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## Sequential batches strategy for the enhancement of protein recovery from salmon frames by proteolysis

**VALENCIA P. (1), SULEIVYS N. (1), SILVANA V. (1), CRISTIAN R. (1), MARLENE P. (1), SERGIO A. (1)**

<sup>1</sup> Universidad Técnica Federico Santa María, Valparaíso, Chile

The aim was to test a new operational strategy consisting in sequential batches where the aqueous phase containing the soluble peptides is withdrawn and the remaining solid phase is submitted to a second batch. The hypothesis is sustained in the fact that peptides inhibit the protease action, thus, the protein extraction should increase when they are withdrawn.

The strategy was tested for the hydrolysis of salmon frame proteins by 13 AU subtilisin per kg at 55°C and pH 6.5 (native) during 2 h in a regular batch. Two sequential batches were operated during 1 h each at the same conditions. After 1 h the reaction mixture was centrifuged and the different phases weighted and analyzed for nitrogen content. The solid phase was hydrolyzed in a second batch during 1 h at the same operating conditions.

The nitrogen extraction was 26.6% ± 0.6 after 2 h of hydrolysis in a regular batch operation. Two sequential batches were operated during 1 h each with the same total protease dose (13 AU/kg) distributed as 75/25, 50/50 and 25/75 percentage in the first/second batch. The nitrogen extraction resulted in 42.9% ± 3.9, 45.9% ± 1.7 and 48.7% ± 0.1 for each protease dose distribution, respectively. These results showed that an increase in nitrogen extraction can be achieved without increasing operation time and protease dose. The sequential batches were also tested without the addition of protease in the second batch. The nitrogen extraction was 43.4% ± 1.5, 43.4% ± 3.3 and 35.3% ± 0.3 for protease dose of 75%, 50% and 25%, added to the first batch and without addition in the second batch, respectively. The adsorption of subtilisin was inferred from results as an explanation for the hydrolysis reaction observed in the second batch.

The nitrogen extraction was significantly increased with the sequential batches strategy without increasing the operating time and protease dose compared to a one batch operation. A higher nitrogen extraction was obtained even without addition of protease in the second batch. The sequential batches is a promising strategy to enhance the efficiency of the enzymatic hydrolysis of byproduct proteins.