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Improvement of microbiological safety and shelf-life of pulse spreads through *sous vide* and high pressure processing

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Abstract. Microbiological quality of *sous vide* treated (80 °C/15 min) and high pressure processed (700 MPa/10 min/20 °C) cowpea (*Vigna unguiculata* (L.) Walp. cv. Fradel) and maple pea (*Pisum sativum* var. *arvense* L. cv. Bruno) spreads in flexible vacuum packaging during 62-day storage at 5 ± 1 °C were assessed. Pulse spreads, made from cooked pulses with salt, citric acid, oil, and seasoning, were filled in PA/PE or PET/ALU/PA/PP flexible film pouches, packaged in vacuum (20 mbar) and hermetically sealed. Microbiological testing was performed by determining total plate count, yeast and mould count on days 0, 15, 29, 42, 50, 57, and 62, and the presence of *B. cereus*, *C. perfringens* and *E. coli* before processing and after 62-day storage. The results showed that total plate count increased significantly after 62-day storage in both *sous vide* (p = 0.011) and high pressure processed (p = 0.017) spreads; the observed over one-log increase was without significant differences between pulse spreads and packaging materials ($p < 0.05$). The admissible level of total plate count ($N_{max} < 3.69$ log CFU g^{-1}) in pulse spreads was not exceeded. The presence of yeasts and moulds, *C. perfringens* and *E. coli* in pulse spreads was not confirmed, whereas *B. cereus* accounted to <10^2 CFU g^{-1} after 62-day storage. The suggested shelf-life of processed pulse spreads is 62 days; except for *sous vide* treated spreads with seasoning in both packaging materials – 57 days. Both processing methods are suitable to ensure the production of high quality pulse spreads with adequately long shelf-life.

Key words: cowpea, maple pea, microbiological quality, pathogens, flexible packaging

INTRODUCTION

Pulse spreads are an innovative product made from rehydrated cooked seeds of pulses (Kirse & Karklina, 2015). They are an alternative to traditional animal-derived spreads, and have all nutritious components of pulses: high quality plant protein, complex carbohydrates, soluble and insoluble dietary fibre, a wide range of minerals and vitamins (Kirse et al., 2016a). However, shelf-life of fresh spreads is less than six days at refrigerator temperature (Kirse & Karklina, 2015). The high moisture content (65–68%), water activity (above 0.97), and the typical pH range (5.2–5.5) of the product makes pulse spreads susceptible to microbial and sensorial changes thus affecting the product shelf-life (Keenan et al., 2011; Yamani & Mehyar, 2011).

Several studies have described the application of *sous vide* treatment (Levkane et al., 2010; Baldwin, 2012; Sansone et al., 2012) and high pressure processing (Balasubramaniam, 2010; Vercammen et al., 2011; Georget et al., 2015) to increase shelf life of foodstuff by inactivating microorganisms and improving microbial safety of food products (Yordanov & Angelova, 2010). Both methods are suitable post-processing methods for shelf-life extension of various ready to eat products. Vacuum packaging in flexible polymer pouches is a fundamental technology of *sous vide* treatment and high pressure processing (Brody et al., 2008), which is carried out to eliminate the risk of recontamination during storage, reduce aerobic bacterial growth and prevent leaking of food constituents during processing, resulting in flavourful and nutritious food with long shelf-life (Church & Parsons, 2000; Seydim et al., 2006).

With regards to *sous vide* treatment, the effect of temperature on microorganisms has been studied extensively (Smelt & Brul, 2014), concluding that thermal treatment at 70 °C for 2 min is sufficient to eliminate non-spore-forming pathogens such as *Listeria monocytogenes*, *Salmonella* and *E. coli* 0157 (Juneja & Snyder, 2007). Literature data suggests that high pressure inactivates microorganisms by interrupting cellular functions responsible for reproduction and survival (Torres & Velazquez 2008); the permeabilization of the cell membrane is reversible at low pressures (up to 200 MPa), but irreversible at higher pressures (Rastogi et al., 2007). Both processing methods, however, cannot eliminate microorganism spores, as spores are heat-tolerant at temperatures below 100 °C (Baldwin et al., 2012) and pressure-tolerant at pressures above 1,000 MPa (Zhang & Mittal, 2008). Current high pressure processed products in the market rely on refrigeration, similar to pasteurization (Ates et al., 2016).

