment.

Maksimenko et al. [50] studied the effect of HHP treatment (100-200 MPa, 10 min, 20 °C) in combination with the sodium chloride (0-2%) and sodium phosphate (0-0.5%) addition on the physico-chemical properties of beef gels (color, WHC, protein composition, texture). Beef gels pressurized at 150-200 MPa showed the synergetic effect in the increasing water content and the reducing cooking losses compared to unpressurized beef gels. The L*, a* and b* color values of raw beef gels slightly decreased upon HHP treatment; however, HHP-induced color changes in raw beef gels did not significantly influence the color of beef gels after heat treatment. Electrophoretic investigation of samples by SDS-PAGE showed that the staining intensity of the α -actinin protein band decreased in the HHP-treated beef gels. The cohesiveness, adhesiveness, gel strength and modulus elasticity of beef gels with low concentration of sodium chloride and/or sodium phosphate after heat treatment were improved as a result of HHP application

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Product	Conditions of the experiment	Result of the experiment	Proposed mechanism	Reference
Low fat and low salt beef sausage	Low fat beef mince was prepared with 0.6%, 0.8%, 1% NaCl and then HHP processed at 0.1–400 MPa/10 °C/ 2 min	Samples with 1% NaCl and treated with HHP at 200 MPa had similar texture characteristics and cooking losses compared to samples with 2% NaCl and without HHP treatment	An increase in solubilization of myosin and actin, improvement in binding of muscle components	Sikes et al. [47]
Low fat and low salt beef gel	Low fat beef gel was prepared with 0–2% NaCl and 0–0.5% phosphate, and HHP pretreated at 0.1–200 MPa/20 °C/10 min	Texture and moisture binding capacities were improved after HHP treatment at 150 MPa	Combined use of moderate HHP and low salt concentration caused modification of protein structure, reduction in concentration of α -actinin and M-protein, improvement in water-protein and protein-protein interactions	Maksimenko et al. [50]
Pork sausage with reduced salt and phosphate content	HHP pre-treatment of mince at 0.1–150 MPa/20 °C/5 min before mixing with 0.5–2.5% NaCl and 0.25% phosphate	HHP treatment at 150 MPa can be used for reduction of salt content up to 1.5% while maintaining acceptable organoleptic and functional properties		O'Flynn et al. [29]
Pork sausage with reduced phosphate content	HHP pre-treatment of mince at 150 and 300 MPa/20 °C/5 min before mixing with 0%, 0.25% or 0.5% phosphate	HHP treatment at 150 MPa has potential for phosphate reduction (0% or 0.25%) without significant changes in functional and consumer properties	HHP caused solubilization and extraction of MP without using food additives, modified protein structure, facilitated binding of meat particles	O'Flynn et al. [51]
Fish (surimi) gel with reduced salt content	Fish gel was prepared with 0.3% NaCl or 3% NaCl and HHP treated at 0.1, 150, 300 MPa/10 °C/10 min	Mechanical and organoleptic characteristics of gel with 0.3% NaCl were improved after applying HHP at 300 MPa and showed the same results compared to gel with 3% NaCl	HHP induced protein denaturation in low salt samples is similar to unfolding of protein structures in the presence of high salt concentration (3%)	Cando et al. [67]

Table 1. Results of the studies on HHP application to develop new products with reduced content of salt, phosphates and/or fat

at 150–200 MPa/ 20 °C/10 min. The authors came to the conclusion that the combined use of the low sodium chloride concentration and HHP at 150–200 MPa before heat treatment allows reducing salt concentration up to 1% with simultaneous maintenance of sensory and texture characteristics. They suggested that an improvement in texture properties and maintenance of WHC in the pressurized samples containing 1% salt is caused by changes in the protein fractional composition: losses of M-protein and aggregation of α -actinin.

