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Effect of high-pressure processing on the shelf life, safety and organoleptic characteristics of lasagne ready meals during storage at refrigeration and abuse temperature

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ABSTRACT

The effect of different pressure levels (500 and 600 MPa for 1 min at ambient temperature) on lasagne ready meal as a means of increasing the safety and shelf life during storage at refrigeration (4 °C) and abuse temperature (8 °C) was investigated. High-pressure processing (500 and 600 MPa for 1 min) was able to significantly reduce the total aerobic and lactic acid bacteria counts and prolong the microbiological shelf life of lasagne at both refrigeration and abuse temperatures. Pressure at 600 MPa was a useful tool to reduce the safety risks associated with *Staphylococcus aureus* and *Listeria monocytogenes*. However, abuse storage temperature facilitated the recovery of *L. monocytogenes* towards the end of storage. Organoleptic evaluation revealed that HPP did not negatively influence the quality attributes of lasagne and prolonged its organoleptic shelf life. HPP treatment can serve as a useful additional step to enhance safety and increase the shelf life of multicomponent ready meals, such as lasagne.
Industrial relevance: The ready meals sector of the food industry has been experiencing increasing growth in the past years. This comprehensive study explored the effects of HPP on a very popular multicomponent ready meal i.e., lasagne after treatment and during storage. The results showed that HPP can be successfully applied to lasagne ready meals to decrease the risk from *S. aureus* and *L. monocytogenes* and also significantly prolong its shelf life without affecting its organoleptic properties. The utilisation of HPP by the industry can significantly increase safety and also provide the opportunity for this product to reach markets further away.

1. Introduction

Over the past years, the ready meals sector has been experiencing increasing growth (Olsen, Sijtsema, & Hall, 2010; Kanatt, Rao, Chawla, Sharma, 2013). Due to the lack of available time faced by consumers and the convenience offered, such products have become very popular (Kanzler, Manschein, Lammer, & Wagner, 2015). In several food products, such as cooked ham, chicken and mortadella, a thermal treatment at the end of the production process is designed to eliminate pathogens, however cross-contamination could take place during handling, slicing and packaging (Patterson, McKay, Connolly, & Linton, 2010; Patterson, Mackle, & Linton, 2011). Foodborne pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, Salmonellae and *Escherichia coli* O157:H7 pose severe safety issues, since they can survive and grow in numerous products (Bover-Cid, Belletti, Garriga, & Aymerich, 2011; Ingham et al., 2005; Kunwar, Singh, Mangla, & Hiremath, 2013; Oh et al., 2007; Okada, Monden, Igimi, & Yamamoto, 2012; Viswanathan & Kaur, 2001) and therefore different strategies to control the growth of such microorganisms should be implemented in the production line. If cross-contamination takes place and the physicochemical characteristics of the food product and/or storage conditions allow the growth of the pathogen then major safety issues can arise. Lasagne is considered a very popular ready meal, the preparation of which usually includes reheating by the consumer. However, if the reheating step is not designed to eliminate foodborne pathogens or if not adequately reheated then pathogenic microorganisms might survive and cause food poisoning. In general, lasagne is comprised of multiple components (e.g., bolognese and béchamel sauce, minced beef, pasta) which are prepared separately and the meal is then assembled manually by food workers, thereby significantly increasing the risk of cross contamination (Durack, Alonso-Gomez, & Wilkinson, 2011). Lasagne meals fall into the category of products that permit migration of bacteria through its multiple layers by diffusion, capillary action or gravitational movement of liquid containing suspended bacteria cells. Therefore, in the case of cross-contamination of any of the individual components, bacteria can occupy the whole volume of the product in a matter of hours (Durack et al., 2011). In order to enhance safety in these types of multicomponent products, decontamination technologies able to deliver a volumetric effect should be considered for incorporation in the production line. A technology that is able to deliver this volumetric decontamination

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effect and has been gaining increasing importance is high-pressure processing (HPP). HPP is a non-thermal processing technology that has been proven effective against pathogenic microorganisms without significantly affecting the quality and nutritional content of food products (Heinz & Buckow, 2010; Rendueles et al., 2011; Wang et al., 2013). HPP can also result in shelf-life prolongation as it can reduce the counts of spoilage microorganisms such as Enterobacteriaceae, *Brochothrix thermosphacta* and lactic acid bacteria (LAB) (Garriga, Grèbol, Aymerich, Monfort, & Hugas, 2004; Jofré, Aymerich, Grèbol, & Garriga, 2009; López-Caballero, Carballo, & Jiménez-Colmenero, 1999; Patterson et al., 2010). In addition to the safety and shelf-life benefits, HPP is able to produce 'clean label' products which are more wholesome, fresh and natural and highly valued by consumers (Mintel, 2008). The majority of existing studies that have explored the effect of pressure treatment have focused on less complicated products (e.g., cooked meat, sausages) and most of them have employed ideal storage conditions (~4 °C). Therefore, in this study we aimed to investigate the effectiveness of high-pressure processing as a means of prolonging the microbiological and organoleptic shelf life of a much more complex, multicomponent food system namely lasagne, at refrigeration and abuse storage temperature, while enhancing microbiological safety by controlling *L. monocytogenes* and *S. aureus*.

2. Materials and methods

2.1. Sample preparation and storage

Lasagne ready meals were produced under commercial conditions by a local food producer and transferred to the laboratory under refrigeration on the day of production. Pieces of lasagne (10 ± 0.2 g) were vertically cut in order to ensure that representative samples containing all ingredients from all layers were obtained. Samples for the shelf life and challenge studies were placed in polyethylene/polyamide vacuum pouches (Somerville Packaging, Lisburn, Northern Ireland) and vacuum packed, to replicate commercial practices. The oxygen permeability of the pouches was 50 cm^3/m^2/24 h at 1 bar, 23 °C and 0% humidity.