In addition to microbial safety, undesirable sensory and physicochemical changes during storage can lead to food products of inadequate quality. Therefore, shelf-life prediction is based on several deterioration factors – microbiological, sensory and physicochemical parameters (Dermesonluoglu et al., 2016). It is important to evaluate shelf-life of innovative products before they can be commercialised. So far there is little to none research on spreads made from pulses, therefore, shelf-life studies are necessary as the growth of microorganisms can vary between food matrices. The aim of this study was to determine microbiological quality of *sous vide* treated (80 °C/15 min) and high pressure processed (700 MPa/10 min/20 °C) cowpea (*Vigna unguiculata* (L.) Walp. cv. Fradel) and maple pea (*Pisum sativum* var. *arvense* L. cv. Bruno) spreads in flexible vacuum packaging during 62-day storage at 5 ± 1 °C followed by shelf-life assessment.

MATERIALS AND METHODS

Materials

Two type's pulses growing in Europe were used to prepare pulse spreads: cowpeas (*Vigna unguiculata* (L.) Walp. cv. Fradel), harvested in 2013, Portugal and maple peas (*Pisum sativum* var. *arvense* L. cv. Bruno), harvested in 2014, Latvia. Additional ingredients for the preparation of spreads were canola oil (Iecavnieks Ltd., Latvia), citric acid (Spilva, Ltd. Latvia), Himalayan salt (Pakistan) and bruschetta (dried tomato, garlic and basil) seasoning (P.P.H. fleischmann schaft®-Polska Sp. z o.o., Poland).

Preparation and packaging of pulse spreads

Each type of pulses was separately soaked in water (with $NaHCO_3$, 21.5 g kg^{-1}) at 20 ± 2 ºC for 15 h, then rinsed and boiled in a pressure cooker (KMZ, USSR) until tender (about 35 ± 5 min plus 15 min for natural pressure release) as described by Kirse & Karklina (2015). Still warm cooked pulses were then grinded in a food processor (Philips HR 7761/00, Philips, The Netherlands) together with additional ingredients. Four different pulse spreads were studied: cowpea spread without and with bruschetta (1%), maple pea spread without and with bruschetta (1%). The physicochemical parameters characterising spreads were as follows: 0.38% salt, 66.9–67.7% moisture, a_w = 0.977-0.978 and pH 5.38–5.49.

Transparent polyamide / polyethylene (PA/PE) film pouches (film thickness 60 ± 3 μm, PTC Ltd., Latvia) and light proof polyethylene terephthalate / aluminium / polyamide / polypropylene (PET/ALU/PA/PP) film pouches (film thickness 80 ± 3 μm, Nordvak Ltd., Latvia) with dimensions 45 × 170 mm were used to package prepared pulse spreads (50 ± 1 g). Two different packaging materials were used to determine whether the additional PET and ALU layers of the light proof film could provide a better quality for pulse spreads during storage. Filled pouches were hermetically sealed under vacuum (20 mbar, sealing time 1.9 s for PA/PE and 3.4 s for PET/ALU/PA/PP) using chamber type vacuum packaging machine (C300, Multivac Ltd., UK).

Processing treatments and storage conditions

Processing treatments of pulse spreads were carried out as described by Kirse et al. (2016c). *Sous vide* treatment was performed by pasteurizing samples for 15 min at 80.0 ± 0.5 °C (core temperature 78.0 ± 0.5 °C was reached within 9 min of treatment) in water bath (Clifton Food Range, Nickel-Electro Ltd., UK) followed by immediate chilling of samples in 2 ± 1 °C ice-water to 5 ± 1 °C temperature.

