

Physicochemical Stability of Pulse Spreads during Storage after *Sous Vide* Treatment and High Pressure Processing

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Abstract—Pulses are high in plant protein and dietary fiber, and contain slowly digestible starches. Innovative products from pulses could increase their consumption and benefit consumer health. This study was conducted to evaluate physicochemical stability of processed cowpea (*Vigna unguiculata* (L.) Walp. cv. Fradel) and maple pea (*Pisum sativum* var. *arvense* L. cv. Bruno) spreads at 5 °C temperature during 62-day storage. Physicochemical stability of pulse spreads was compared after *sous vide* treatment (80 °C/15 min) and high pressure processing (700 MPa/10 min/20 °C). Pulse spreads were made by homogenizing cooked pulses in a food processor together with salt, citric acid, oil, and bruschetta seasoning. A total of four different pulse spreads were studied: Cowpea spread without and with seasoning, maple pea spread without and with seasoning. Transparent PA/PE and light proof PET/ALU/PA/PP film pouches were used for packaging of pulse spreads under vacuum. The parameters investigated were pH, water activity and mass losses. Pulse spreads were tested on days 0, 15, 29, 42, 50, 57 and 62. The results showed that *sous-vide* treatment and high pressure processing had an insignificant influence on pH, water activity and mass losses after processing, irrespective of packaging material did not change ($p>0.1$). pH and water activity of *sous-vide* treated and high pressure processed pulse spreads in different packaging materials proved to be stable throughout the storage. Mass losses during storage accounted to 0.1% losses. Chosen *sous-vide* treatment and high pressure processing regimes and packaging materials are suitable to maintain consistent physicochemical quality of the new products during 62-day storage.

Keywords—Cowpea, flexible packaging, maple pea, pH, water activity.

I. INTRODUCTION

PULSES or grain legumes (*Fabaceae*) – crops harvested solely for the dry grain, are among the most extensively used foods in the world [1]. Contrary to other legumes, pulses are mainly cultivated for human consumption, yielding from one to twelve seeds within a pod [2]. Pulses are an especially important component of human diet in the developing countries, where other sources of protein are limited (or non-

existent). Pulses are an inexpensive and nutritious plant based food, which contains high quality dietary protein and fiber. Pulses are also a good source of complex carbohydrates, B group vitamins, minerals, oligosaccharides and phenolic compounds [3], [4].

The frequent consumption of pulses is considered to be as an effective tool to decrease the risk of cardiovascular disease [5] as well as overweight and obesity [6].

Despite the advantages of pulse consumption, literature reports [7] quite low pulse consumption in the Western world at less than 3.5 kg per capita per year, whereas in other parts of the world, annual pulse consumption can range from 10 to 40 kg per capita. The three main reasons for not eating pulses are a) lack of knowledge of how to prepare them, b) a poor understanding of the health benefits and c) concern over side effects such as bloating and flatulence [8].

As plant-based spreads are becoming important for health-conscious people who are seeking attractive products from other sources than dairy and meat [9], pulse spreads have proved themselves as an excellent meat alternative (for vegetarians and vegans) and addition to everyday products (for omnivores) which is highly acceptable by consumers [10], having the same constituents – high protein and dietary fiber content, low energy density, slowly digested carbohydrates – as grain legumes [11].

As consumers prefer products without preservatives [12] but with adequately long shelf-life, several additional treatment methods must be considered. *Sous-vide* treatment is a pasteurization technology, where products are packaged in flexible packaging and then heat treated in water (bath) or air (oven) at a controlled temperature and time regime [13], followed by rapid cooling to avoid the risk of microbial growth. High pressure processing is a novel processing technology which can be carried out at ambient temperature thus preserving the foodstuff with minimal effects on taste, texture, appearance, or nutritional value [14], [15].

Such factors as pH, water activity (a_w) and mass losses through packaging materials represent physicochemical parameters of products; significant changes in these parameters during storage indicate quality inconsistencies. The growth intensity of microorganisms is determined by pH value. pH values greater than 4.6 make food products susceptible to bacterial spoilage and the possible growth of pathogens [16]. A decrease in pH value over time indicates the growth of proteolytic microorganisms, whereas an increase in pH value indicates the growth of amylolytic microorganisms

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[17], [18]. The water activity values which cause the susceptibility to spoilage by bacteria, pathogens, yeasts and molds of foods is above 0.85 [19]. Mass losses through packaging depend on the chosen packaging materials based on the barrier properties. All packaging materials are somewhat water-permeable and during storage water migration through packaging is possible, thus resulting in product mass losses. Significant mass losses during storage can be observed if the chosen packaging material has weak barrier properties; this is an indication that current packaging material is poorly suitable for processing and storage of the selected food products [20].

