

High pressure processing for pea spread shelf life extension: a preliminary study

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Abstract The effects of high pressure processing – HPP – (500 and 700 MPa/10, 20 and 30 min/20 °C) on the microbiological quality and colour of maple pea spread were compared to thermal processing (sous vide 80 °C/15 min). Microbiological quality during 15 days at 4 °C was evaluated. Pea spread was filled in polyamide/polyethylene film pouches, packaged in vacuum and hermetically sealed. Pea spreads were made of cooked peas ‘Bruno’ (*Pisum sativum* var. *arvense* L.), to which salt, citric acid, oil and herb spice were added. Total plate count was determined on Plate Count agar, *Enterobacteriaceae*- on Violet Red Bile agar with Glucose, coliforms- on Endo agar. Decontamination of seasonings was carried out using UV-C treatment and sterilization. Identification of bacteria was completed with API biochemical test system. Colour analysis was carried out in L*a*b* colour space. Total plate count in untreated pea spread was 3.41 log₁₀ CFU g⁻¹, all processed samples showed significantly reduced microbial contamination (p<0.05). Coliforms were not detected and *Enterobacteriaceae* count was <10¹ CFU g⁻¹ after two week storage at +4.0±0.5 °C. Spores of *B. subtilis* and *B. licheniformis* were found in all pea spread samples; source of these bacteria was herb spice. UV-C treatment showed considerable decrease in microbiological contamination of seasonings, however, sterilization did not destroy spores. HPP did not influence pea spread colour (p>0.05). HPP (at 700 MPa) demonstrates improvement in pea spread shelf life compared to untreated sample and thermal processing, and is suitable for pea spread shelf-life extension.

Keywords maple pea spread, high pressure processing, sous vide, microbiological contamination, herb spice

1. INTRODUCTION

1.1 Legume spreads

The growth in the number of vegetarians, meat avoiders and meat reducers has stimulated the use of plant based ingredients which can extend meat products while providing an economical, functional, and high-protein meat substitutes. Plant based meat alternatives are successful because of their healthy image (cholesterol free), meat-like texture, and lower cost (Asgar et al., 2010).

Commercially available legume spreads are an innovative product and an alternative to traditional animal-derived spreads or pates. Most well-known vegetable protein spread is hummus. Legumes are one of the most reliable sources of good quality protein and dietary fibre. Plant based spreads could positively influence the low legume consumption in the Western world which is less than 3.5 kg per capita per year (Mudryj et al., 2012). Kirse and Karklina (2015) concluded that some of the main reasons for low legume consumption in Latvia are hard-to-cook phenomenon, meteorism and time consuming preparation which can be avoided with legume spreads. Maple peas (*Pisum sativum* var. *arvense* L.), a local legume growing in Europe and one of the staple foods in Latvian cuisine, has the potential for innovative product development to satisfy the daily needs for protein and fibre.

Shelf-life of maple pea spread without preservation techniques (e.g. water activity reduction, changes in pH, heat application) is less than six days (Kirse and Karklina, 2015) therefore innovative preservation methods must be considered as consumers increasingly demand convenience foods of the highest quality in terms of natural flavour and taste, and which are free from additives and preservatives (Rastogi et al., 2007).

1.2 Preservation techniques

Sous-vide cooking can be defined as the cooking of raw materials under controlled conditions of temperature and time, inside heat stable vacuumized pouches or containers followed by rapid cooling. *Sous vide* technology could be a reasonable choice, as it allows to obtain products with an extended shelf-life and a quality similar to that of fresh food (Baldwin, 2012). Nonetheless, Knockaert et al. (2011) among others established that thermal treatment can have a detrimental effect on texture, colour, flavour and nutritional value of foods.

High pressure processing (HPP) is a minimal processing technology, which serves as a cold-pasteurisation that eradicates microorganisms regardless of the geometry of the product and without the use of preservatives (Zhang and Mittal, 2008), thus making this technology accepted as safe and consumer friendly (Rastogi et al., 2007). Food treated by HPP has been shown to keep its original freshness, flavour, taste, and colour. According to Patras et al. (2009), while the structure of high-molecular-weight molecules such as proteins and carbohydrates can be altered by HPP, smaller molecules such as volatile compounds, pigments, vitamins, and other compounds connected with the sensory, nutritional, and health promoting are unaffected.