High pressure processing was performed by pascalising samples for 10 min at 700 MPa pressure in 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 with isopropanol, water mix (1:2) as the pressure transmitting liquid. High pressure processing was carried out at an ambient temperature (20.0 ± 1.0 °C), however, during processing temperature rise was observed due to the pressure increase in the vessel and reached a maximum of 40 °C; the pressure drop lowered the temperature to 10 °C. Processing regimes were chosen according to the previous investigations where *sous vide* treatment for 15 min at 80 °C (Kirse et al., 2016b) and high pressure processing for 10 min at 700 MPa (Kirse et al., 2015) showed the highest potential for shelf-life study based on lower microbial count and resource efficiency.

Untreated and processed samples were stored in a commercial display cooler (Snaige Ltd., Lithuania) with tempered glass door under daylight luminescence with radiant fix at 400 to 1000 lx (measured by LX-107 Portable Digital Light meter, Lutron Electronic Enterprise Co., Ltd., Taiwan) for 62 days at 5 ± 1 °C temperature.

Microbiological analysis

Microbiological testing of pulse spreads during storage was carried out according to preparation of test samples, initial suspension and decimal dilutions for microbiological examination (ISO 6887-4:2003). Peptone water (0.1% w v^{-1}) tenfold dilution aliquots were obtained after homogenization in a stomacher (BagMixer400, Interscience, USA) for 10 seconds. Triplicate plates were prepared using pour plate method for enumeration of total plate count (aerobic and facultative anaerobic, mesophilic bacteria, hereafter referred to as TPC) on Plate Count Agar (Ref. 01-161, Scharlau, Spain, incubation at 30 °C for 72 h) and yeasts and moulds on Malt extract agar (Ref. 01-111, Scharlau, Spain incubation at 27 °C for 48 h). Standard methods were used to determine the presence of such pathogens as *Bacillus cereus* (ISO 7932:2004), *Clostridium perfringens* (ISO 7937:2004) and *Escherichia coli* (ISO 7251:2005). After incubation, the colonies were counted using automated colony counter aCOLyte (Topac Inc., USA) and reported as colony forming units (CFU).

Microbiological analysis of total plate count, and yeast and mould count were carried out during storage at days 0, 15, 29, 42, 50, 57, and 62. Pathogens were determined in pulse spreads before processing and after 62-day storage. Data are expressed as log colony forming units per gram of product (log CFU g^{-1}).

Statistical analysis

The processing of obtained data was performed using mathematical and statistical methods with 'Microsoft Office Excel v16.0' (Microsoft Corp., Redmond, WA); differences among results were analysed using one-way analysis of variance. Each sample was analysed in triplicate and results were given as mean ± standard deviation. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Microbiological parameters that determine the quality of pulse spreads are total plate count (TPC), count of yeasts and moulds, presence and count of pathogens (*E. coli, B. cereus, C. perfringens*), which have been defined by the European Commission (EC Regulation No 2073/2005) and the Cabinet of Ministers of the Republic of Latvia (Regulation No 461/2014). CM Regulation No 461/2014 on microbiological criteria for vegetable jams, purées and similar products suggest total plate count of such products below 5×10^3 CFU g^{-1} (3.69 log CFU g^{-1}). As shown in the previous research, untreated maple pea spread with bruschetta reached the admissible level (3.69 log CFU g^{-1}) in less than four days of refrigerated storage (Kirse & Karklina, 2015; Kirse et al., 2016b).

In the present research, a similar trend was observed for the other three pulse spreads (cowpea spread, cowpea spread with seasoning and maple pea spread); the initial shelf-life of untreated pulse spreads was 3 to 5 days of refrigerated storage. Additionally, both *sous vide* and high pressure processed pouches ballooned after 1-week storage at room temperature (20 ± 1 °C) and TPC exceeded the admissible level (3.69 log CFU g^{-1}), therefore pulse spreads were only stored at 5 °C temperature. The short shelf-life of pulse spreads suggests that post-processing methods and appropriate packaging solutions should be considered to reduce the total number of microorganisms and avoid accelerated deterioration, thus extending shelf-life of the new products.

