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MICROBIOLOGICAL QUALITY OF SOME MEAT PRODUCTS SUBJECTED TO HIGH PRESSURE PROCESSING

CALITATEA MICROBIOLOGICĂ A UNOR PRODUSE DIN CARNE SUPUSE PROCESĂRII LA PRESIUNE ÎNALTĂ

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ABSTRACT | **REZUMAT**

High Pressure Processing (HPP) is a non-thermal process capable of inactivating and eliminating pathogenic and spoilage microorganisms. This new technology has enormous potential in the food industry by controlling food spoilage, improving food safety, and extending product shelf life, while preserving the characteristics of fresh foods, without preservatives, and minimally processed. Like other food processing methods, such as thermal processing, HPP has somewhat limited applications, as it cannot be universally applied to all types of foods, such as certain dairy products and animal-derived products and low-acid foods. Here, we discuss the effects of high-pressure processing on microbial food safety and, to a lesser extent, food quality. All samples analysed showed negative microbiological results. These findings demonstrate that HPP conditions of 600 MPa for 3 minutes, commonly used in the food industry, provide an adequate safety margin for controlling the relevant vegetative pathogens in meat products. The results of this study can be utilised by food processors to support validation studies and may be useful for the future development of safe spheres for the HPP industry.

Keywords: high-pressure processing, *Escherichia coli, Salmonella* spp., *Listeria monocytogenes*

Procesarea la presiune înaltă (HPP) este un proces netermic capabil să inactiveze și să elimine microorganismele patogene și de alterare a alimentelor. Această tehnologie nouă are un potențial enorm în industria alimentară, controlând deteriorarea alimentelor, îmbunătățind siguranța alimentelor și prelungind perioada de valabilitate a produsului, păstrând în același timp caracteristicile alimentelor proaspete, fără conservanți, procesate minim. Ca și în cazul altor metode de procesare a alimentelor, cum ar fi procesarea termică, HPP are aplicații oarecum limitate, deoarece nu poate fi aplicat universal tuturor tipurilor de alimente, cum ar fi unele produse lactate și produse de origine animală și alimente cu conținut scăzut de acid. In acest studiu au fost analizate efectele prelucrării la presiune înaltă asupra siguranței alimentelor microbiene și, într-o măsură mai mică, a calității alimentelor. Toate probele analizate, au avut rezultate negative, din punct de vedere microbiologic. Aceste constatări demonstrează că HPP la 600 MPa timp de 3 minute asigură o marjă de siguranță adecvată pentru controlul agenților patogeni în produsele din carne. Rezultatele acestui studiu pot fi utilizate de procesatorii de bucătărie pentru a sprijini studii de validare și pot fi utile pentru crearea viitoare a unor sfere sigure pentru industria HPP.

Cuvinte cheie: procesare la presiune înalta, *Escherichia coli, Salmonella* spp., *Listeria monocytogenes*

According to data from the Taiwan Food and Drug Administration, approximately 2,000 cases of food poisoning are reported annually due to the accidental consumption of food products contaminated with pathogenic microorganisms. Most cases of foodborne illnesses result from the consumption of food products such as processed seafood, fruits, or plant-based products, which are eaten raw by a large portion of consumers. Traditional high-temperature pasteurisation is not suitable for preserving the fresh flavours of these raw materials. With the lack of proper pasteurisation, there is a higher risk of ingesting pathogenic microorganisms when consuming such products. This demonstrates the need for diverse pasteurisation methods in the food industry to ensure the safety of all products. Additionally, modern dietary concepts, which emphasise healthier eating, encourage consumers to increase their intake of whole or unprocessed foods (12). Therefore, researchers in food processing hope to fully or partially replace high-temperature pasteurisation with physical, biological, or chemical methods for food preservation. With the development of non-thermal pasteurisation technologies, the flavour of food products can be preserved because the mild processing methods reduce the use of chemical additives and improve the retention of the natural state and freshness of food. In recent years, the development of non-thermal processing technologies has received significant attention from the food processing industry; indeed, non-thermal processing associations worldwide now organise annual symposia to discuss advancements in various coun-

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tries regarding progress in processing technologies, such as high-pressure processing (HPP), pulsed electric field processing, pulsed light processing, electron beam processing, and plasma processing. However, HPP is the only non-thermal processing technology that has been successfully commercialised in the last decade. HPP involves applying ultra-high pressure (100–600 MPa) for pasteurisation. Since it preserves the natural flavours and nutritional values of raw ingredients, HPP has been recognised as a mild processing technology that can achieve both food safety and flavour preservation (12).

