PUBLICATIONS

Waglay, A., Karboune, S., & Alli, I. (2014). Potato protein isolates: Recovery and characterization of their properties. *Food Chemistry, 142*, 373-382.

Waglay, A., & Karboune, S. (2015). Potato Proteins. In L. Kaur, & J. Singh. *Advances in Potato Chemistry*. Maine, USA: Elsevier Inc. *(Accepted*)

Waglay, A., Karboune, S., & Khodadadi, M. (2015). Investigation and Optimization of a Novel Enzymatic Approach for the Isolation of Proteins from Potato Pulp. *LWT- Food Science and Technology*. (*Accepted*)

Waglay, A. & Karboune, S. (2015). A Novel Enzymatic Approach Based on the Use of Multi-Enzymatic Systems for the Recovery of Enriched Patatin and Protease Inhibitors Extracts from Potato Pulp. *Food Chemistry* (*To be submitted*)

Waglay, A., Achouri, A., Zareifard, M. R., & L'Hocine, L. (2015). Pilot Plant Extraction of Potato Proteins and their Structural and Functional Properties. *Food Research International* (*Submitted*)

Waglay, A. & Karboune, S. (2015). Formulation of a Functional Cookie Enhanced with Potato Protein Isolates: Textural and Sensory Evaluations. *Journal of Agricultural and Food Chemistry*. (*To be submitted*)

Waglay, A. & Karboune, S. (2015). Enzymatic Generation of Selected Peptides from Potato Protein Isolates and Characterization of their Structural Properties. *Biotechnology Progress*. (*Submitted*)

CONFERENCE PRESENTATIONS

Waglay, A., Karboune, S, and Alli, I. (2012). Investigation of Selected Approaches for the Extraction of Potato Proteins and Characterization of their Functional Properties. IFT12 Annual Meeting. Las Vegas, Nevada, USA. June 25-28, 2012.

Waglay, A., Khodadadi, M., & Karboune, S. (2013) Investigation and Optimization of a Novel Enzymatic Approach for the Extraction of Protein from Potato Pulp. IFT13 Annual Meeting, Chicago, USA, July 13- July 17, 2013.

Waglay, A. & Karboune, S. (2014) A Novel Enzymatic Approach based on the Use of Multi-Enzymatic Systems for the Recovery of Enriched Patatin and Protease Inhibitors Extracts from Potato Pulp. IFT14 Annual Meeting & Food Expo, New Orleans, USA, June 21- June 24, 2014.

Waglay, A., Achouri, A., Karboune, S., Zareifard, M. R., & L'Hocine, L. (2015) Pilot Plant Extraction of Potato Proteins and their Application as a Value Added Ingredient. ICEF12, Quebec, CA, June 14- June 18, 2015.

Waglay, A. & Karboune, S. (2015). Formulation of a Functional Cookie Enhanced with Potato Protein Isolates: Textural and Sensory Evaluation. IFT15 Annual Meeting & Food Expo, Chicago, USA, July 11- July 14, 2015.

PATENT

Process for obtaining protein-enriched preparations from potato. International Patent Application. PCT/CA2015/050510.

SCHOLARSHIPS AND AWARDS

Schulich Graduate Fellowship

Macdonald Class of '44 Rowles Graduate Bursary

Graduate Excellence Fellowship

Provost Graduate Fellowship

GRADUATE AND POSTDOCTORAL STUDIES

MCGILL UNIVERSITY

FINAL ORAL EXAMINATION

FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

OF

AMANDA WAGLAY

DEPARTMENT OF FOOD SCIENCE AND AGRICULTURAL CHEMISTRY

POTATO PROTEINS: ISOLATION, CHARACTERIZATION, AND THEIR APPLICATION FOR THE ENZYMATIC GENERATION OF PEPTIDES AND FOR THE DEVELOPMENT OF A REDUCED-GLUTEN COOKIE

DATE: Thursday, August 6th, 2015
TIME: 9:15 AM

MACDONALD STEWART BUILDING, ROOM MS2-022
McGill University, Macdonald Campus

COMMITTEE:

Dr. J. Aldridge	(Pro-Dean, Institute of Parasitology)
Dr. V. Yaylayan	(Chair, Department of Food Science)
Dr. S. Karboune	(Supervisor, Department of Food Science)
Dr H. Ramaswamy	(Internal Examiner¸ Department of Food Science)
Dr. I. Alli	(Internal Member, Department of Food Science)
Dr. V. Orsat	(External Member, Department of Bioresource Engineering)

Dr. Martin Kreiswirth, Dean of Graduate and Postdoctoral Studies
Members of the Faculty and Graduate Students
are invited to attend