A preliminary study on the shelf-life extension of maple pea (*Pisum sativum* var. *arvense* L.) spread using *sous vide* treatment (80 °C/15 min) suggested the possible shelf-life of pea spread up to 96 days based on microbiological quality (Kirse et al., 2016b); the presence on anaerobic pathogens was not confirmed. However, microorganism growth during storage after *sous vide* treatment showed that microorganisms adapt to the environment in 50 to 60 days, and then a more rapid growth can be observed. Therefore, the investigated storage time for the processed pulse spreads in the current research was 62 days (proportional to the start of the accelerated growth).

A previous study showed that mean microorganism count in untreated maple pea spread with seasoning accounted to 3.41 log CFU g^{-1} (Kirse et al., 2015). The current study established that TPC in untreated spreads ranged from 3.40 to 3.48 log CFU g^{-1} without significant differences among samples ($p > 0.05$). An over 1.5-log reduction in

TPC compared to untreated spread samples was observed for pulse spreads after processing without significant differences between pulse spreads and samples in both packaging materials ($p < 0.05$). The presence of yeasts and moulds was not confirmed in any *sous vide* and high pressure processed samples.

TPC dynamics in *sous vide* treated pulse spreads during storage in different flexible packaging films showed that TPC after 62-day storage was significantly higher than immediately after processing ($p = 0.011$) (Fig. 1). Initial TPC in spreads packed in PA/PE after *sous vide* treatment was between 1.67 to 1.77 log CFU g^{-1} whereas in spreads packed in PET/ALU/PA/PP – between 1.62 to 1.71 log CFU g^{-1}. After storage over one-log increase of TPC in spreads was observed with mean counts ranging from 2.81 to 2.95 log CFU g^{-1}, analysis of variance of the data indicated no significant differences among pulse spreads and samples in both packaging materials ($p < 0.05$); TPC did not exceed admissible level ($N_{max} < 3.69$ log CFU g^{-1}) for any of the samples.

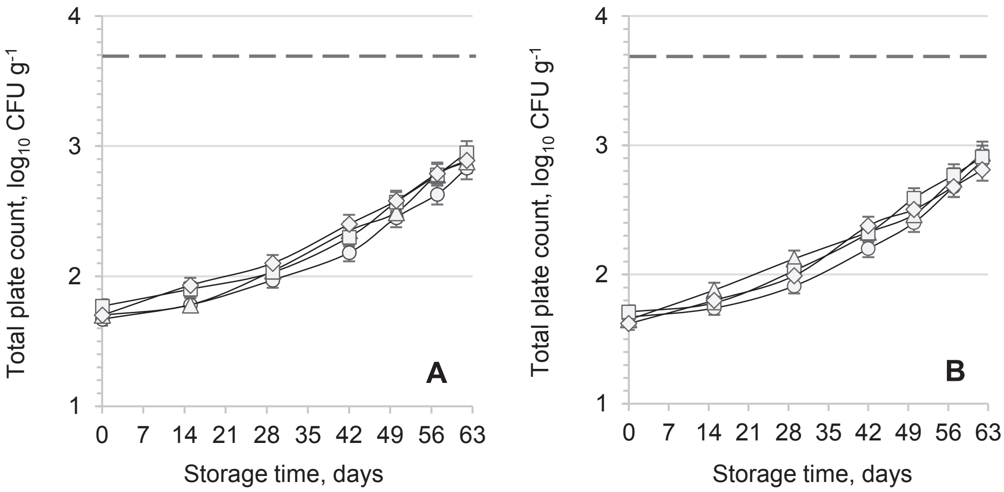

Figure 1. TPC dynamics during storage of *sous vide* treated cowpea spread (○), cowpea spread with bruschetta (△), maple pea spread (□) and maple pea spread with bruschetta (◇) packed in PA/PE (A) and PET/ALU/PA/PP (B). The dashed line (– –) denotes the admissible level of TPC < 3.69 log CFU g^{-1} for vegetable purées (CM Regulation, 2014).