Listeria monocytogenes is a heterogeneous species comprising 13 serotypes, which can be divided into four genetic groups. Serotypes 1/2b, 3b, 4b, 4d, and 4e belong to group I, while serotypes 1/2a, 1/2c, 3a, and 3c are included in group II. Serotypes 4a and 4c belong to group III and are rarely isolated from humans. Serotypes 1/2a and 1/2b are most commonly isolated from food, while 4b is isolated from clinical cases (20). According to European Commission Regulation (EC) No. 2073/15 of November 2005, *Listeria monocytogenes* is considered one of the criteria for food safety. Its presence should be monitored in products where its growth is possible before these products enter the market. In such products, *Listeria monocytogenes* should not be present in 25 g (25 ml) of a sample (6). Furthermore, during the shelf life of a product, the number of these bacteria in a sample should not exceed 100 CFU/g (CFU/mL) of product, regardless of whether the product promotes or does not promote the growth of these bacteria. Food testing is usually limited to detecting the presence or determining the number of this pathogen and does not include testing, among other things, for growth determination after storage, which is particularly important due to the possibilities associated with the growth of *Listeria monocytogenes* during storage (27).

Listeria monocytogenes is responsible for causing listeriosis, a zoonosis characterised by a very severe course, especially in people from the so-called high-risk group, which includes the elderly, pregnant women, newborns, and immunocompromised individuals (20). Listeriosis is characterised by a severe course (meningitis, endocarditis, encephalitis, sepsis, spontaneous abortions, stillbirths) and a high mortality rate of 20-30% (23, 26). In the treatment of listeriosis, antibiotics are used to inhibit the infection caused by *L. monocytogenes*. This bacterium is considered susceptible to a broad spectrum of antibiotics that exhibit bactericidal activity against Gram-positive bacteria, including tetracyclines, ampicillin, penicillin G, imipenem, amoxicillin, sulphonamides, aminoglycosides, macrolides, chloramphenicol, and glycopeptides (9). The ubiquitous distribution of Salmonella in the natural environment and its prevalence in the global food chain, along with its physiological adaptability and virulence, make it a significant human bacterial pathogen. Its potential severe economic impact on the food industry underlines the need for continuous vigilance and strict controls at all levels of food production (7). Human salmonellosis generally manifests in two types of disorders: typhoid fever caused by serotypes of *Salmonella enterica*, including *S.* Typhi, *S.* Paratyphi A, *S.* Paratyphi B, and *S.* Paratyphi C, and gastroenteritis caused by non-typhoid Salmonella (NTS) serotypes such as S. Enteritidis and S. Typhimurium (11). The European Food Safety Authority (EFSA) Biological Hazards Group (BIOHAZ) concluded that *Salmonella* and *Enterobacter sakazakii* are the microorganisms of greatest concern for food safety (6). EFSA recommended monitoring and controlling enterobacteria both in the production environment and in the final product (10). According to European Commission Regulation (EC) No. 2073/15 of November 2005, *Salmonella* spp. should not be present in 25 g (25 ml) of a sample (6). Research on microorganisms as hygiene indicators in food is commonly used to obtain information about production, storage, transport, and processing conditions of food (14). Since they are usually present in the intestinal microbiota of warm-blooded animals, microorganisms of the species *E. coli* are important hygiene indicators, as they suggest direct or indirect contamination with faecal material (15). The food industry needs reliable tests for detecting and quantifying *E. coli* in animal-derived products (8). Several detection methods are based on specific characteristics of *E. coli*, such as detecting glucuronidase activity (17). The enzyme β-D-glucuronidase (GUD) is produced by 94-96% of *E. coli* strains. Therefore, the presence of GUD is useful for identifying this species. This enzymatic identification technique is the basis for Petrifilm™ EC (3M Microbiology, St. Paul, MN, USA). In Petrifilm™ EC, *E. coli* colonies usually appear blue, associated with gas production. This test is useful for confirming suspect cultures, as it is very practical and readily available, allowing a direct count of *E. coli* in the food sample (18). According to European Commission Regulation (EC) No. 2073/15 of November 2005, the accepted limits for *E. coli* vary, depending on the product, and are expressed in CFU/g (6).

The study evaluates the effects of high-pressure processing on microbial food safety and, to a lesser extent, food quality.

MATERIALS AND METHODS

Samples of Food Products

Thirty samples of each of the following products were used: "country ham", "cabanos sausages", "semi -smoked sausages", "turkey headcheese", and "rustic salami," obtained from a meat product factory and subjected to high-pressure processing. The products were transported in a refrigerated box at 4°C to several private laboratories for analysis.