The main purpose of packaging is to ensure quality during shelf-life and throughout storage. Packaging that is effective prevents the transmission of oxygen, as well as light and water vapor, and microbial growth therefore limiting deterioration of packaged goods [21].

In order to evaluate physicochemical stability of pulse spreads the aim of the research was to investigate the influence of *sous vide* treatment and high pressure processing on pH, water activity and mass losses of cowpea (*Vigna unguiculata* (L.) Walp. cv. Fradel) and maple pea (*Pisum sativum* var. *arvense* L. cv. Bruno) spreads in different flexible packaging materials during 62-day storage at 5 °C temperature.

II. MATERIALS AND METHODS

Experiments were carried out at the Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture in 2016.

A. Raw Materials

Pulse spreads were made from two pulses growing in Europe: Cowpeas (*Vigna unguiculata* (L.) Walp. cv. Fradel), harvested at Universidade de Trás-os-Montes e Alto Douro in 2015, Portugal and maple peas (*Pisum sativum* var. *arvense* L. cv. Bruno), harvested at Institute of Agricultural Resources and Economics in 2015, Priekuli, Latvia. Additional ingredients were used to prepare pulse spreads: 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).

B. Preparation of Pulse Spreads

Pulse spreads were made by soaking each type of pulses in water (with added NaHCO₃, 21.5 g kg⁻¹) at 20 ± 2°C for 15 h separately, then rinsing and boiling in a pressure cooker (KMZ, USSR) until tender (about 35±5 min plus 15 min for natural pressure release) [22]. Still warm cooked pulses were then grinded in a food processor (Philips HR 7761/00, Philips, The Netherlands) together with salt and citric acid, seasoning was added to the pulse paste; oil was added at the end of mixing in the food processor. Four types of pulse spreads were made: cowpea spread, cowpea spread with seasoning, maple pea spread and maple pea spread with seasoning. Pulse spreads with seasoning contained 10 g bruschetta seasoning per 1000 g spreads. The amount of salt did not exceed 0.4 g

per 100 g spread. The experimental design is summarized in Fig. 1.

C. Packaging of Pulse Spreads

Each type of pulse spreads was packaged into two different packaging materials – transparent polyamide/polyethylene (PA/PE) film pouches (film thickness 60±3 µm, PTC Ltd.) and light proof polyethylene terephthalate/aluminum/polyamide/polypropylene (PET/ALU/PA/PP) film pouches (film thickness 80±3 µm, Nordvak Ltd., Latvia). The dimensions for each pouch were 45 mm x 170 mm; the amount of spread per pouch was 50±1 g.

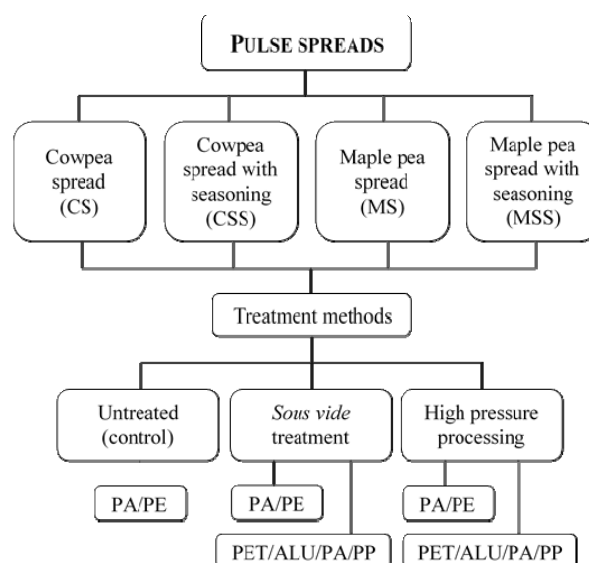


Fig. 1 Structure of performed experiments

After filling, pulse spread 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) and subjected to additional treatment. Additional processing regimes were chosen based on previous research data [22] where optimal regimes were determined.