Available studies on the microbiological quality of products after *sous vide* treatment have been focussed mostly on meat processing. An investigation on *sous vide* processed beef (97 °C/11 min) carried out by Paik et al. (2006) found that samples inoculated with *B. cereus* and *C. perfringens* spore cocktail did not exceed the guidelines of bacterial loading after 60 days at 4 °C. Similar findings were given by Can & Harun (2015) who concluded that *sous vide* treated (90 °C/20 min) chicken meatballs maintain consistent microbiological quality for 70 days at 2 °C. Pino Hernández et al. (2017) pointed out that microbial load in *sous vide* treated (60 °C/9.48 min) fish fillets remained within the thresholds established by Brazilian laws during 63-day storage at 1 °C temperature. Whereas Levkane et al. (2010) showed that *sous vide* technology (65 °C/20 min) of potato salad with meat in mayonnaise was suitable to maintain microbiological quality during 52-day storage at 4 ºC and 10 ºC temperature. The results of the present study are consistent with the findings of the above-mentioned investigations.

Total plate count dynamics in high pressure processed pulse spreads during storage in different flexible packaging films showed a similar trend to *sous vide* treated spreads – after 62-day storage TPC was significantly higher than in samples immediately after processing (p = 0.017) (Fig. 2). Initial TPC in spreads packed in PA/PE after high pressure processing was between 1.78 to 1.87 log CFU g^{-1} whereas in spreads packed in PET/ALU/PA/PP – between 1.75 to 1.87 log CFU g^{-1}. After storage over one-log increase of TPC in spreads was observed with mean counts ranging from 2.84 to 2.98 log CFU g^{-1} without significant differences between pulse spreads and samples in both packaging materials ($p < 0.05$); TPC did not exceed admissible level ($N_{max} < 3.69$ log CFU g^{-1}) for any of the samples.

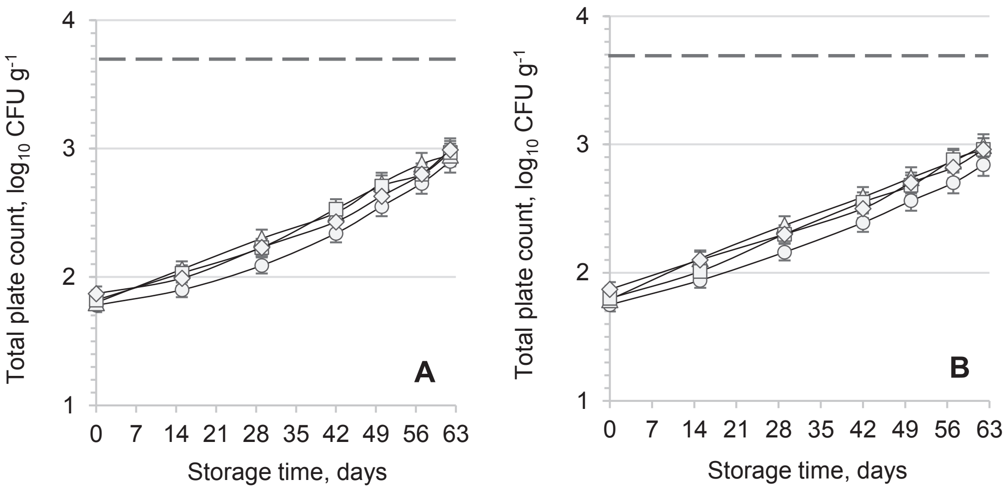

Figure 2. TPC dynamics during storage of high pressure processed cowpea spread (○), cowpea spread with bruschetta (△), maple pea spread (□) and maple pea spread with bruschetta (◇) packed in PA/PE (A) and PET/ALU/PA/PP (B). The dashed line (– –) denotes the admissible level of TPC < 3.69 log CFU g^{-1} for vegetable purées (CM Regulation).