HPP: Operating Principle

The operating principle of HPP is as follows: food products that have been hermetically sealed are placed in a thermally insulated, airtight vessel and subjected to ultra-high pressure (100–600 MPa) transmitted through a liquid medium (usually water), which provides a pasteurisation effect by applying high pressure uniformly and instantaneously. According to the compression heating principle, an increase in pressure by 100 MPa results in a temperature increase of approximately 3°C in the water. The initial pasteurisation temperature is controlled in the range of 5-10°C, meaning that when the pressure rises to 600 MPa, the water temperature will not exceed 30°C. Thus, HPP reduces the impact of temperature on the components of the food. Two basic principles, namely the isostatic principle and Pascal's principle, govern the uniform application of pressure to food products within the airtight vessel. According to the isostatic principle, when pressure is applied to a liquid medium in a closed environment, equal pressure is exerted on objects at any point in the medium, regardless of the shape or size of the object. Pascal's principle states that the change in pressure caused by the application of an external force to a fluid at rest in a closed container is transmitted uniformly and without loss to every part of the fluid and to the walls of the container (5).

Therefore, the effects of high-pressure pasteurisation are not influenced by the shape and size of food packaging; food products of different volumes can be processed within the same batch. Additionally, preservatives are not necessary to maintain microbial safety in food products. HPP is superior to traditional thermal processing technologies in the following aspects: (1) it can be performed at ambient temperature, eliminating the energy consumption required for heating and subsequent cooling; (2) food products remain in their final packaging during HPP and do not come into direct contact with the processing equipment, which prevents secondary contamination post-pasteurisation and allows for the recovery and reuse of the pressure-transmitting medium. Since HPP offers the advantages of reduced energy consumption and low contamination, it is a relatively environmentally friendly processing technology (19).

The development of HPP products has diversified in recent years, with HPP being applied to the development of healthy foods. HPP has been used to enhance the functional components of food products, to develop beverages containing pressure-tolerant Bacillus probiotics, and to reduce the content of additives in food products, which increases the diversity of product types and enhances the added value of products. Although HPP has already been widely applied in food pasteurisation, the high investment costs of HPP equipment result in higher production costs compared to traditional high-temperature pasteurisation, making the average unit price of HPP products higher than that of non-HPP products. Therefore, recent developments in the application of HPP have primarily focused on improving health benefits for consumers. Adkins et al. reported the use of HPP in cancer immunotherapy to kill tumour cells for the generation of whole-cell and dendritic cell-based vaccines (1). In a review by Pottier et al. (2017), the use of high pressure as a barrier technology was highlighted for reducing food additives and salt, as well as its potential to improve digestibility and reduce allergenicity (21). This suggests that high pressure not only serves as a pasteurisation technology but can also potentially be used to develop healthier food products. The nutritional value of food products can be enhanced during the pasteurisation process, allowing consumers to increase their nutrient intake without having to change their eating habits.

Microbiological methods

The following methods were used for the determinations of *Salmonella spp*., *Listeria monocytogenes*, and *E. coli* β-glucuronidase-positive count:

- Detection of *Salmonella spp* in 25g: PN-EN ISO 6579-1:2017-04+A1:2020-09 (4).
- Detection of *Listeria monocytogenes* in 25g: PN-EN ISO 11290-1:2017-07 (3).
- Determination of *E. coli* β-glucuronidase-positive count: PN-ISO 16649-2:2004 (2).

RESULTS AND DISCUSSION

Microbiological Determinations

Samples of "country ham," vacuum-packed, made from pork meat and sourced from various batches, were analysed for the detection of *Listeria monocytogenes* and *Salmonella spp.* were negative, as these

Table 1

Results of microbiological determinations

Product	Requested exam	Limits	Results	Conclusions
Country-style bacon	*Salmonella* spp.	Not detected/25g	Not detected/25g	satisfactory
	Listeria monocytogenes	Not detected/25g	Not detected /25g	satisfactory
	β-glucuronidase-positive *E. coli*	<1,0x101 ufc/g.	<1.0x101 CFU/g.	satisfactory
Cabanos sausages	*Salmonella* spp.	Not detected/25g	Not detected /25g	satisfactory
	Listeria monocytogenes	Not detected/25g	Not detected/25g	satisfactory
Semi-smoked sausages	*Salmonella* spp.	Not detected/25g	Not detected/25g	satisfactory
	Listeria monocytogenes	Not detected/25g	Not detected/25g	satisfactory
Rustic salami	*Salmonella* spp.	Not detected/25g	Not detected/25g	satisfactory
	Listeria monocytogenes	Not detected/25g	Not detected/25g	satisfactory
Turkey drumstick	*Salmonella* spp.	Not detected/25g	Not detected/25g	satisfactory
	Listeria monocytogenes	Not detected/25g	Not detected/25g	satisfactory

pathogens were not detected in 25 g/sample. Regarding the determination of β-glucuronidase-positive *E. coli*, all the analysed samples showed results below $1.0x10^1$ CFU/g. For the samples of "cabanos sausages", "semi-smoked sausages", "rustic salami", and "turkey drumstick", tests for the detection of *Salmonella spp.* and *Listeria monocytogenes* were conducted, with all results being negative in 25 g/sample (Table 1).

