LWT - Food Science and Technology 65 (2016) 197–205

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Investigation and optimization of a novel enzymatic approach for the isolation of proteins from potato pulp

Amanda Waglay, Salwa Karboune*, Maryam Khodadadi

Department of Food Science and Agricultural Chemistry, McGill University, 21111 Lakeshore, Ste. Anne de Bellevue, Quebec, H9X 3V9, Canada

ARTICLE INFO

Article history:
Received 20 April 2015
Received in revised form
18 July 2015
Accepted 27 July 2015
Available online 29 July 2015

Keywords:
Potato pulp
Enzymatic isolation
Patatin
Protease inhibitors
Optimization

ABSTRACT

Potato proteins have been associated with several functional and beneficial properties. A novel enzymatic approach, based on the use of combination of polysaccharide degrading biocatalysts, was investigated for the isolation of non-denatured proteins from potato pulp. The removal of starch was found to be essential for the effectiveness of the enzymatic approach, as protein recovery increased from 47% to 63–75% with starch removal. Contrary to endo-arabinase (Arase), endo-1,4-β-galactanase (GLase) and polygalacturonase MI (PGase) improved the protein recovery. A 5-level, 5-variable central composite rotatable design was performed using as independent variables temperature (°C; 30.0, 33.8, 37.5, 41.3, 45.0), time (h; 1.50, 9.00, 16.5, 24.0, 31.5), potato pulp concentration (kg/m^3; 80.0, 110, 140, 170, 200), PGase units (U; 1.5, 11.0, 20.5, 30.0, 39.5), and GLase units (U; 1.5, 11.0, 20.5, 30.0, 39.5); and the responses were protein recovery yield, recovered patatin concentration, and recovered protease inhibitors concentration. For yield and recovered patatin, the most significant interaction was that between temperature and units of PGase with an antagonistic relationship. The most significant interaction for recovered protease inhibitors was found to be that between pulp concentration and temperature, showing a positive correlation. Comparison of predicted and experimental values validated the established predicted models, which can be used to identify the conditions for the isolation of potato proteins with selected composition.

1. Introduction

Globally, potatoes are one of the staple crops, used for human consumption, industrial processing, and/or agricultural regimes (Kamnerdpetch, Weiss, Kasper, & Scheper, 2007). Industrial processing of potatoes generates low value by-products known as potato fruit juice and potato pulp, which contain a crude protein content of 50 g/100 g (Vikelouda & Kiosseoglou, 2004) and 74 g/100 g (dry weight), respectively (Kamnerdpetch et al., 2007). These proteins have been shown to be of high nutritional quality as they contain a high proportion of lysine, an essential amino acid often lacking in vegetable and cereal proteins (Ralet & Guéguen, 2001; van Koningsveld et al., 2001).

Potato proteins are commonly divided into three main fractions namely, patatin, protease inhibitors and high molecular weight proteins (Bártova & Bárta, 2009; Pots, De Jongh, Gruppen, Hamer, & Voragen, 1998; Ralet & Guéguen, 2001). Together, these fractions have been reported to possess several beneficial characteristics such as lower allergenicity (Moreno, 2007), high antioxidative activity (Kudo, Onodera, Takeda, Benkeblia, & Shiomi, 2009; Liu, Han, Lee, Hsu, & Hou, 2003), and abilities to modulate lipid metabolism (Liyanage et al., 2008). Along with these health promoting qualities, potato proteins, more specifically patatin, exhibit foaming and emulsifying abilities. Indeed, potato protein isolates create more stable foams when compared to β-lactoglobulin and β-casein (van Koningsveld et al., 2002). In addition, emulsions formed with potato protein isolates prevail those obtained with soy protein isolates (van Koningsveld et al., 2006).

In terms of structure, patatin is present in a dimer in native form (Ralet & Guéguen, 2000); however, once denatured, it breaks down to its monomer units consisting of a single polypeptide with up to two carbohydrate chains resulting in isoforms with molecular weights ranging from 40 to 45 kDa (Pots, Gruppen, Hessing, van

Abbreviations: Termamyl, α-amylase *Bacillus licheniformis*; GLase, endo-β-1,4galactanase; PGase, polygalacturonase M1; ARase, endo-arabinase; CCRD, central composite rotatable design; RSM, response surface methodology; U, enzymatic units.

* Corresponding author.
E-mail address: salwa.karboune@mcgill.ca (S. Karboune).

http://dx.doi.org/10.1016/j.lwt.2015.07.070
0023-6438/

Boekel, & Voragen, 1999). Contrarily, the protease inhibitors, represent a heterogeneous group, which varies according to their molecular mass, inhibitory activities, and amino acid sequence (Pouvreau et al., 2001).

Until now, the application of potato proteins has been quite limited due to the lack of non-denaturing extraction techniques. Industrially, the conventional extraction technique used is a combination of severe thermal (100 °C) and acidic treatment (pH 4.5–5.0) (Knorr, Kohler, & Betschart, 1977). These conditions lead to the extensive denaturation of the proteins as patatin has been shown to begin unfolding at 45 °C (Waglay, Karboune, & Alli, 2014), whereas the protease inhibitors denature between 55 and 70 °C (van Koningsveld et al., 2001). Therefore, in order to explore the techno-functionalities and the health benefits associated with potato proteins, improvement of the extraction method is required. Investigation of the release of cell wall protein from potato, using several glycosyl-hydrolyzing enzymes, revealed that the degradation of the galacturonide linkages are crucial for the protein recovery (Strand, Rechtoris, & Mussell, 1976). To our knowledge no studies have taken advantage of this subtractive approach to isolate plant proteins with retained functionality for their use as health promoting ingredients.