ABSTRACT

Potato proteins were isolated from imitation by-products potato fruit juice (PFJ) and potato pulp, using several conventional extraction techniques namely: thermal/acidic combination, acidic precipitation, salt precipitation, ethanol precipitation, ammonium sulphate ($(NH_4)_2SO_4$) saturation, carboxymethyl cellulose complexation and a novel enzymatic approach. Of the conventional methods assessed $(NH_4)_2SO_4$ precipitation resulted in the highest protein recovery yield of 98.6%, with isolates enriched in non-denatured patatin, the main storage protein found in potatoes (*Solanum tuberosum*) as expressed by best preservation of its lipid hydrolase activity. The novel enzymatic approach began with the use of pure enzymes, specifically endo-polygalacturonase M1(PGase) and endo-β-1,4-galactanase, for the opening of the pectin network and the isolation of potato proteins with a recovery yield of 73.9%. The protein recovery yield and recovered patatin was found to be antagonistically affected by temperature and units of PGase whereas the recovered protease inhibitors was governed by pulp concentration and temperature. Similar high yields, were achieved with a more industrial appealing approach using multi-enzymatic systems. Of those assessed, Depol 670L (DEP) and Ceremix 2XL (CER) were examined and optimized for their ability to recover protein extract enriched with patatin (up to 60.0%) and protease inhibitor recovery (up to 72.0%), respectively. DEP-based enriched patatin extracts (0.51 μmol/(min. mg patatin)) greatly preserved the lipid acyl hydrolase activity, when compared to the employed industrial extraction thermal/ acidic (<0.04 μmol/(min. mg patatin)). Whereas CER-based enriched protease inhibitor extracts (834.4 mg protease/g extract) resulted in higher preservation of trypsin inhibition when compared to the industrial technique (363.5 mg protease/g extract).

These extraction techniques were further scaled-up in a pilot plant facility, to compare their efficiencies and extracts recovered, in terms of thermal denaturation, protein profile, and functionalities. Two of these processes were based on the isolation of potato proteins from PFJ by (1) subsequent-ultrafiltration steps or (2) 60% $(NH_4)_2SO_4$ saturation. The other process was performed on potato pulp, (3) involving the use of a multi-enzymatic system (Depol 670L) to degrade the plant cell wall releasing the proteins. The need for an extracting agent was deemed necessary as the protein recovery increased from 3.79 g/Kg potato (ultrafiltration) to 7.27 and 5.63 g/Kg for $(NH_4)_2SO_4$ precipitation and the use of DEP, respectively. The DEP-based protein concentrate had improved functionality in terms of emulsifying activity index and foam expansion.

Potato protein isolate was used as a starting material to generate peptides using four selected proteases namely, Novo Pro-D, Alcalase, Flavourzyme, and Papain. The processes were evaluated in terms of the degree of hydrolysis and end-product profile. Both, Flavourzyme and Papain exhibited a high catalytic efficiency for the hydrolysis of potato proteins, due to their dual catalytic nature possessing both endo-protease and exo-peptidase activity. This dual nature was found to greatly impact end-product generation, and the presence of the protease inhibitors present in the isolate was found to have an inhibiting effect on both endo- and exo-modes. Peptide mapping was used for the identification of the generated peptides as well as for the determination of their parent potato protein fraction. Flavourzyme-based hydrolysates, contained more known exclusive unique peptides common to patatin, whereas Papain-based hydrolysates were more specific to the protease inhibitors.

Potato protein concentrate and isolate extracted by ultrafiltration (PPC) and 60% $(NH_4)_2SO_4$ precipitation (PPI) were further incorporated into a reduced gluten cookie. PPI resulted in cookies which were more texturally preferred by 25 semi-trained panelists as well as had higher fracturability, when compared to cookies prepared with PPC. A central composite rotatable design was performed with 2-variables namely, rice flour proportion and protein enrichment and 5-levels. The design was evaluated by 70 semi-trained panelists on a 5-point hedonic scale for mean quantitative descriptive scores and mean liking based scores. The attributes, which were found to be statistically relevant, were crispness, aftertaste and overall liking. Consumers generally preferred the cookie control made of wheat flour alone; however, the formulation composed of 67.88% rice flour/ 32.12% wheat flour with 7.20% potato protein isolate enrichment resulted in acceptable reduced-gluten cookies based on desirability plots, generated from mean liking scores. The instrumental textural analyses showed that cookies enriched with PPI were harder and more flexible; this was well correlated with the consumer's preference for the desirable crispness and adhesiveness, when compared to control rice flour.