According to the European Commission Regulation (EC) No. 2073/15 of November 2005, the results for E. coli, Salmonella spp., and Listeria monocytogenes are within the permissible limits (6).

Effects of High Pressure on Microorganisms

Food pasteurisation technologies are primarily evaluated based on the tolerance of indicator pathogens; corresponding pasteurisation parameters are established depending on the environmental conditions in which the products are distributed. For example, *Clostridium botulinum* is used as an indicator pathogen to establish operational parameters for autoclave pasteurisation. Although high pressure is capable of eliminating pathogens, food products contain a wide variety of microbes with different physiological characteristics, leading to very diverse pressure tolerance characteristics among these microbes. Therefore, cumulative results from extensive microbial pressure tolerance experiments are necessary to select pressure-tolerant indicator pathogens to ensure that reliable high-pressure pasteurisation parameters are set (24). For example, the pressure tolerances of common pathogens generally follow these patterns, which must be well understood when conducting high-pressure pasteurisation studies: prokaryotic microbes > eukaryotic microbes; gram-positive bacteria > gram -negative bacteria; cocci > bacilli. Since HPP is a continuous pasteurisation process, processing time is a key factor influencing production. Although productivity can be increased by reducing the pasteurisation time for each batch of products, this raises the risk of inadequate pasteurisation. Therefore, pH, water activity, and the components of food products must be considered when determining high-pressure pasteurisation conditions. When selected conditions are unfavourable for microbial growth, pasteurisation efficiency can be increased, and pasteurisation time can be reduced. Thus, the combination of HPP with additive barriers can act synergistically to inactivate microorganisms, such as a low pH value increasing HPP's inactivation efficiency against pathogenic bacteria in salads (22). However, there is a wide variety of microbes with different physiological characteristics in food products, which leads to varying pressure tolerance characteristics among different microbes. An increase in treatment pressure can result in varying degrees of influence on microbes. Therefore, research units and competent health authorities must establish relevant pasteurisation process conditions and methods for verifying the pasteurisation performance of equipment to ensure the safety of HPP products. Currently, only a few countries have formulated relevant rules or regulations based on the results of microbial pressure tolerance experiments. The operating specifications for HPP products issued by the United States Department of Agriculture (USDA) recommend using *Escherichia coli* as an indicator pathogen and a reduction of at least 5 logs of the indicator pathogen with any combination of time/pressure to ensure microbial safety (25). The competent health authority in Canada, Health Canada, has issued recommendations regarding pressure and the duration of pressure maintenance for individual products. For example, a minimum pressure of 600 MPa for a minimum duration of 3 minutes is required for effective reduction of *Listeria monocytogenes* in ready-to-eat meat products. Since HPP cannot effectively inhibit the growth of microbial spores, the use of HPP for pasteurizing products distributed at ambient temperature is not recommended. Currently, a large number of commercially mass-produced HPP food products are distributed and sold through cold chain systems in many countries, but HPP products distributed at ambient temperature are not available due to the inability of the pressure treatment to completely eliminate microbial spores. Certain bacteria (*Bacillus* and *Clostridium*) form spores as a survival strategy in response to unfavourable environmental conditions. Since the resistance of these spores to stress is much higher than that of the vegetative cells of microbes, pressure treatment alone cannot effectively eliminate microbial spores (13).

Therefore, HPP products must be distributed through cold chain systems, as HPP cannot currently achieve commercial sterility. Recent studies on the development of HPP food products that can be distributed at ambient temperature have shown that a pressure of 600 MPa combined with a moderate temperature is necessary to achieve a sterility equivalent to or greater than 12D in canned products and to ensure the quality of food products at ambient temperature (12).

CONCLUSIONS

The study results demonstrate that the HPP conditions of MPa for 3 minutes, commonly used in the food industry, meet the performance standard and the HA CCP regulation for meat products to control E. coli, Salmonella, and L. monocytogenes. Out of 150 samples analysed, 100% were within the limits set by Regulation (EC) No. 2073/2005 of the Commission of November 15, 2005, regarding microbiological criteria for foodstuffs. HPP has significant potential and has been successful in the food industry as a new technology that can achieve the same food safety standards as pasteurisation while simultaneously meeting the demand for fresh and minimally processed foods. The application of HPP can inactivate microorganisms and enzymes and can modify structures, with minimal or no effect on the sensory quality of the food. HPP extends shelf life while preserving organoleptic qualities through the inactivation of microorganisms.

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