



# Potential of high isostatic pressure and pulsed electric fields to improve mass transport in pea tissue



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## ABSTRACT

The aim of this study was to investigate and evaluate the potential of high isostatic pressure (HP) and pulsed electric fields (PEF) for implementation in innovative legume processing concepts. Whole peas were subjected to HP (400 MPa, 40 °C, 10 min) and PEF (5 kV cm<sup>-1</sup>, 125 kJ kg<sup>-1</sup>) treatments. The impact on the pea tissue was assessed by means of microscopic changes, release of protein and oligosaccharides during treatment and rate of subsequent drying and rehydration steps. HP and PEF resulted in altered cell wall structures and improved mass transport in comparison to the control samples (20 °C, 10 min). Effects on the extent of diffusion depended on the molecular size of the cell compounds. More than 10% of raffinose equivalent sugars were removed during treatments while preserving more than 99.9% of nutritionally valuable protein. Hence, both emerging technologies may be implemented in pea processing for a targeted reduction of flatulence causing oligosaccharides. The drying and rehydration rates of whole peas were increased to a higher extent by HP and PEF than by conventional thermal treatments (80 °C, 10 min). These innovative pre-treatments may therefore be applied to enhance the efficiency of industrial mass transfer processes or to decrease the preparation time of meals with whole dried peas and thus increase the flexibility for the consumer.

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## 1. Introduction

A growing demand for vegan proteins and gluten-free products as well as the objective of an eco-friendly and sustainable cultivation of arable land led to an increasing interest in novel legume foods. The World Health Organization even advises people to, inter alia, increase the consumption of legumes to prevent overweight and obesity as well as lower their risk for non-communicable diseases (WHO, 2013). The contribution of peas, beans and lentils to the protein intake in Europe, and particularly in Germany, is very low compared to most regions of the world, where grain legumes are an important staple food (FAO, 2013). In a survey, disfavour of the characteristic taste, flatulence and laborious preparation were mentioned as the main reasons for the low consumption of legume dishes (Klemcke et al., 2013). Intensive processing and isolation of individual components may partially solve these problems, but in particular, the well-balanced combination of protein, slowly digestible starch and dietary fibres in whole grain legumes is held responsible for an increased satiety and a positive effect on weight management (McCrorry et al., 2010). Hence, new products are required that contain legumes in the form of whole seeds or wholemeal flour and are contemporaneously well accepted by the consumer and preferably convenient. These novel foods cannot be developed solely on the basis

of conventional processing. They also demand the application of innovative concepts and technologies, like high isostatic pressure (HP) or pulsed electric fields (PEF).

High isostatic pressure is mainly used as a gentle approach for the preservation of high value heat sensitive products. Yeasts, moulds and most of the food-borne pathogenic vegetative bacteria can be inactivated at pressures between 300 and 600 MPa (Smelt, 1998), as well as several food relevant enzymes (Seydlerhelm et al., 1996). Additionally, this pressure range may be applied aiming at the disintegration of plant materials. Possible mechanisms reported for the disintegration of microbial and plant cells are a pressure-induced denaturation of membrane proteins (Kato et al., 2002; Ulmer et al., 2002; Winter & Jeworrek, 2009), increased activity of degrading enzymes (Angersbach et al., 2002), cell wall stress, excessive mechanical strain of the membrane (Hartmann & Delgado, 2004), as well as altered membrane fluidity and permeability due to phase transition of the phospholipid bilayer (Follonier et al., 2012; Winter et al., 2007).

The main mechanism of action of a pulsed electric field treatment is the permeabilization of biological cells due to an instantaneous increase of the electric potential applied on the cell membrane. The elastic properties cannot withstand the attraction forces of the charges if a critical value is exceeded. Consequently, a pore formation is induced, leading to a loss of the membrane's semipermeability (Barsotti & Cheftel, 1999). Improvement of mass transfer processes like solid–liquid separation, extraction and drying processes is thus one main field of

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application for pulsed electric fields (Toepfl et al., 2005). Due to the pulsed operation mode, intensive warming of the samples can be avoided and the quality of heat sensitive foods may be preserved.

The aim of this study was to figure out whether high pressure and pulsed electric fields as cell disintegration tools can contribute to an improved legume processing and may lead to the creation of innovative products tailored to consumer preferences. Their potential as pre-treatments for drying and rehydration processes or for the targeted removal of oligosaccharides was determined. Comparison with a conventional thermal treatment was performed to evaluate the uniqueness of the emerging processes.

## 2. Materials and methods

### 2.1. Materials

Dry yellow peas, cultivar Salamanca, were provided by Norddeutsche Pflanzenzucht Hans-Georg Lembke KG (Holtsee, Germany). Peas were stored in plastic bottles at room temperature until use. Seeds were swollen before treatment for  $20 \pm 0.25$  h with tap water in a ratio of 1:3 (w/w) at room temperature. Broken or incompletely swollen peas, distinguished by their wrinkled hull, were sorted out and not used for subsequent experiments.

Rapeseed, cultivar Lorenz, was obtained from Norddeutsche Pflanzenzucht Hans-Georg Lembke KG (Holtsee, Germany).