Sous vide and HPP inactivates vegetative microorganism cells, however, bacterial endospores are resistant to pasteurizing (temperature <100°C) and high pressure (survival at >1000 MPa) (Balasubramaniam and Farkas, 2008; Knockaert et al. 2011), therefore both methods can be used to minimize contamination of vegetative microorganisms in packaged products.

The aim of this study was to investigate the effect of HPP and subsequent storage period (15 days) at 4 °C on microbiological quality and colour in maple pea spread, compared to the same untreated and *sous vide* processed spread.

2. MATERIALS AND METHODS

2.1 Preparation of maple pea spread

The following materials were used for pea spread preparation: maple peas 'BRUNO' (*Pisum sativum* var. *arvense* L.) grown and harvested in 2014 at State Priekuli Plant Breeding Institute (Latvia), 'EXTRA VIRGIN' canola oil (Iecavnieks Ltd., Latvia), citric acid (Spilva Ltd., Latvia), Himalayan salt (Pakistan) and herb (sun-dried tomato, garlic and basil) spice 'BRUSCHETTA' (P.P.H. fleisch mannschaft@Polska Sp. z o.o., Poland).



Fig. 1: Vacuum packaged pea spread in transparent polymer pouches before preservation treatments.

Maple pea spread was made of ground re-hydrated cooked maple peas (93%), to which salt (0.3%), citric acid (0.2%), oil (5.9%) and herb spice (1%) were added (Kirse and Karklina, 2015). Pea spread was vacuum packaged (Fig. 1) in two layer transparent laminate

polyamide/polyethylene film pouches (45 mm x 170 mm, film thickness 45 µm) with barrier properties and hermetically sealed by chamber type machine *Multivac C300*, each sample was 50±1 g.

2.2 HPP and *sous vide* treatment

HPP of pea spread was carried out using Iso-Lab High Pressure Pilot Food Processor (S-FL-100-250-09-W, Stansted Fluid Power Ltd., Essex, UK) in a 2.0 L pressure vessel. An isopropanol, water mix (1:3 w/v) was used as the pressure transmitting liquid. Pea spread pouches were placed in pressure vessel and treated at 500 MPa with 10 and 20 min dwell time, and at 700 MPa with 10, 20 and 30 min dwell time. The experiment was carried out at room temperature which increased due to pressure increase in the vessel and maximally reached 40–42 °C during pressurization at 700 MPa.

Sous vide treatment of pea spread was carried out in *Clifton Food Range* water bath. Samples were pasteurized for 15 min at +80.0±0.5 °C temperature, which corresponds to the core temperature of the packaged pea spreads +76.0±1.0 °C. After heat treatment, packages were immediately chilled to sample temperature +4.0±0.5 °C in +2±1 °C cold ice-water. This heat treatment regimen was chosen based on previous experiments as the optimal *sous vide* regimen for pea spreads.

Samples were stored in a commercial cooler ELCOLD at +4.0±0.5 °C (temperature recorded by Greisinger MINILog) for 15 days under fluorescent light (OSRAM Lumilux De Luxe) with radiant fix at 100–800 lux (measured by Light meter LX-107). Samples were analysed in triplicate on days 0, 7 and 15.

Sample abbreviations were: control – untreated pea spread, SV – *sous vide* treatment at 80 °C/15 min, HP – high pressure pasteurization where the first number describes pressure (MPa) and the second number describes treatment time (min).

2.3 Decontamination of seasonings

Seasonings (citric acid, salt and herb spice) were decontaminated for 30 min under UV-C germicidal lamp (254 nm / 30 Watt) in a laminar flow cabinet (*S-KR 130*, Kojair, Finland). Decontamination of herb spice was also performed in STERINOVER horizontal autoclave (Lagarde, France) for 15 min at +121±2 °C.

2.4 Microbiological analysis

Microbiological testing of pea spreads was completed within one hour after processing; testing of seasonings was performed before and after decontamination.

