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A novel enzymatic approach based on the use of multi-enzymatic systems for the recovery of enriched protein extracts from potato pulp

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ABSTRACT

Ten commercial available multi-enzymatic systems have been explored for the efficient recovery of patatin and protease inhibitors from potato pulp. Their enzyme activity profile was characterized, where corresponding enzyme activity profile led to similar protein recovery yield. Of those assessed, Depol 670L (DEP) and Ceremix 2XL (CER) were efficient for the recovery of protein extract enriched with patatin (up to 60.0%) and protease inhibitors (up to 72.0%), respectively. The efficiency of DEP was significantly dependent on the interactive effect of enzymatic units and incubation time (p-value <0.015), while CER was found to be affected by the quadratic effect of both variables (p-value <0.022–0.052). The enriched patatin DEP-based protein extract possessed higher lipid acyl hydrolase activity emphasizing its preservation. The enriched protease inhibitors CER-based protein extract resulted in higher trypsin inhibiting activity, when compared to the industrial method. DEP and CER-based protein extracts were structurally characterized by peptide mass mapping.

1. Introduction

Potato crops are the second highest protein-providing crop per hectare grown (0.3 kg protein/ha) when compared to wheat (0.69 kg protein/ha), rice, and corn. Potato proteins are of great interest as food ingredients due to their high nutritional quality (Bártova & Bárta, 2009), their antioxidant potential (Kudo, Onodera, Takeda, Benkeblia, & Shiomi, 2009), and their techno-functional properties (Creusot, Wierenga, Laus, Giuseppin, & Gruppen, 2010). The potato processing industry releases two by-products rich in proteins namely, potato fruit juice (PFJ) and potato pulp, in large quantities. Recovering proteins from these by-products constitutes a great avenue as they are coupled with high polluting capacity (Waglay, Karboune, & Alli, 2014).

Potato proteins comprise three main fractions, namely, patatin, protease inhibitors, and high-molecular-weight proteins (Van Koningsveld et al., 2006). Patatin is the major storage protein found in the tuber, which represents approximately 40% of the protein. It is known as a glycoprotein, studied as a homogeneous group of isoforms possessing molecular weight ranging from 39 to 45 kDa (Pots, Gruppen, Hessing, van Boekel, & Voragen, 1999). The use of patatin as a food ingredient is of great interest due to its nutritional quality, which is similar to egg protein (Ralet & Guéguen, 2001), its antioxidative activity (Liu, Han, Lee, Hsu, & Hou, 2003), its anti-proliferative activity against mouse melanoma B16 (Sun, Jiang, & Wei, 2013), and its lower allergenicity (Koppelman et al., 2002). In addition, patatin offers many techno-functional properties, such as foam forming, gelling and emulsifying properties (Creusot et al., 2010; Ralet & Guéguen, 2001; Van Koningsveld et al., 2006). These properties are primarily governed by patatin's low denaturation temperature of 50–55 °C (Pots, De Jongh, Gruppen, Hamer, & Voragen, 1998). Therefore, mild reaction conditions are necessary to preserve patatin and take advantage of its functional and beneficial properties.

On the other hand, the protease inhibitors are a heterogenous group varying according to their molecular mass ranging from 5 to 25 kDa, amino acid composition, and their inhibitory activity (Pouvreau et al., 2001). Six main groups of protease inhibitors have been identified, including potato inhibitor I, potato inhibitor II, potato aspartate protease inhibitors, potato cysteine protease inhibitor, potato Kunitz-type protease inhibitor, and potato carboxypeptidase inhibitor (Pouvreau et al., 2001). Each group has been shown to possess several beneficial properties, such as the protease inhibitor I and II, potato cysteine inhibitors, and Kunitz-type have been shown to possess anti-microbial activity (Kim et al., 2005, 2013). Contrarily, the potato carboxypeptidase and potato cysteine inhibitors have been shown to inhibit tumor cell growth and decrease reactive oxygen species, respectively (Blanco-Aparicio et al., 1998). Unlike patatin, protease inhibitors exhibit higher denaturating temperatures of 55–70 °C and form unstable foams and emulsions (van Koningsveld et al., 2001).

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In our previous (Waglay et al., 2014) and other studies (Bártova & Bárta, 2009; Knorr, Kohler, & Betschart, 1977; van Koningsveld et al., 2001), the efficiency of selected extraction techniques to isolate potato proteins has been studied and compared. Thermal/acidic precipitation as the industrially employed technique resulted in high yields (>85%), with limited applications due to loss of functionalities (Knorr et al., 1977). Ferric chloride has been shown to have a strong affinity towards potato proteins, resulting in protein recovery yields ranging from 75.2 to 86.5% (Bártova & Bárta, 2009; Waglay et al., 2014). Ammonium sulfate saturation resulted in a positive concentration-dependent yield recovery, with a corresponding negative impact on purification factor (Waglay et al., 2014). Complexation with carboxymethylcellulose led to the recovery of protein extracts with enhanced functionality (Vikelouda & Kiosseoglou, 2004). However, limited patatin extraction was obtained, due to the strong pH adjustment necessary for protein–polysaccharide interaction (Waglay et al., 2014).

