Development of protein hydrolysates from green lentil flour using enzymatic and/or fermentation processes (WP1)

Quiroga, E. ; Gibon, E. ; Brans, A. ; Vandevenne, M. ; Matagne, A.
Centre d'Ingénierie des protéinés, Faculté de Sciences, Liège Université

Abstract

Bioactive peptides (BP) has been a novel source of molecules capable to modulate the body in response to disease or other ailments. This has increased the interest in their research to give alternatives to the pharmaceutical-dependant patients.
To find these peptides, protein from a known source, usually from food products, is extracted and then hydrolysed to obtain a mix of possible BP's. Our current focus is to obtain these hydrolysates from green lentil flour, a novel protein source. At the moment, we are working to improve the extraction procedure to increase the available protein for the hydrolysis process, which as well needs to be optimized.

Introduction

In the past decade, a noteworthy trend has emerged—the growing awareness among consumers of the influence of diet on health. This has spurred a surge in natural product and supplement demand for a new sense of wellness.
Bioactive Peptides (BP's), chains of amino acids derived from protein hydrolysis, have gained prominence for their health benefits, fuelling a thriving industry focused on using them for preventive and therapeutic purposes.
Selecting the right protein source is pivotal in uncovering novel BP's. While traditional sources include animal and plant proteins, there's a rising interest in non-conventional sources like seafood, food by-products, waste, and insects.
Our current research delves into the potential of green lentil flour as an unconventional protein source. This promises to open new opportunities where the next *"nutraceutical"* can be obtained.

Methods

Commercially available green lentil flour was obtained from Sol Natural (Spain). According to their nutritional values, their protein content in it is 25 g of protein in 100 g of flour. All the reagents utilized in the extraction and hydrolysis are selected if they are safe to use in the food industry.

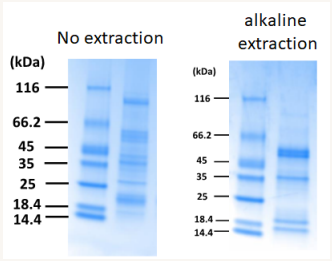
Concept Test

To test the viability of the source, 0.5% (w/w) of the green lentil flour was resuspended in water and left to incubate at 25 °C overnight (16-18 hours). After that, the pH was raised to 11.5 for protein extraction, and the insolubles were discarded after centrifugation at 4000 RCF for 20 minutes. The proteins then were precipitated using Isoelectric precipitation by bringing the pH to 4.5 and centrifugating to 4000 RCF for 20 minutes. The protein pellet was resuspended in Phosphate buffer at pH 7.5.
The protein solution was evaluated using SDS-PAGE and Spectroscopy, and the samples hydrolysed with commercial protease.

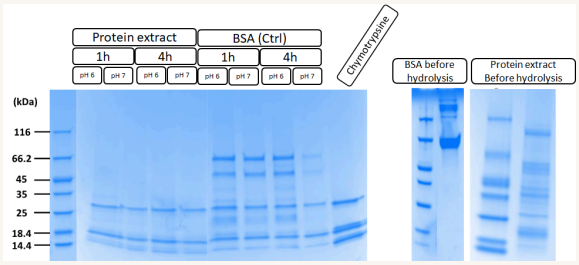
Protein extraction optimization

We evaluate changes in different parts of the process to improve the yield of the extraction. The protocol adjustments were tested using a 10% (w/w) lentil flour sample, altering the duration of incubation, shaking, temperature, buffer, additives, and other steps.

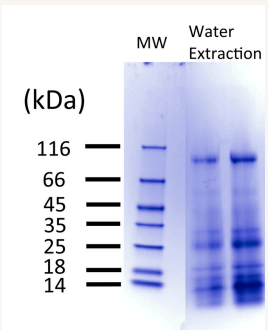
Results



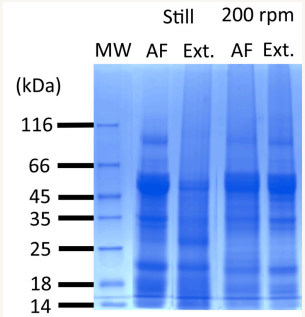
SDS-PAGE of the protein extraction for the proof of concept.
The increase of pH during the extraction caused a lost of protein diversity, in contrast to what has been reported in literature, where the method has recoveries of 20% to 60% depending on the source, in this case we recovered just 0.4%



SDS-PAGE of the protein hydrolysis for the proof of concept.
The hydrolysis was too effective and the protein extract was hydrolysed to fragments below 14 kDa, making them undetectable by the current conditions of the gel. Although the control presents a wider size distribution, the hydrolysis should be optimized for the protein extracted of the green lentil flour to obtain a wider distribution of peptide sizes.



SDS-PAGE for water extraction of 10% flour
The protein extraction was done in water at pH 9, overnight and without agitation. SDS-PAGE done on the fraction precipitated using isoelectric precipitation.
Recovery of 6% of protein



SDS-PAGE for Phosphate Buffer pH 7.5 of 10% flour
The protein extraction was done with 100 mM Sodium phosphate, pH 7.5, overnight at 25 °C. One sample was shake at 200 rpm constantly and the other was without shaking during the incubation phase. AF means Acidic Fraction, and Ext. is Extracted.
Recovery of 6% by the Still Method and 62,7% by the Shaked method

Conclusions

- We need to optimize the extraction protocol to obtain the maximum yield possible. We believe that we can reach higher recovery rates using enzymatic-assisted extractions.
- Hydrolysis requires an optimization to secure a wide distribution of peptide sizes, to increase the changes to find viable BP's

Acknowledgments

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