O'Flynn et al. [29,51] reported that low salt sausages (salt content reduction from 2.5% to 1.5%) can be produced using meat pretreated with HHP at 150 MPa, 20 °C. These parameters allowed maintaining familiar sensory and functional properties of sausages. Moreover, the use of HHP processing improved rheological and texture properties, reduced cooking losses.

HHP pre-treatment can be successfully adapted to obtain low phosphate and low fat meat products. This was confirmed by studies that showed that HHP caused solubilization of myofibrillar proteins without the additional introduction of phosphate additives. Changes in the protein structure caused by HHP allow obtaining minced meat products with improved functionality [37]. The use of HHP processing at 150 MPa for 5 min. has a large potential for reducing phosphate levels up to 0.25% in low fat sausages without significant changes in their functional characteristics. The authors [53] reported that sausages with 0.25% phosphate content had improved firmness, adhesiveness, cohesiveness, and consistency, when pork meat batters were processed at 150 or 300 MPa/20 $^{\circ}$ C/5 min, compared to sausages with 0.5% phosphate content that were not treated with high pressure.

Maksimenko et al. [50] also reported that beef gels prepared with 1% NaCl without the addition of phosphate and fat that were HHP pre-treated at 150 MPa/20 °C/10 min, demonstrated improved texture and functional characteristics after heat treatment compared to the unpressurized samples with 2% salt + 0.5% phosphate content. In cooked sausage production without phosphates addition, pressurization at 100 MPa/20 °C/5 min improved functionality of meat proteins and allowed obtaining a product with the corresponding structure [36].

Based on similar considerations, HHP processing was used for development of gel-type fish products. Cando et al. [67] found that HHP processing at 300 MPa/10 °C/10 min improved the mechanical and sensory properties of gel with reduced salt concentration (0.3%) and stabilized protein structures in these gels to the same extent as in the samples with 3% salt content. The process of gel formation with participation of muscle tissue proteins from fish and slaughter animals is different; thus, conditions of HHP exposure are not the same.

Therefore, investigations carried out on various raw material types showed the possibility of managing the functional properties of muscle proteins. However, not only physical parameters are important in HHP technology. Along with them, salt and phosphate concentrations should be regarded as the main factors influencing the gel formation of meat proteins. In other words, prudent combination of recipe selection and HHP technology allows significant reduction in used salt and phosphate food additives.

Use of high pressure technology to increase safety and maintain quality of meat products

Another important direction for the application of HHP technology is an? increase in the microbiological safety of meat products. This direction considers the use of significantly higher ranges of HHP than parameters recommended for solubilization and the increase of functional characteristics of MP.

Several studies [68] showed that exposure of meat products with the HHP of up to 600 MPa for 3 min can inactivate dangerous pathogenic microorganisms (L. monocytogenes, E. coli, Salmonella and others), as well as the majority of mold species. To suppress vital activities of molds, treatment at 400 MPa is considered sufficient; however, it is noted that higher HHP level and longer exposure are required for killing yeast spores [69]. Effectiveness of HP treatment of meat products depends on existing microbial species, chemical composition, pH, and water activity of a product. HHP over 800 MPa causes the destruction of microbial cell membranes and intracellular protein structures leading to the disorder of homeostasis and cell death. The application of HHP in combination with other types of technological processing (blanching, dehydration, roasting, freezing-thawing, and others), opens wide possibilities for creating combined technologies to improve microbiological safety and product stability [69].

pH values higher than 6.2, which are typical for DFD meat, on one hand, ensure high functional characteristics of raw meat material, and, on the other hand, prevent predicting a possibility of its long term storage in the chilled form. Investigations of chilled DFD beef samples treated with HHP at 800–1000 MPa for 5 min and stored at a temperature of 0 to +4 °C in a vacuum package showed that samples had high sensory characteristics that corresponded to fresh DFD meat after 60-day storage. With that, microbiological indices in HHP-treated beef were lower than the normative values even after 60 days of storage [70].