D. Sous Vide Treatment

Pulse spreads were pasteurized by *sous vide* treatment in Clifton Food Range water bath (Nickel-Electro Ltd., UK). *Sous vide* treatment was carried out by pasteurizing samples for 15 min at 80.0±0.5 °C temperature (core temperature 76.0±1.0 °C.) which was followed by immediate chilling of samples in +2±1 °C cold ice-water to 5.0±1.0 °C temperature [22].

E. High Pressure Processing

Pulse spreads were subjected to high pressure (Fig. 2) 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. An isopropanol, water mix (1:2) was used as the pressure transmitting liquid [22]. Pouches of pulse spreads were placed in the pressure vessel and treated at 700 MPa with

10 min dwell time at ambient temperature.



Fig. 2 Pulse spreads after high pressure processing in flexible transparent PA/PE packaging material pouches

F. Storage of Processed Pulse Spreads

Untreated (control) vacuum packaged samples of all four pulse spreads, *sous vide* treated and high pressure processed samples were stored at identical conditions (temperature recorded by MINILog, GHM Messtechnik GmbH Standort Greisinger, Germany) 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.

G. Determination of pH, Water Activity and Mass Losses During Storage

Standard methods were used to determine changes in pH, water activity and mass losses of pulse spreads during 62-day storage. pH was determined with JENWAY 3510 pH-meter using JENWAY (3 mol/KCl) electrode. Water activity was determined with *AquaLab LITE* (accuracy of $\pm 0.015 a_w$, Decagon Devices Inc., USA). Mass losses through packaging materials were determined by weighing the same two samples of each spread type over 62-day period using electronic scale *Precisa* (accuracy of ± 0.01 g, *Precisa Gravimetrics AG*, Switzerland). These parameters were determined on day 0 (after processing), 15, 29, 42, 50, 57 and 62.

H. Software and Data Processing

The processing of obtained data was performed using mathematical and statistical methods with Microsoft Office Excel v16.0; differences among results were analyzed using one-way analysis of variance. Each sample was analyzed in triplicate (except for mass losses), and the values were reported as means of the measurements \pm standard deviation. For the interpretation of the results it was assumed that $\alpha = 0.05$ with 95% confidence and differences among results were considered significant if p -value $< \alpha_{0.05}$.

III. RESULTS AND DISCUSSION

In order to evaluate the influence of treatment technologies and packaging materials foodstuff, it is important to compare physicochemical parameters. pH values of untreated pulse spreads are shown in Table I. Spreads made from cowpeas had higher pH value compared to spreads made from maple peas. Cowpea spread had a noticeably higher pH value (5.491 ± 0.002) than the rest of the spreads; however, the difference was not significant ($p > 0.1$). The influence of *sous vide* treatment, high pressure processing, and chosen packaging materials on pH of all investigated pulse spreads was not significant ($p > 0.1$) on day 0, compared to untreated samples, as well as among treatments and packaging materials for each spread type. Considerable differences between water activity in untreated samples of pulse spreads after *sous vide* treatment and high pressure processing in different packaging materials were not found ($p > 0.1$).

Fig. 3 shows pH values of *sous vide* treated pulse spreads packed in PA/PE and PET/ALU/PA/PP pouches during 62-day storage at 5 ± 1 °C. A slight pH decrease was observed in all *sous vide* treated pulse spreads. The most noticeable decrease in pH was observed for cowpea spread with seasoning packed in PET/ALU/PA/PP (from pH 5.373 to 5.243) and cowpea spread packed in PET/ALU/PA/PP (from pH 5.458 to 5.316), especially after day 49.

TABLE I
 MEAN VALUES, STANDARD DEVIATION AND RESULTS OF STATISTICAL ANALYSIS (ONE-WAY ANOVA) OF pH AND WATER ACTIVITY OF UNTREATED AND PROCESSED PULSE SPREADS IN DIFFERENT PACKAGING MATERIALS