90 ml 0.1% sterile peptone water was added to 10 g sample of processed pea spread in a stomacher bag; then the sample was homogenized with a stomacher BagMixer400 (Interscience, USA) for 10 seconds. Serial dilutions in 0.1% sterile peptone water were pour-plated in triplicate for determination of aerobic and facultative anaerobic, mesophilic bacteria (hereafter referred to as TPC – total plate count) on Plate Count agar (Ref. 01-161, Scharlau, incubation at 30 °C for 72 h), for coliforms on Endo agar (Ref. 01-589, Scharlau, incubation at 37 °C for 24 h) and for *Enterobacteriaceae* on Violet Red Bile agar with Glucose (Ref. 01-295, Scharlau, incubation at 37 °C for 24 h). After incubation, the colonies were counted using automated colony counter aCOLyte (Topac Inc., USA) and reported as colony forming units (CFU). Data are expressed as log₁₀ CFU g⁻¹ indicating the amount of cells per gram of product inside a pouch.

Microbiological safety of pea spreads was evaluated according to the guidelines on microbiological contamination of food stuffs:

- Regulation No 461/2014 (2014) describes vegetable products which have been pasteurised and/or sterilized, therefore TPC during storage for *sous vide* and HPP pea spreads is set at $<5 \cdot 10^3$ CFU g⁻¹ (3.69 log₁₀ CFU g⁻¹);
- according to Gilbert et al. (2000) pea spread is included in savoury group and TPC during storage for untreated pea spread is set at $<10^5$ CFU g⁻¹ (5.00 log₁₀ CFU g⁻¹);
- admissible count for coliforms is set at <20 CFU g⁻¹ and for *Enterobacteriaceae* $<10^2$ CFU g⁻¹ (Gilbert et al., 2000).

Identification of microorganisms was carried out by cultivating selected colonies on Plate Count Agar using streak plate method. Gram staining was performed followed by catalase and oxidase tests. Bacterial identification was completed by the API biochemical test system using API 50 CHB kit (bioMérieux, France).

2.5 Colour analysis

Colour changes in pea spread samples were measured in CIE L*a*b* colour system using Colour Tec PCM / PSM (Accuracy Microsensors Inc., USA). Colour values were recorded as L* (lightness) – the vertical co-ordinate that runs from L* = 0 (black) through grey to L* = 100 (white); a* (redness) – the horizontal co-ordinate that runs from -a* (green) through grey to +a* (red); and b* (yellowness) – another horizontal co-ordinate that runs from -b* (blue) through grey to +b* (yellow).

The measurements were repeated in tenfold on randomly selected locations at the surface of each sample. Colour difference (ΔE^*) was calculated according to equation (1) to describe the colour change of *sous vide* and HPP treated pea spread samples compared to untreated sample:

$$\Delta E^* = \sqrt{(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2} \quad (1)$$

where, ΔE^* – total colour difference; L*, a* and b* – colour values of sample after additional treatment; L*₀, a*₀ and b*₀ – colour values of untreated sample.

2.6 Software and data processing

The obtained data processing was performed with statistical software 'R 3.0.2' and 'Microsoft Office Excel 14.0'; differences among results were analysed using one way analysis of variance and Tukey's test. The results were expressed as mean ± standard deviation. Differences among results were considered significant if p-value $<\alpha_{0.05}$.

3. RESULTS AND DISCUSSION

3.1 Microflora in pea spreads and seasonings

TPC in untreated pea spread sample was significantly different ($p < 0.05$) compared to pea spreads after *sous vide* treatment and HPP (Fig. 2). None of the samples exceeded either of the defined admissible TPC at day 0.

A 1 log reduction could be achieved with *sous vide* treatment and HPP at ≥ 500 MPa (with dwell time ≥ 20 min at 500 MPa HPP).

Microbial contamination in SV sample decreased from 2.56×10^3 CFU g⁻¹ to 50 CFU g⁻¹ and in HP samples at 700 MPa (HP 700_10, HP 700_20, HP 700_30) from 2.56×10^3 CFU g⁻¹ to <100 CFU g⁻¹ after pasteurization. Significant differences were not found among these samples during 2 week storage ($p = 0.832$). HP

samples at 700 MPa and SV sample showed considerably lower microbiological contamination ($p < 0.05$) compared to HP (500 MPa) samples.

