Food Research International 77 (2015) 753–761

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Influence of high isostatic pressure on structural and functional characteristics of potato protein

Anne K. Baier *, Dietrich Knorr

TU Berlin, Department of Food Biotechnology and Food Process Engineering, Koenigin-Luise-Str. 22, 14195 Berlin, Germany

ARTICLE INFO

Article history:
Received 19 February 2015
Received in revised form 15 May 2015
Accepted 27 May 2015
Available online 28 May 2015

Keywords:
High isostatic pressure
Potato protein
Protein structure
Solubility
Foaming properties

ABSTRACT

Potato protein possesses promising nutritional and techno-functional properties, but distinct heat sensitivity. Therefore, the potential of high isostatic pressure as an alternative preservation and modification method was investigated. Pressures of 200, 400 and 600 MPa were applied at isothermal conditions of 20 and 40 °C to dispersions made of potato protein concentrate and isolated patatin for dwell times of 10 min. Process induced changes in solubility, foaming properties and selected structural characteristics were compared to results of pure thermal treatments from 20 to 80 °C. Potato protein solubility in neutral solutions made of concentrate was reduced to 21% after heating to 70 and 80 °C whereas it only decreased to 74% after pressurization at 600 MPa. Processing of isolated patatin at pH 6 and pH adjustment from 7 to 6 after processing reduced protein solubility to 12% for heat treatments and to 55 and 89%, respectively, for pressure treatments indicating different denaturation or aggregation mechanisms. Hydrogen bonds and hydrophobic interactions were involved in pressure induced aggregation, whereas aggregates formed during heat treatments were primarily stabilized by hydrophobic interactions. The surface hydrophobicity of soluble protein increased by factor 2.5 to 4.5 after heat treatments and by factor 1.3 at maximum after pressure treatments. High pressure processing provides therefore a good alternative to conventional heat pasteurization as initial potato protein quality may be preserved to a higher extent. Foam stability was increased to 177% by pressure treatments, but this modification was not long-term stable. Applying high pressure with the aim of a functional modification therefore requires further investigations.

1. Introduction

In recent years, high isostatic pressure (HP) came into focus of the food industry as an alternative preservation method for heat sensitive products. Pressures of 300 to 600 MPa can be applied to inactivate yeasts, molds and most of the food pathogen vegetative bacteria (Smelt, 1998). The suitability of HP processing for a shelf-life increase of protein rich products depends on the protein's individual pressure sensitivity. Several research activities focused on pressure effects on food proteins and divergent results were published concerning the degree of structural changes and the influence on protein functionality (Chapleau & de Lamballerie-Anton, 2003; Galazka, Dickinson, & Ledward, 1996, 1999; Ibanoglu & Karatas, 2001; Molina, Papadopoulou, & Ledward, 2001; Pittia, Wilde, Husband, & Clark, 1996; Torrezan, Tham, Bell, Frazier, & Cristianini, 2007 among others).

The volume of a protein is given by the sum of the constitutive volume of its atoms, the volume of void spaces due to imperfect packaging of the amino acid residues and the volume decrease resulting from hydration of peptide bonds and charged amino acid residues (Kauzmann, 1959). A rise in pressure will reduce the volume of a system by chemical reaction or closer packaging of the molecules according to the principle of le Chatelier (Heremans, 1982). Almost all globular proteins show a positive compressibility indicating the presence of internal cavities, but no correlation between the protein's compressibility and their pressure sensitivity was found (Gekko & Hasegawa, 1986). Accordingly, a protein's behavior under pressure can hardly be derived from known structural characteristics and therefore requires individual testing.