A novel enzymatic approach was proven to be efficient for the isolation of proteins with retained functionality from potato pulp (Waglay, Karboune, & Khodadadi, 2015). This two-step enzymatic approach begins by removing starch using the amylase Termamyl, followed by degrading the cell wall pectin network by glycosyl-hydrolases for an efficient recovery of proteins. Starch removal was found to allow for easier access of the polysaccharide-hydrolyzing enzymes to plant cell wall components. The combined use of endo-1,4-β-galactanase and endo-polygalacturonase MI as mono-component biocatalysts improved the potato protein recovery, while endo-arabinanase did not contribute significantly to this recovery. In order to broaden this enzymatic approach and make it more industrially appealing, the use of multi-enzymatic products, which contain several glycosyl-hydrolases activities, has been explored. The efficiency of ten multi-enzymatic systems, namely, Gamanase 1.5L (GAMase), Depol 670L (DEP), Ceremix 2XL (CER), Hemicellulase CE-1500 (HEMase), Iogen HS 70 (IOG), Viscozyme (VIS), Pectinex Ultra SPL (PEC), Newlase II (NEWase), Diazyme L-200 (DIA), and Laminex DG (LAM) to recover the proteins was assessed using potato pulp as a starting material. The effect of the enzyme activity profile of the multi-enzymatic systems on the protein recovery yield, the extracted amount of patatin, and protease inhibitors was discussed. It is hypothesized that multi-enzymatic systems with the same enzyme activity profile can lead to similar protein recovery efficiency. Using DEP and CER-based recovery processes, the effects of endo-β-1,4-galactanase unit level and incubation time on the protein recovery and fractionation were further investigated using response surface methodology (RSM). RSM is a useful empirical modelling method, taking into account mathematical and statistical techniques. The objective is to establish the relationship between a response and different variables to yield an optimum level for the best system performance (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008). RSM has been used extensively in the literature, such as by Zhu, Han, and Chen (2010), where they determined the optimum levels of different variables to optimize the microwave-assisted extraction of astaxanthin from *Phaffia rhodozyma*, and Bang, Kim, and Kim (2016) optimized the small-scale Lecitase Ultra-catalyzed hydrolysis of phosphatidylcholine. The improved biocatalytic approach is expected to preserve the structure of potato proteins and their functional and beneficial effects, hence increasing their applications.

2. Materials and methods

2.1. Materials

Fresh potatoes of Russet Burbank variety were purchased from a local supermarket. Sodium metabisulfite, sulfuric acid (H_2SO_4), trifluoroacetic acid, hydrochloric acid were purchased from Sigma Chemical Co. (St Louis, MO). Bradford reagent and SDS-PAGE Broad Molecular Weight standard were obtained from Bio-Rad (Hercules, CA). Bovine serum albumen (BSA), Tris base and potassium phosphate dibasic were acquired from Fisher Scientific (Fairlawn, NJ). Potassium phosphate monobasic was purchased from MP Biomedicals, LLC (Solon, OH). GAMase from *Aspergillus niger* and CER from *Bacillus* spp. were obtained from Novo Nordisk Bioindustrial (Bagsvaerd, Denmark), while DEP from *Trichoderma reesei*, was provided from Biocatalyst Limited (Cardiff, UK). VIS and PEC from *Aspergillus aculeatus* were obtained from Novozymes Canada (Ottawa, Canada), HEMase and LAM from *Trichoderma reesei* were obtained from Genencor International (Palo Alto, CA). IOG from *Rhizopus niveus* was obtained from Iogen Bio-Products, while NEWase from *R. niveus* was provided by Amano Enzyme (Chipping Norton, UK); DIA from *A. niger* was obtained from Solvay Enzyme Products Incorporated. Potato protein extract enriched with patatin was provided by Solanic (Veendam, The Netherlands).

2.2. Preparation of potato pulp

Potato pulp was prepared with potatoes of Russet Burbank variety. The potatoes were washed and finely chopped into 0.5 g/mL samples. The potato pieces were ground with a mortar and pestle for 1 min with 1.315 mM sodium metabisulfite. The ground pieces were homogenized using a Waring commercial blender on low speed for 1 min.

2.3. Characterization of enzyme activity profile of multi-enzymatic system

2.3.1. Proteolytic activity assay

The presence of proteolytic activity in the multi-enzymatic systems was assessed using potato proteins as substrate. Potato protein extracts (1%, *w/v*) in potassium phosphate buffer (50 mM, pH 6.5) were added to the multi-enzymatic systems at appropriate dilutions to yield a final concentration of 0.5% (*w/v*). Reaction mixtures were incubated for 20 min at 40 °C. A blank substrate and blank enzyme were run in parallel. The released amino acids were quantified using ninhydrin assay. Reaction mixture (200 μL) were mixed with 100 μL of 10 mM sodium cyanide suspended in 2.65 M sodium acetate buffer at pH 5.4 in a ratio 1:49 (*v/v*) and 100 μL of 3% (*w/v*) ninhydrin solution in 2-methoxyethanol. The ninhydrin reaction mixture was incubated for 15 min at 100 °C. After cooling to room temperature with the addition of 1 mL *n*-propanol diluted with distilled water (1:1 *v/v*), the absorbance of the mixture was measured at 570 nm. Standard curve was developed using leucine as a free α-amino group.

2.3.2. Glycosyl-hydrolase activity assay

Selected multi-enzymatic systems were assessed for their levels of endo-polygalacturonase, endo-1,4-β-galactanase, rhamnogalacturonase, and endo-α-1,5-arabinanase activities, using orange polygalacturonic acid, potato galactan, soybean rhamnogalacturonan, and sugar beet arabinan as substrates, respectively. The reaction was initiated by adding 25 μL of multi-enzymatic system at appropriate dilutions to 0.475 mL of substrate solution (0.25%, *w/v*, for polygalacturonic acid and 0.55%, *w/v*, for galactan, rhamnogalacturonan, and arabinan) in 50 mM sodium acetate buffer at pH 5.0. Standard dinitrosalicylate methods were used to quantify the selected enzymatic activities. Absorbance of the mixture was measured at 540 nm. Standard curves were constructed using galacturonic acid, galactose, rhamnose, and arabinose as standard. One unit of activity was defined as the amount of enzyme which releases one μmol of the corresponding glycoside end per min of