TPC in pea spread without additional treatment exceeded the admissible level ($N < 10^5$ CFU g⁻¹) for ready-to-eat spreads in less than seven days of storage at refrigerator temperature. TPC in all processed samples did not exceed the admissible level ($N < 5 \cdot 10^3$ CFU g⁻¹) for vegetable spreads after 2 weeks. Coliforms were not present in any samples, *Enterobacteriaceae* count was $< 10^1$ CFU g⁻¹.

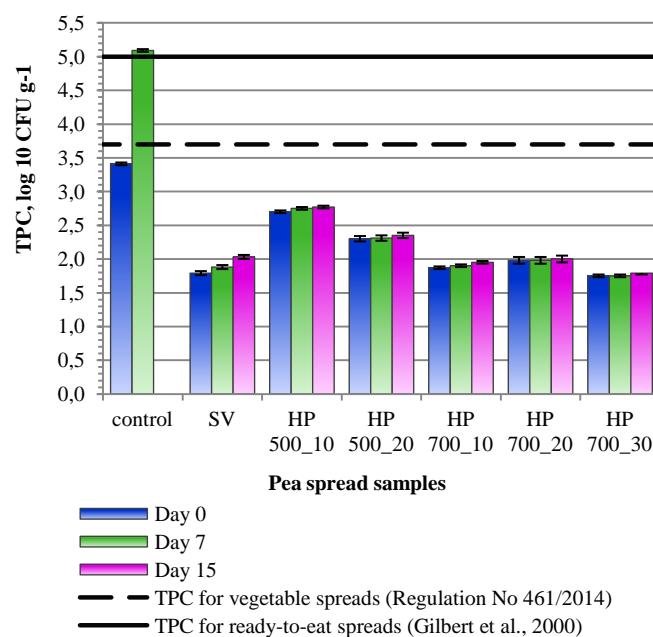


Fig. 2: The influence of different treatments on TPC dynamics in maple pea spread during storage at $+4.0 \pm 0.5$ °C temperature.

Sous vide treatment and HPP demonstrates improvement in pea spread shelf life compared to control sample. HPP at 700 MPa is suitable for pea spread shelf-life extension; however, long-term shelf-life research is required.

It is critical to store processed pea spreads at refrigeration temperatures, because microbial contamination of pea spread samples SV and HP 700_10 which were stored at room temperature ($+20 \pm 0.5$ °C) was not significantly different ($p = 0.341$) and exceeded admissible TPC in 8 days (≥ 3.7 log₁₀ CFU g⁻¹).

Bacteria found in all pea spread samples showed similar morphological characteristics. As *sous vide* and HPP are suitable for destroying vegetative cells, these microorganisms were thought to be potential spore forming bacteria. Gram staining proved them to be catalase positive gram positive bacteria of *Bacillus* spp. API biochemical identification showed two different bacteria species – *Bacillus subtilis* and *Bacillus licheniformis*. Deák and Farkas (2013) reported that *B. subtilis* and *B. licheniformis* are spore forming bacteria commonly found in many spices, and these spores can withstand pasteurization and pressure up to 1500 MPa (Zhang and Mittal, 2008).

Peas cooked in a pressure cooker (at 80 KPa) are practically sterile, therefore another ingredient – seasonings – were subjected to microbiological testing (before and after decontamination treatment) as possible source of contamination with spore forming bacteria.

UV-C treatment is performed at low temperatures and classified as a non-thermal process, which does not leave any residue in the treated products. Koutchma et al. (2009) described that UV-C radiation effectively and rapidly inactivates pathogen microorganisms by transferring the electromagnetic energy from a source through a photochemical reaction within the nucleic acids of the microorganisms. Erdogdu and Ekiz (2011) noted that spices (cumin seeds) maintained their colour, and no significant weight and volatile compound losses were observed after UV-C treatment.

UV-C treatment established over 2 log reduction for salt and citric acid (Fig. 3). Contamination in herb spice 'BRUSCHETTA' was maintained at a high level after UV-C treatment, therefore sterilization was carried out. Sterilization reduced bacterial load to $2.01 \log_{10}$ CFU g^{-1} ; however, organoleptic evaluation showed significant losses of colour, flavour and appearance, as heat treatment affects the sensitive flavour components (Deák and Farkas, 2013). The authors also showed that plate count per g of aerobic mesophilic bacteria in seasonings are generally between 3.5–8.4. \log_{10} CFU.