Food proteins from vegetable sources are becoming increasingly important due to economic, ecological and health aspects. Considerable amounts of protein occur as a by-product of the potato starch industry and its recovery would fulfill the requirements for an efficient and sustainable usage of the entire raw material. Proteins present in potato fruit juice can be divided into patatin, protease inhibitors and other proteins, including lectins and several endogenous enzymes. Patatin, a glycoprotein with an isoelectric point between 4.5 and 5.2, accounts for 38% of the protein present in potato fruit juice (Alting, Pouvreau, Guiseppin, & van Nieuwenhuijzen, 2011). It usually appears as a dimer that dissociates into monomers with a molecular weight of 43 kDa in the presence of SDS (Racusen & Weller, 1984). The heterogeneous group of protease inhibitors forms the majority of the protein and consists of several subgroups with different molecular sizes (Alting et al., 2011). Promising

* Corresponding author.
E-mail address: anne.k.baier@tu-berlin.de (A.K. Baier).

http://dx.doi.org/10.1016/j.foodres.2015.05.053
0963-9969/

nutritional and functional quality has been ascribed to native potato protein (Holm & Eriksen, 1980; Kapoor, Desborough, & Li, 1975; Ralet & Gueguen, 2001) but common processing includes a combined heat and acid treatment that limits its use to animal feeding (Knorr, Kohler, & Betschart, 1977; Wojnowska, Poznanski, & Bednarski, 1981). Membrane processing and adsorption techniques delivered products of satisfying quality (Bisschops & Giuseppin, 2008; Giuseppin, Van Der Sluis, Laus, & Laus, 2008; Wojnowska et al., 1981) but problems may arise from the absent inactivation of microorganisms and enzymes. Pasteurization with high isostatic pressure may be an option for processing high quality potato protein and may thereby contribute to an increased usage of this eco-friendly protein by the food industry and a more holistic process development and product utilization in starch manufacturing.

The aim of this study was to investigate the influence of different pressure–temperature combinations on potato protein structure and functionality. Pure thermal treatments were performed to compare induced changes and to evaluate the potential of high pressure as an alternative preservation or modification method.

2. Material and methods

2.1. Material

Potato protein concentrate was recovered from fresh juice of potatoes, cultivar Belana. The juice was centrifuged at 10,000 *g* and 20 °C for 10 min to remove remaining starch granules and concentrated in an ultrafiltration unit (Proscale, Merck Millipore, Darmstadt, Germany) with a modified polyether sulfone membrane (Pellicon 2, 10 kDa cut off, Biomax membrane cassette, Merck Millipore, Darmstadt, Germany) at a maximum feeding pressure of 6 bar. The concentrated juice was dried in a laboratory spray drier (Mini Spray Dryer B-191, Büchi Labortechnik GmbH, Essen, Germany) at a maximum drying temperature of 80 °C. The concentrate possessed a protein concentration of 0.61 g/g dry weight and a moisture content of 10%. Commercially available potato protein isolate (SOLANIC 206P, Mat: 102425667, Batch: 145498) was composed of protein fractions with molecular weights higher than 35 kDa, mostly of patatin. The protein content of the isolate was at least 0.92 g/g dry weight, the ash content was below 0.05 g/g dry weight and the moisture content was between 6 and 8%. All protein products were vacuum-packed and stored at temperatures between 2 and 6 °C until use.

2.2. High pressure and heat treatments

Initial solutions were prepared by dissolving the protein samples in tap water tempered to 20 ± 2 °C to reach a final protein concentration of 1% (w/w). The dispersions were continuously stirred for 30 min on a magnetic stirrer. The pH value was adjusted to pH 7 or 6 with 0.1 M hydrochloric acid or 0.1 M sodium hydroxide. The dispersions were centrifuged for 10 min at 10,000 *g* and 20 °C (F14-6x250y, Sorvall RC 6+, Thermo Fisher Scientific, Waltham, Massachusetts, USA) before treatments to remove possible insoluble contaminants. Soluble solids were analyzed at 20 °C in a digital refractometer (PFM 80, Bellingham and Stanley, Turnbridge Wells, UK). Results were given in °Brix corresponding to % of soluble solids.