Similar to *sous vide* treatment, studies on the microbiological quality of products after high pressure processing as the post-processing treatment have been focussed on products of animal origin. Hygreeva & Pandey (2016) reviewed the studies of the last decade on high pressure processing of processed meat products, concluding that depending on the chosen processing regimes (400–600 MPa/3–10 min) and storage conditions (2–8 °C) high pressure processing ensures consistent microbiological quality throughout storage (40 to 120 days). Masegosa et al. (2015) found that pressure treatment (600 MPa/5 min) significantly reduced the mesophilic counts of ready-to-heat vegetable meals after processing and maintained their microbial stability throughout 20-day storage at 4 °C. Whereas, a study on the effect of different pressure levels (500 and 600 MPa/1 min) on lasagne ready meal indicated that high-pressure processing significantly reduced the total aerobic and lactic acid bacteria counts and prolonged the microbial shelf-life of lasagne for 56 days (Stratakos et al., 2015). The results of TPC during storage of high pressure processed pulse spreads are consistent with the findings of the above described investigations.

The number of microorganisms during storage below the admissible level does not guarantee that the product is free from pathogens. Therefore, possible presence of pathogens was determined in all samples of pulse spreads stored at 5 ± 1 °C for both processing methods (Table 1). EC Regulation No 2073/2005 on microbiological criteria for vegetables, fruits and products thereof defines that the count of *E. coli* must be below 100 CFU g^{-1} (n = 5, c = 2, m = 100 CFU g^{-1}, M = 1000 CFU g^{-1}), whereas CM Regulation No 461/2014 denotes that pathogens must be absent in 1 g of vegetable jams, purées and similar products. Results showed that the presence of such pathogens as *E. coli* and *C. perfringens* in pulse spreads before preservation treatment and after *sous vide* and high pressure processing with sequential storage for 62 days was not confirmed in any of studied packaging materials.

Table 1. Pathogens in *sous vide* and high pressure processed pulse spreads, CFU g^{-1}

Microorganisms	Samples*	Before processing	After 62-day storage	
			sous vide treatment	high pressure processing
Bacillus cereus	CS	2.00×10^2	3.60×10^1	4.10×10^1
	CS_B	2.41×10^2	6.80×10^1	6.70×10^1
	MS	2.02×10^2	5.80×10^1	5.70×10^1
	MS_B	1.96×10^2	2.30×10^1	3.20×10^1
Escherichia coli	CS CS_B MS MS_B	not detected	not detected	not detected
Clostridium perfringens	CS CS_B MS MS_B	not detected	not detected	not detected

* CS – cowpea spread, CS_B – cowpea spread with bruschetta,
MS – maple pea spread, MS_B – maple pea spread with bruschetta

Contamination level of *B. cereus* in pulse spreads after spread preparation was below 2.41×10^2 CFU g^{-1}, whereas after 62-day storage *B. cereus* accounted to < 10^2 CFU g^{-1}, indicating that chosen processing methods had a positive effect on *B. cereus* reduction in all samples. Insignificant differences were found between pulse spreads in different packaging materials ($p < 0.05$).

B. cereus is the dominant aerobic bacterium in cooked, pasteurized and chilled products, because of the probable survival of its spores during the pasteurization step after packing (Paik et al., 2006). Literature data suggests that only *B. cereus* levels between 10^5 and 10^8 cells and/or spores produce toxins that cause vomiting or diarrhoea (Ceuppens et al., 2015). The diarrhoeal toxin is produced in the temperature range of 10–43 °C, with an optimum of 32 °C whereas the production of emetic toxin is within the temperature range of 12–37 °C, with more toxin produced at 12–15 °C compared to higher temperatures (Finlay et al. 2000, Banerjee & Sarkar, 2004). During storage, the growth of *B. cereus* was not observed and *B. cereus* should not be a hazard when refrigeration is properly maintained throughout the shelf-life of pulse spreads, in order to limit the germination and multiplication of spores (Deák & Farkas, 2013). However, even these low levels of *B. cereus* are not allowed according to the CM Regulation No 461/2014.