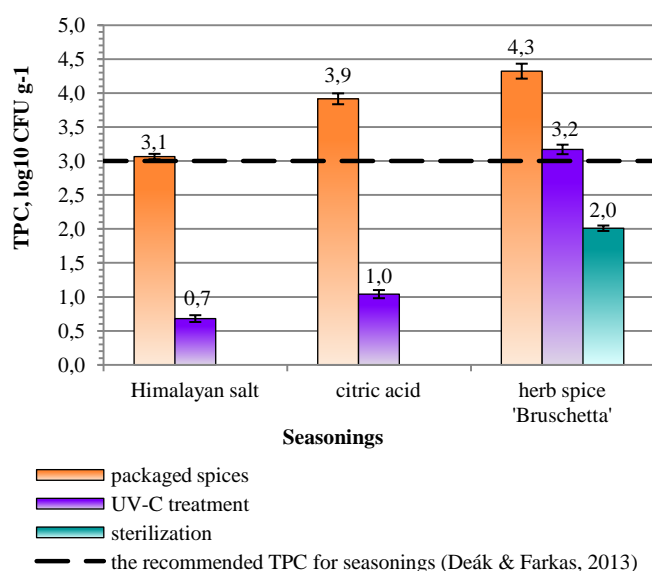


Fig. 3: Decontamination influence on microbiological load in seasonings.

Overall, UV-C treatment showed significant reduction compared to the initial microbial contamination in salt and citric acid ($p=0.009$), and herb spice ($p=0.033$). Spores of *B. subtilis* and *B. licheniformis* were found in herb spice samples after UV-C treatment and sterilization.

3.2 Pea spread colour analysis

Food colour usually is the first quality parameter evaluated by consumers. Total colour change (ΔE) indicates the magnitude of colour difference between processed and unprocessed samples. Cserhalmi et al. (2006) suggested differences in perceivable colour (ΔE) be classified analytically as not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3.0), well visible (3.0–6.0), and great (6.0–12.0).

Significant colour changes ($p=0.008$) between pea spreads with different treatments (Fig. 4) were observed. Untreated pea spread had the lightest colour. Pasteurised (*sous vide*) pea spread showed a well visible colour change (6.40) when compared to untreated pea spread, while HP samples showed not noticeable differences

(≤ 0.36). Andrés et al. (2016) reported similar findings on total colour difference after HPP.

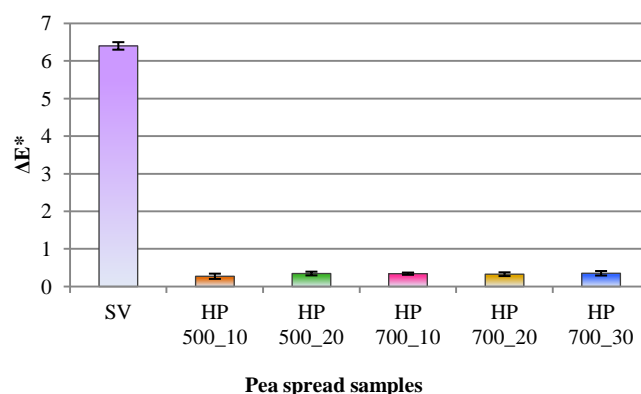


Fig. 4: Influence of treatment on total colour difference of pea spreads.

Food colour is critical in the acceptance of products, therefore HPP is preferable to *sous vide* processing for maple pea spreads.

4. CONCLUSIONS

HPP demonstrates improvement in pea spread shelf life compared to untreated sample and thermal processing, and is suitable for pea spread shelf-life extension (at 700 MPa).

Microbiological contamination of HPP samples at 700 MPa was below $2.00 \log_{10}$ CFU g^{-1} during two week storage at $+4.0 \pm 0.5$ °C temperature.

Spores of *B. subtilis* and *B. licheniformis* were found in all pea spread samples; source of these bacteria was herb spice.

UV-C treatment showed considerable reduction in microbiological load of seasonings. Sterilization did not destroy bacteria spores in herb spice.

5. HPP did not influence pea spread colour ($p>0.05$) contrary to *sous vide* processing.

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