Treatment parameters were chosen within the pressure and temperature range commonly used for pasteurization of liquid products. A constant dwell time of 10 min was applied either for HP as for heat 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, Institute of High Pressure Physics, Warsaw, Poland). Samples were double-packed in bags made of pressure-stable foil (Whirl Pak, Nasco, Fort Atkinson, Wisconsin, USA) and vacuum sealed (PlusVac 23, Komet Maschinenfabrik GmbH, Plochingen, Germany). 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 20 or 40 °C before experiments with a connected water bath (DC10-K20, Thermo Haake GmbH, Karlsruhe, Germany) to avoid temperature inhomogeneities during treatment. Samples were given into the high pressure unit directly before the treatment and subjected to pressures of 200, 400 or 600 MPa for dwell times of 10 min. Initial temperatures of sample and pressure transmitting medium necessary to achieve isothermal treatments at 20 or 40 °C were figured out by recording temperature profiles with a Thermo-Egg (Knoerzer et al., 2010, see Table 1).

For heat treatments, a self-constructed capillary heating system was used. Samples were transported by a peristaltic pump (SCI Q 323, Watson Marlow, Falmouth, UK) with a flow rate of 1.25 mL/s. Liquid was pumped through two metal heater spirals whereof the first was placed in a water bath (Polystat cc3, Huber GmbH, Offenburg, Germany) adjusted to treatment temperature and the second one in an oil bath filled with silicon oil (M40.165.10, Huber GmbH, Offenburg, Germany). Settings of the oil bath necessary to obtain the target temperature were determined empirically. Samples were filled into test tubes after passing the second heating element, incubated in the water bath at treatment temperature and rapidly cooled after dwell time in an ice bath.

All HP and heat treatments were performed at least in duplicate.

2.3. Protein solubility

Samples were centrifuged for 5 min at 10,000 *g* at ambient temperature (Biofuge pico, Heraeus, Osterode, Germany) and the protein content in the supernatant was determined via Biuret reaction. 200 μL of adequate diluted sample was mixed with 800 μL of Biuret reagent containing 9.4 mM copper sulfate (Merck KG, Darmstadt, Germany), 28.56 mM potassium–sodium tartrate (Merck KG, Darmstadt, Germany) and 831 mM sodium hydroxide (Merck KGaA, Darmstadt, Germany). The absorbance was measured at a wavelength of 540 nm (Lambda 25 Perkin Elmer, Waltham, Massachusetts) after 20 min of incubation at 37 °C. All samples were analyzed in triplicate. Deionized water was used as blank. A calibration curve ranging from 2 to 10 mg/mL was prepared with bovine serum albumin (Fluka, Buchs, Switzerland) in deionized water.

2.4. Dynamic light scattering

Samples were characterized using the Zetasizer Nano ZS (Malvern Instruments GmbH, Herrenberg, Germany) and the corresponding software. Samples were centrifuged for 5 min at 500 *g* at ambient temperature to remove large particles that undergo sedimentation during measurement. The supernatant was analyzed in semi-micro cuvettes (VWR International Ltd., Darmstadt, Germany) at a temperature of 25 °C. All samples were analyzed at least in triplicate.

2.5. Aggregate characterization

The analytical procedure was adapted from solubility tests for whey protein gels (Schmitt et al., 2010) and extruded meat analogues (Liu & Hsieh, 2008). 100 mM phosphate citrate buffer pH 7 was supplemented with 8 M urea (Merck KG, Darmstadt, Germany), 3% SDS (w/v, Carl Roth

Table 1
Sample temperatures and start temperatures in the pressure-transmitting medium before pressure build-up necessary to achieve isothermal dwell times at 20 or 40 °C.

Treatment temperature in °C	Treatment pressure in MPa	Sample temperature in °C	Start temperature in °C
20	200	6.0	18
	400	6.0	12
40	200	27.5	38
	400	20.0	32
	600	20.0	25