Parameters	Pulse spread samples	Processing treatment and packaging material					p-value
		I	II	III	IV	V	
pH	CS	5.491 \pm 0.002	5.466 \pm 0.004	5.458 \pm 0.002	5.498 \pm 0.002	5.503 \pm 0.002	$p = 0.721$
	CSS	5.398 \pm 0.002	5.383 \pm 0.003	5.373 \pm 0.002	5.424 \pm 0.001	5.407 \pm 0.002	$p = 0.687$
	MS	5.420 \pm 0.001	5.411 \pm 0.002	5.415 \pm 0.001	5.434 \pm 0.001	5.438 \pm 0.003	$p = 0.910$
	MSS	5.379 \pm 0.003	5.376 \pm 0.002	5.396 \pm 0.003	5.356 \pm 0.001	5.382 \pm 0.001	$p = 0.805$
Water activity (a_w)	CS	0.977 \pm 0.001	0.979 \pm 0.003	0.979 \pm 0.002	0.976 \pm 0.004	0.977 \pm 0.002	$p = 0.982$
	CSS	0.977 \pm 0.001	0.976 \pm 0.004	0.977 \pm 0.002	0.977 \pm 0.002	0.976 \pm 0.002	$p = 0.991$
	MS	0.977 \pm 0.002	0.977 \pm 0.002	0.979 \pm 0.002	0.977 \pm 0.001	0.977 \pm 0.001	$p = 0.977$
	MSS	0.978 \pm 0.001	0.979 \pm 0.002	0.979 \pm 0.002	0.977 \pm 0.002	0.977 \pm 0.002	$p = 0.980$

CS – cowpea spread, CSS – cowpea spread with seasoning, MS – maple pea spread, MSS – maple pea spread with seasoning

I – control, II – *sous vide* treatment, PA/PE, III – *sous vide* treatment, PET/ALU/PA/PP, IV – high pressure processing, PA/PE, V – high pressure processing, PET/ALU/PA/PP

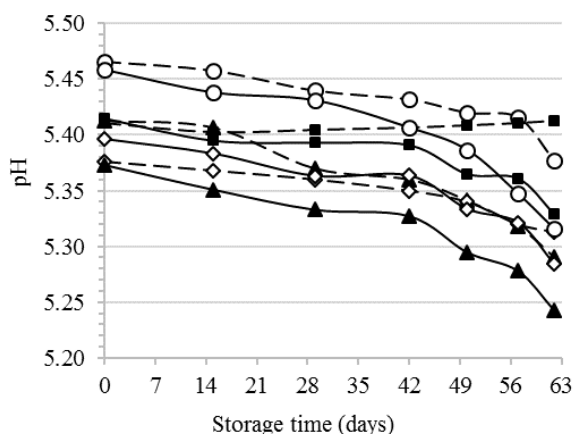


Fig. 3 pH value of *sous vide* treated cowpea spread (○), cowpea spread with seasoning (▲), maple pea spread (■) and maple pea spread with seasoning (◇) during storage. Dashed line represents spreads in PA/PE packaging; straight line - in PET/ALU/PA/PP packaging

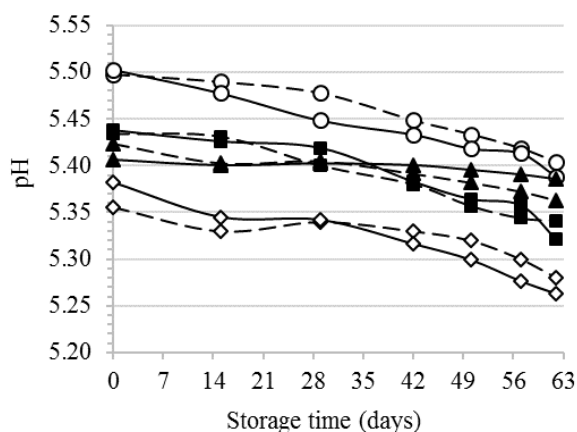


Fig. 4 pH value of high pressure processed cowpea spread (○), cowpea spread with seasoning (▲), maple pea spread (■) and maple pea spread with seasoning (◇) during storage. Dashed line represents spreads in PA/PE packaging; straight line - in PET/ALU/PA/PP packaging

Significant differences in pH value were not observed between *sous vide* treated pulse spreads in different packaging materials during storage ($p > 0.05$).

A similar trend of pH changes was observed for high pressure processed pulse spreads (Fig. 4) as all pH curves showed a slight decrease. The highest decrease was detected for cowpea spread packed in PET/ALU/PA/PP (from pH 5.503 to 5.389) and maple pea spread with seasoning packed in PET/ALU/PA/PP (from pH 5.382 to 5.263), especially after day 42. Significant differences in pH values among investigated high pressure processed pulse spread samples after 62-day storage were not found ($p > 0.05$).