Three different treatments were used:

(i) Control (C): untreated vacuum-packed samples.
(ii) High-pressure processing (HPP 500): samples were vacuum packed and pressure treated at 500 MPa for 1 min.
(iii) High-pressure processing (HPP 600): samples were vacuum packed and pressure treated at 600 MPa for 1 min.

Samples were stored at 4 and 8 °C for a period of 56 days (samples were tested during storage at regular time intervals).

2.2. Physicochemical characteristics

The pH of the lasagne, after mixing with deionised water (1:1), was measured using a Jenway pH Meter Model 3505. The moisture content was calculated by drying the sample in an oven at 104 °C until a constant weight was reached. Lastly, water activity (a_w) was measured by means of a Hygrolab 3 a_w meter (Rotronic instruments, Crawley, UK). For all measurements three replicate samples from different production runs were characterised.

2.3. Shelf-life assessment

Lasagne samples were opened aseptically and the contents placed in a sterile stomacher bag with a mesh insert (Interscience, St. Nom La Breteche, France) and a 10^{-1} dilution prepared in maximum recovery diluent (MRD) (Oxoid, CM733, Basingstoke, U.K.) using a variable diluter (Baby Gravimat, Interscience, St. Nom La Breteche, France). The diluted sample was homogenised in a stomacher (Seward, Worthing, UK) for 1 min. Suitable decimal dilutions were prepared in MRD. Each sample was plated in duplicate. For enumeration of total aerobic counts (TAC) an aliquot of 100 μl of each of the 10-fold dilutions was spread plated on plate count agar (Oxoid code, CM0463, Oxoid) and incubated at 30 °C for 48 h. LAB were enumerated by pour plating on De Man, Rogosa, Sharpe (MRS) agar (Oxoid code CM1153) and incubating at 30 °C for 72 h. *Pseudomonas* spp. were enumerated by spread plating on *Pseudomonas* agar base (Oxoid code CM0559) supplemented with *Pseudomonas* CFC selective supplement (Oxoid code SR0103), and incubating at 30 °C for 48 h. Enterobacteriaceae were enumerated by pour plating on violet red bile glucose agar (VRBGA) (Oxoid code CM1082) and incubating at 37 °C for 24 h.

2.4. Inoculum preparation and enumeration of L. monocytogenes and S. aureus

Before inoculation, the absence of *L. monocytogenes* and *S. aureus* cells was confirmed on the different batches of lasagne. Each 5 strain cocktail of the two pathogenic microorganisms was inoculated onto the lasagne separately in two different challenge studies. The cocktail of *S. aureus* consisted of NCTC 8325, ATCC 27664, NCTC 10656, NCTC 105657 and NCTC 10652. The *L. monocytogenes* cocktail consisted of LR102, VI 51028, 0227-359, 0113-131 and VI 51010 strains. For each *S. aureus* and *L. monocytogenes* strain used, a loopful of a fresh tryptone soya agar (Oxoid code CM0131) + 0.6% yeast extract (Oxoid code LP0021) (TSAYE) slope culture was inoculated into 10 ml of brain–heart infusion broth (BHI) (Oxoid code CM1135) and incubated at 37 °C for 24 h. Subsequently 100 μl of a 10^{-4} dilution of this broth was inoculated into another 10-ml BHI broth and incubated at 37 °C for 48 h, until the stationary phase of growth was reached. The final 10 ml cultures were centrifuged at 3600 ×*g*, for 30 min, washed twice in phosphate-buffered saline (PBS) and the pellet re-suspended in a final volume of 10-ml PBS to give approximately 10^8 CFU/ml. The suspensions of all 5 strains for each pathogenic microorganism were combined and mixed well. The combined suspensions were inoculated (100 μl) onto different lasagne samples (10 g), at a level of approximately 5 log CFU/g. Three samples in total for each of the 3 different treatments and each pathogenic microorganism were opened aseptically and the contents were transferred to a sterile stomacher bag with a filter insert (Interscience, St. Nom La Breteche, France). A 10^{-1} dilution of the sample was prepared in maximum recovery diluent (MRD) (Oxoid code CM733). The dilution was homogenised for 1 min in a Seward stomacher. If necessary, further 10-fold dilutions were prepared in 9-ml MRD.

2.5. High-pressure processing

The pressure treatment of lasagne was performed with the use of a commercial-scale Avure Quintus high-pressure press (Avure Technologies, U.S.A.), with a pressure vessel 19 cm in diameter and 1.27 m in length, of 35-L volume. The pressure transmission fluid used was potable water. The pressure come-up times were 115 and 138 s for 500 and 600 MPa, respectively. Decompression times were 14 and 17 s, for 500 and 600 MPa, respectively. The initial temperature of the water was approximately 18 °C and the final temperature due to adiabatic heating was 29 and 31 °C for 500 and 600 MPa, respectively. The samples were pressure treated at 500 and 600 MPa with a hold time at pressure of 1 min.

2.6. Enumeration of L. monocytogenes and S. aureus

For enumeration of *L. monocytogenes* an aliquot of 100 μl of each of the 10-fold dilutions was spread plated on Oxoid chromogenic Listeria agar (OCLA) (Oxoid, code CM1084B) supplemented with OCLA selective supplement (Oxoid code SR0226E) and Brilliance Listeria differential supplement (Oxoid code SR0228E) and incubated at 37 °C for 48 h.