The present study is aimed at the development of a novel enzymatic approach for the isolation of non-denaturing and highly functional proteins from potato pulp. This approach relies on the degradation of the polymers surrounding the proteins for efficient release of proteins from the cell network with minimal deleterious effects. It will begin by removing starch to allow for easier access of the polysaccharide hydrolyzing biocatalysts, namely polygalacturonase M1 (PGase), endo-β-1,4-galactanase (GLase), and endo-arabinase (ARase) to the plant cell wall components. The effects of reaction parameters (temperature, time, pulp concentration, units of PGase and GLase) on the protein recovery yield and the extracted amount of protease inhibitors and patatin were investigated using response surface methodology (RSM). The relationships between these selected parameters were examined. Preserving the proteins structure and their functional and beneficial effects is expected to increase their application.

2. Materials and methods

2.1. Preparation of potato pulp

Potato pulp was prepared with potatoes of Russet Burbank variety. The potatoes were washed and finely chopped into 500 kg/m^3 samples. The potato pieces were ground with a mortar and pestle for 1 min with 0.25 kg/m^3 sodium metabisulfite. The ground pieces were homogenized using a Warring commercial Blender at low speed for 1 min.

2.2. Starch removal

Two selected α-amylases (Sigma Chemical Co.) from *Bacillus licheniformis* (Termamyl) and *Bacillus* sp. were evaluated for the removal of starch. Dried potato pulp (150 kg/m^3) was consistently weighed and suspended in 2.12 kg/m^3 potassium phosphate buffer at pH 6.5. Selected units of α-amylase were added to the potato pulp suspension to yield 0–2.98 U/mg pulp. Reactions were carried out at 40 °C with constant stirring at 220 rpm for 16 h. The remaining starch was determined using potassium iodide colorimetric method.

2.3. Enzymatic approach for protein recovery

Destarched potato pulp was (25–160 kg/m^3) suspended in 8.20 kg/m^3 sodium acetate buffer pH 5.0. The enzymatic reactions were initiated by adding ARase (0.1–0.3 U/mg pulp, Megazyme), PGase (0.008–0.5 U/mg pulp, Megazyme) and GLase (0.008–0.5 U/mg pulp, Megazyme) from *Aspergillus niger* to the destarched potato pulp suspension. The reaction mixtures were incubated at 40 °C for selected reaction times of 6–48 h. Variant enzyme/pulp ratios (0.008–0.5 U/mg potato pulp) and pulp concentration (28.6–200.0 kg potato pulp/m^3) generated by RSM, were investigated. After incubation, the reaction mixtures were vacuum filtered (1.2 μm) and the supernatant containing proteins were recovered. The protein content of the recovered pulp after enzymatic treatment and supernatant were determined using Leco® TruSpec N (Leco Corporation, St-Joseph, Michigan). Two blanks, without potato pulp or without enzymes, were conducted in tandem of the trials. All assays were run in duplicate. The protein yield was estimated as the recovered proteins in the supernatant over the initial proteins present in potato pulp, multiplied by 100.

2.4. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Protein extracts were analyzed by SDS-PAGE according to the method of Laemmli (1970) using 50 and 150 kg/m^3 acrylamide content in the stacking and resolving gels, respectively. Sample loading was achieved in a mini protein gel apparatus (Bio-Rad) with a 1.5 mm-thick gel. The electrophoresis was conducted at a constant voltage of 120 mV. The analyses of the electrophoretic patterns to obtain the protein profiles were carried out using Red Imaging system equipped with Alpha-View SA Software. The recovered patatin and protease inhibitors are expressed as g of extracted patatin or protease inhibitor per g of initial pulp protein and were calculated according to the following calculation:

$$\text{g patatin/g pulp protein} = ((\text{g recovered potato proteins}) * (\text{relative proportion patatin}/100))/\text{g pulp} \quad (1)$$

2.5. Structural characterization of protein extracts using fluorescence spectroscopy

The fluorescence spectra of protein extracts were recorded using a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon system). The spectra were recorded at pH 4 and 9 while varying temperatures from 25 to 85 °C with a 5 °C interval increase. Excitation was at 295 nm and the resulting emission was measured at 305–450 nm with a scan speed of 120 nm/min. Both the excitation and emission slits were set at 3.5 nm. Protein extracts were prepared using a conventional thermal/acidic (pH 4.8) extraction method and enzymatic extraction method (150 kg pulp/m^3, 0.994 U/mg pulp Termamyl, 5.56 U/g pulp each of PGase and GLase). Following enzymatic hydrolysis, protein extracts were dialyzed for 48 h against Millipore water using a 2000 Da molecular weight benzoylated dialysis tubing to remove any interfering sugars.

2.6. Experimental design

Optimization of the protein yield (%), the recovered patatin (g per g of potato pulp protein), and the recovered protease inhibitors (g per g of potato pulp protein) was investigated using response surface methodology (RSM). A five-level, five variable central composite rotatable design (CCRD) was used. The five independent variables with their corresponding levels consisted of x_1 temperature (30.0, 33.8, 37.5, 41.3, 45.0 °C), x_2 time (1.5, 9.0, 16.5, 24.0, 31.5 h), x_3 pulp concentration (80.0, 110, 140, 170, 200 kg/m^3), x_4