It can be concluded that chosen thermal treatment and high pressure processing regimes, which are necessary to maintain microbiological and sensory quality of pulse spreads during storage [22], [23], do not influence pH of pulse spreads

significantly during 62-day storage. This shows that consistent product quality is maintained after additional processing.

Water activity of *sous vide* treated and high pressure processed pulse spreads packed in PA/PE and PET/ALU/PA/PP during 62-day storage at 5 ± 1 °C did not show a significant increase or decrease ($p > 0.1$), and was within the range of standard deviation for each type of pulse spread as shown in Table I. This means that additional processing does not influence the susceptibility to spoilage by microorganisms of pulse spreads, however, spoilage is possible throughout storage period. Strict temperature conditions must be ensured during storage, as temperature above 5 °C can cause rapid product microbial deterioration [13], [23].

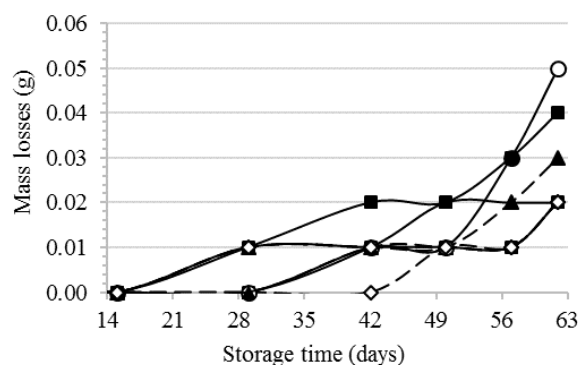


Fig. 5 Mass losses through packaging of *sous vide* treated cowpea spread (○), cowpea spread with seasoning (▲), maple pea spread (■) and maple pea spread with seasoning (◇) during storage. Dashed line represents spreads in PA/PE packaging; straight line - in PET/ALU/PA/PP packaging

Mass losses through product packaging are characterized by the loss of moisture (water). Fig. 5 shows mass losses through packaging of *sous vide* treated pulse spreads packed in PA/PE and PET/ALU/PA/PP during 62-day storage at 5 ± 1 °C. Mass losses from day 0 to day 14 were not observed in all samples of pulse spreads. After day 14, a slight mass loss increase in all samples was found. The highest value of mass losses was 0.05 g for cowpea spread packed in PA/PE and 0.04 g for maple pea spread packed in PA/PE, compiling to 0.1% losses.

A similar trend of mass losses was observed for high pressure processed pulse spreads (Fig. 4) as all samples did not show mass losses during the first 14 days of storage. The highest decrease of mass losses through packaging materials was observed for cowpea spread packed in PET/ALU/PA/PP (0.05 g) and maple pea spread with seasoning packed in PET/ALU/PA/PP (0.04 g), also compiling to 0.1% losses.

Both investigated packaging materials, especially with aluminum layer (PET/ALU/PA/PP), have high barrier properties [24], therefore the slight decrease in mass losses for all samples (even if at 0.1% loss) could be ascribed to human error. Significant differences in mass losses through packaging among investigated *sous vide* treated and high pressure processed pulse spread samples after 62-day storage were not found ($p > 0.05$).

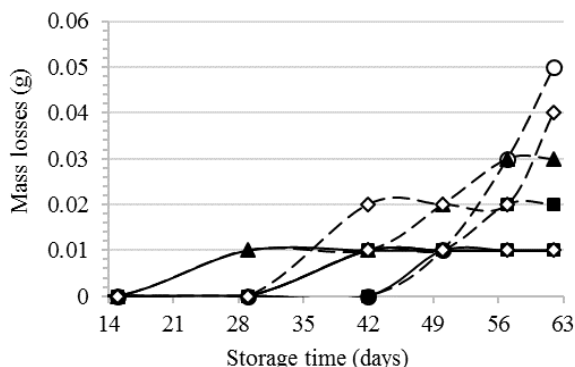


Fig. 6 Mass losses through packaging of high pressure processed cowpea spread (○), cowpea spread with seasoning (▲), maple pea spread (■) and maple pea spread with seasoning (◇) during storage. Dashed line represents spreads in PA/PE packaging; straight line - in PET/ALU/PA/PP packaging

IV. CONCLUSIONS

It is possible to maintain consistent pulse product quality during 62-day storage after *sous vide* treatment and high pressure processing in both types of flexible vacuum packaging based on the values of pH, water activity and mass losses.

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