### 2.2. High pressure, heat and pulsed electric field treatments

High pressure treatments were conducted in a laboratory system with indirect pressure generation and a vessel volume of 750 mL (High Pressure Single Vessel Apparatus U4000, Unipress, Warsaw, Poland). A 1:1 mixture of deionized water and 1,2-propanediol (Sigma-Aldrich Corporation, St. Louis, Missouri) was used as pressure-transmitting medium. The vessel was tempered to 40 °C before experiments with a connected water bath (DC10-K20, Thermo Haake GmbH, Karlsruhe, Germany) to avoid temperature inhomogeneities during treatments. Peas and tap water as treatment medium were filled in a ratio of 1:1 (w/v) into bags made of pressure-stable polyamide/polyethylene foil. Vacuum-sealing (PlusVac 23, Komet Maschinenfabrik GmbH, Plochingen, Germany) was conducted to minimize the presence of air bubbles with differing compression characteristics. Bags were given into the high pressure unit directly before treatment and subjected to a pressure of 400 MPa for 10 min. Initial sample and medium temperatures necessary to achieve isobaric and isothermal conditions of 40 °C during treatment in the used high pressure system were 20 °C and 32 °C, respectively.

For the heat treatments, peas were given into plastic bags. Tap water tempered to 80 °C was added in a ratio of 1:1 (w/v). Temperature was kept at 80 °C for 10 min in a water bath (C20, Lauda, Lauda-Königshofen, Germany). Afterwards bags were cooled to ambient temperature with running tap water.

Pulsed electric field treatments were performed discontinuously in a self-constructed PEF unit of the TU Berlin. Three capacitors connected in series with a respective capacity of 1  $\mu\text{F}$  were charged by a high voltage generator (ALE802, lambda Emi, Neptune, New Jersey, United States) with a maximum voltage of 25 kV. Current, voltage and pulse width were monitored by a TDS 220 oscilloscope (Tektronix, Beaverton, USA). Discharging of the capacitors at the desired voltage was enabled by a tungsten spark gap with adjustable electrode spacing. Peas were treated in a prismatic treatment chamber made of polyoxymethylene with fixed stainless steel electrodes of 10 mm distance and a sample volume of 200 mL. 100 g of swollen peas was filled up with tap water tempered to 20 °C. 667 pulses at an electric field strength of 5 kV  $\text{cm}^{-1}$  and a pulse width of 10–15  $\mu\text{s}$  were applied with a frequency of 2 Hz resulting in a total energy input of 125 kJ  $\text{kg}^{-1}$ . The temperature increase

detected after treatment was 11.8 K at maximum. Peas remained in the treatment chamber and medium for a total incubation time of 10 min.

The parameters for high pressure, heat and pulsed electric field treatments were selected by means of preliminary experiments. Swollen peas incubated for 10 min in tap water of 20 °C in a ratio of 1:1 (w/v) served as control sample.

The peas were separated from medium with a sieve after the respective treatments. They were dried manually with paper tissue and delivered to further analyses. Treatment media were frozen and kept at  $-20$  °C until analyses. All treatments were performed at least in duplicate.

### 2.3. Characterization of pea tissue

#### 2.3.1. Microscopy of tissue sections

Tissue sections of untreated and treated peas were analysed under the microscope to illustrate treatment effects on a cellular level. Histological sections were cut and microscopied at the Zentraleinrichtung Elektronenmikroskopie (ZELMI) of the TU Berlin. Cutting thickness of the sections was 10  $\mu\text{m}$ . The cell walls were stained with toluidine blue for an improved visualization. Embedding of pea tissue in cold-curing resin (Technovit 7100—the sliceable, Heraeus Kulzer GmbH & Co KG, Wehrheim Germany) was performed to prepare uniform and thin specimens. All fixation, dehydration and embedding steps were performed in polyethylene embedding moulds (Histoform S) according to the enclosed manual.

#### 2.3.2. Specific volume

The specific volume of the peas after drying and rehydration was determined via seed displacement according to Street (1991). Rapeseed was chosen for filling empty spaces between the peas due to its small particle size and its homogeneous bulk density. Measurements were performed in duplicate.

#### 2.3.3. Germination ability

The treatment impact on the germination ability was evaluated by placing 10 randomly chosen peas on water agar (1% (w/v), Oxoid, Hampshire, England) for 48 h and assessing the germination ability by visual criteria. Each sample was analysed in triplicate.

#### 2.3.4. Moisture content

Moisture content of the dry, swollen and rehydrated samples was quantified via oven drying and subsequent differential weighing. 1 to 2 g of peas was dried in glass bowls for 48 h ( $\pm 4$  h) at 105 °C in a drying oven (Heraeus Instruments UT6060, Hanau, Germany). The weight changes were determined after 30 min of cooling in an exsiccator, filled with silica gel as drying agent (Carl Roth GmbH & Co KG, Karlsruhe, Germany). Samples were analysed in triplicate.

## 2.4. Characterization of moisture transport

### 2.4.1. Drying rate

The drying rate of pea tissue was detected with a moisture analyser (Sartorius MA35, Sartorius AG, Göttingen, Germany). 5 randomly selected peas (approximately 2.5 g) were dried at 50 °C for 180 min. Mass changes were recorded by a connected PC and the software Termite (CompuPhase Automatisering, Bussum, Netherlands). Re-starting the drying process after 90 min was necessary due to the running time limitation of the moisture analyser. The moisture content was given as  $m_M/m_{DW}$  where  $m_M$  and  $m_{DW}$  are the masses of moisture and dry weight in g, respectively. The drying rate at time  $t$  was calculated as  $\Delta_{t_2-t_1}(m_M/m_{DW})/(t_2-t_1)$  with  $t_1 = t_2 - 0.2$  min.

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