HHP treatment at 800 MPa for 5 min. increased the sanitary-hygienic condition of chilled beef packed under vacuum in a polymer package 48 hours after slaughter and also prevented protein decomposition and facilitated retardation of fat oxidation processes in meat. The beef samples corresponded to the regulatory requirements for microbiological safety after 60-day storage [71].

Investigations of the microbiological indices of mechanically deboned chicken meat treated with HHP (250 MPa for 15 min.) during storage at a temperature of 4 ± 2 °C showed the possibility of the two-fold increase in product shelf-life compared to control samples not treated with HHP. It was observed that HHP treatment did not affect the nutritional value of the product, and sensory indices corresponded to the initial characteristics. It was shown that HHP treatment used for raw meat materials is highly technological and easily incorporated in the process of meat product manufacture. With that, HHP at 250 MPa allowed increasing not only sanitary indicators but also functional-technological characteristics of raw materials [72].

HHP can significantly retard oxidative processes, which occurrence can significantly influence meat and meat product storability. The research [70] showed that processing at 800 MPa and 1000 MPa enabled reducing the peroxide values in chilled beef samples by 9 and 4.5 times compared to untreated samples. However, an increase in pressure of up to 1000 MPa was undesirable as oxidative processes in a product enhanced compared to the samples treated at 800 MPa. During storage of packed chilled beef for 30 and 60 days, the samples pressurized at 800 MPa had the peroxide value on the 30th day two times lower and on the 60th day four times lower than in the unpressurized samples [70].

The research [71] also showed low growth dynamics of the acid value and peroxide value in the samples of beef shoulder packed in a polymer package in 500 g pieces and pressurized at 800 MPa for 5 min. The acid value and peroxide value in pressurized beef on the 60th day of storage were six and four times lower, respectively, compared to the control sample.

HHP treatment of chilled meat activates its natural antioxidant systems, in which an important role is given to such compounds as vitamin E, polyunsaturated fatty acids and several amino acids (histidine, leucine, threonine, valine, and glutamic acid), and others. During HHP impact on muscle tissue, the lysosome membranes containing enzymes are destroyed and cathepsins are actively released. The process of enzyme release accelerates autolysis and amino acids with antioxidant properties are actively formed, which explains the enhancement of meat antioxidant properties [73].

Vacuum packed meat processed with HHP at 800 MPa is less subjected to changes in protein substances during storage, which is indicated by the results of the detection of amino-ammonia nitrogen (AAN). The AAN content in processed samples on the 15th, 30th and 60th days of storage was 6, 12 and 10 times lower, respectively [73].

Therefore, analysis of the results obtained in the investigation of meat treated with HHP at 800 MPa and higher indicates that the use of this technology allows extending the shelf-life of perishable products such as meats, which presents a huge interest in the field of the development and preservation of food reserves.

Conclusion

Based on the data stated above, it is necessary to emphasize that the application of HHP technology for meat product processing at different parameters has wide prospects for use. For example, raw meat material processing at 100–200 MPa directly before the conventional heat treatment can become one of the possible methods for managing the functional-technological characteristics of the meat system. This treatment can be regarded as compensation for the addition of the reduced amount of sodium chloride

and sodium phosphate, as well as for the low fat content in mince in manufacturing the comminuted or gel-type meat products. With that, it will ensure the maintenance of sensory and consumer characteristics of finished products as well as an improvement in product composition regarding healthy nutrition.

On the other hand, the application of HHP in a range of 400 MPa to 800 MPa in meat processing is no less promis-

ing for increasing its microbiological safety, resistance to oxidative spoilage and an increase in shelf-life without using food preserving agents.

The considerable promise of these two directions in the HHP application is so evident that the development of new technological processes combining different stages of raw material and food system processing with HHP technology at different parameters is expected.

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All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

The authors declare no conflict of interest.

Received 19.02.2020 Accepted in revised 18.06.2020 Accepted for publication 25.06.2020