Microbiological parameters determine whether food products are safe for consumption, yet product flavour quality drives consumer acceptance and demand as argued by Singh-Ackbarali & Maharaj (2014), therefore sensory and physicochemical parameters need to be taken into consideration when predicting shelf-life of pulse spreads. Sensory properties of pulse spreads (overall appearance, aroma, mouthfeel and taste) during 62-day storage were evaluated by six experts and each spread sample was assigned a quality number (QN) on a 5-point scale (5 – very good quality to 1 – unsatisfactory quality) as described by Kirse et al. (2016d). The quality of *sous vide* treated pulse spreads was very good (QN = 4.96–4.75) up to day 29 regardless of packaging film material; cowpea spread and maple pea spread maintained good quality during 62-day storage in both chosen packaging films, whereas spreads with bruschetta maintained good quality up to day 57 and average quality – after day 57. High pressure processed cowpea spreads maintained very good quality during the whole storage time (QN = 4.99–4.79), besides packaging film materials and seasoning had insignificant influence on the quality of cowpea spreads. Maple pea spread (MS), however, maintained good quality after day 57; maple pea spread with bruschetta (MS_B) in PA/PE packaging maintained good quality, but in PET/ALU/PA/PP packaging – very good quality (Kirse et al., 2016d). With regards to physicochemical parameters, *sous vide* and high pressure processing had an insignificant influence on pH, water activity, mass losses (Kirse et al., 2016c) and nutritional value of pulse spreads (Kirse & Karklina, 2016) after processing and throughout storage, irrespective of packaging materials ($p > 0.1$). Considering that *sous vide* and high pressure processing maintained consistent physicochemical and microbiological quality of pulse spreads during 62-day storage, shelf-life prediction was based on the results of sensory evaluation.

As defined in EU legislation, Commission Regulation (EC) No. 2073/2005 defines shelf-life as 'either the period corresponding to the period preceding the 'use by' or the minimum durability date'. In Commission Regulation (EC) No. 1169/2011 minimum durability date is defined as the date 'until which the foodstuff retains its specific properties when properly stored' and should be preceded by the words 'best before ...' (when the date includes an indication of the day) or 'best before end ...'; whereas in cases where foodstuffs are highly perishable from the microbiological point of view and are 'therefore likely after a short period to constitute an immediate danger to human health, the date of minimum durability shall be replaced by the 'use by' date'. Any special storage conditions (e.g., temperature not to exceed 5 °C) must be specified on the packaging when using 'best before' date. Robertson (2010) reported that such practice allows manufacturers to set the quality standard of the food, as the product will still be acceptable to many consumers after the 'best before' date has passed. Based on the results of quality changes during storage the suggested shelf-life of processed pulse spreads is given in Table 2.

Processed pulse spreads should be given the 'minimum durability date' and packaging should contain 'best before ...' label, as both processing methods were able to suppress the survival of investigated microorganisms in pulse spreads and total plate count did not exceed the admissible level at day 62.

Table 2. Suggested shelf-life of pulse spreads

Processing technology	Packaging	Pulse spreads*			
		CS	CS_B	MS	MS_B
Sous vide treatment	PA/PE	62 days	57 days	62 days	57 days
	PET/ALU/PA/PP	62 days	57 days	62 days	57 days
High pressure processing	PA/PE	62 days	62 days	62 days	62 days
	PET/ALU/PA/PP	62 days	62 days	62 days	62 days

* CS – cowpea spread, CS_B – cowpea spread with bruschetta,
MS – maple pea spread, MS_B – maple pea spread with bruschetta

CONCLUSIONS

Sous vide (80 °C/15 min) and high pressure processing (700 MPa/10 min/20 °C) significantly reduced the total microorganism count in cowpea and maple pea spreads thus maintaining consistent microbiological quality for 62 days at 5 °C. The presence of such pathogens as *E. coli* and *C. perfringens* in pulse spreads was not confirmed, whereas the level of *B. cereus* spores found in all spread samples did not possess harm to consumers. Considering microbiological, sensory and physicochemical quality changes during storage, the suggested shelf-life of processed pulse spreads is 62 days, except for *sous vide* treated spreads with bruschetta in both packaging materials – 57 days. Both processing methods are suitable to ensure the production of high quality pulse spreads with significantly longer shelf-life compared to untreated spreads.

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