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Isolation and characterization of biologically active potato proteins and their enzymatic hydrolyzates

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Introduction

About 25 % of cultivated potatoes in Germany are used for industrial starch production. During this process potato fruit juice occurs as a waste fraction which contains nutritionally valuable components though, e.g. approximately 2 % [w/w] of physiologically active proteins for example. Industrial recovery of this fraction is accomplished by heat coagulation and results in denatured low-grade product. It is not suitable for human consumption and therefore placed on market as low value animal feed.

A novel membrane adsorber technology based on the principles of ion exchange chromatography enables the biotechnological processing of potato fruit juice (PFJ). Under gentle conditions the isolation of native and therefore bioactive protein fractions with valuable functional properties succeeds.

A commercialization as high valuable ingredients for nutraceuticals, athlete and baby food, dietary and pharmaceutical products is conceivable.

Further enzymatic hydrolyzation gives information about gastric digestibility and offers the opportunity to generate specific biologically active peptides .

Potato proteins

Potato (Solanum tuberosum) is a major world crop of which more than 300 million tonnes are produced worldwide annually. It is the most important vegetable in European countries today. Potatoes are used for several purposes, including human consumption, industrial processing (potato starch, alcohol, etc.) and recultivation. Fresh potato contains about 17-21 % starch, 0.5-2.0 % proteins, mineral compounds, organic acids and ash. Therefore, it can be used as a raw material for starch production. Furthermore, the tuber contains a higher proportion of the essential amino acid lysine than most cereal proteins, but is deficient in the sulfur-containing amino acids, methionine and cysteine.

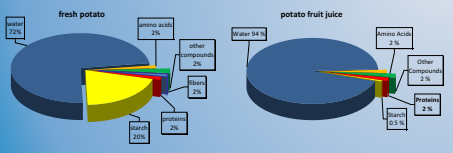


Figure 1: Composition of fresh potato and potato fruit juice (PFJ)

Potato protein is classified in three categories. Among the first category is the main storage glycoprotein patatin that shows a lipid transferase and lipid hydrolase activity.

A multitude of protease inhibitors presents the second category: A heterogeneous class of small, heat stabile high-cystein proteins that differ in molecular weight, amino acid sequence and inhibitor activity.

The third category comprises high molecular proteins such as polyphenoloxidases (PPO), protein kinases and phophorylases.

Tab. 1: Composition of potato protein

Protein	Proportion of soluble protein [%]	Mol.-Wt [kDa]
Major potato protein: Patatin	40-60	
patatin isoforms	40-60	40-44 (dimer 80-88)
Minor potato proteins: Protease Inhibitors	20-30	
Potato Inhibitor II (PI-2)	22	10.2 (dimer 20.4)
Potato Cysteinyl Protease Inhibitor (PCPI)	12	20.1-22.8
Potato Aspartyl Protease Inhibitor (PAPI)	6	19.9-22
Potato Inhibitor I (PI-1)	4.5	7.8
Potato Kunitz Protease Inhibitor (PKPI)	4	20.2
Other Serine Protease Inhibitors (OSPI)	1.5	21-21.8
Potato Carboxypetidase Inhibitor (PCI)	1	4.3
Other potato proteins in PFJ	20-30	
Polyphenoloxidases (PPO)	no info	60-69
Phosphorylase isoenzyms	no info	180-600
Starch Synthases/Protein Kinases	no info	140
Lectin	1	65,5

Application of membrane adsorbers for biotechnological processing of PFJ

The membrane adsorber technology combines the advantages of conventional ion exchange chromatography columns regarding separation power and capacity with the advantages of membrane technology. The favourable membrane pore structure avoids diffusion limitations resulting in higher throughput and decreased process time (Fig. 2)

Figure 2: Comparison of conventional beads and membrane adsorber

The biotechnological processing of complex media like PFJ demands competitive downstreaming strategies for effective separation and purification of biologically active protein fractions. Traditional chromatographic methods often require several steps resulting in time consuming and costly procedures. In contrast, protein purification via membrane adsorbers offers the advantage of fast and effective isolation under gentle conditions.

Figure 3 shows the downstreaming process scheme in pilot plant scale employing mambrane adsorber technology. Patatin can be recovered by anion-exchange membranes (Q-membrane adsorber), whereas protease inhibitors bind on cation-exchange-membranes (S-membrane adsorber). Further ultra-/diafiltration and protein drying yield powdery patatin with a purity of up to 88 % and protease inhibitors with a purity of up to 77 % in their biologically active form. The chromatographic process is monitored by measuring UV-absorbance and conductivity simultaneously (Fig. 4).

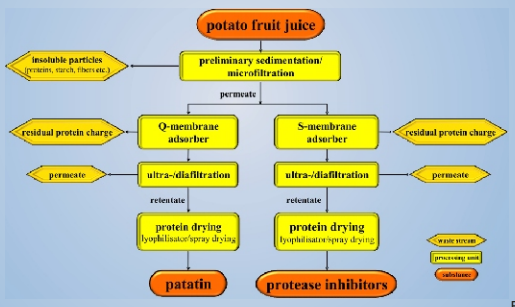


Figure 3: Flow-chart of PFJ processing

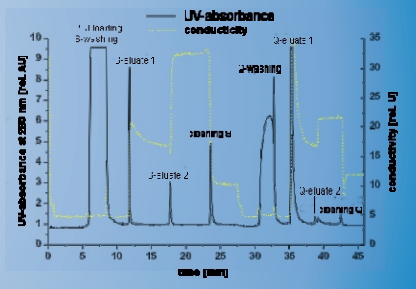
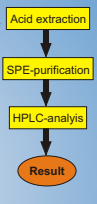


Figure 4: Separation process monitored by measuring UV-absorbance and conductivity

Glycoalcaloid & PPO

For the profitable use of the potato protein fractions as dietary supplements or pharmaceuticals the toxic glycoalkaloids of the obtained samples have to be examined. The most important representatives in the potato are -solanine and -chaconine. The extraction process developed for powdery potato protein samples is divided into acetic acid extraction, SPE-purification and HPLC analysis with simple UV-detection. Quantitative glycoalkaloid determination is possible down to a low ppm-range of 20 ppm.



Enzymatic browning of potato proteins by polyphenol oxidases (PPO) is a main cause of quality loss. The amount of PPO in the obtained potato protein fractions is analysed by an enzymatic assay using catechol as substrate. The proceeding degradation of ascorbic acid can be measured photometrically at 265 nm.

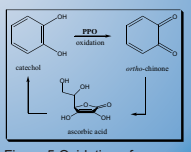


Figure 5:Oxidation of catechol catalysed by PPO

Enzymatic patatin digestion

The main method for altering food proteins is the enzymatic hydrolysis. It is applicable under mild conditions and thus, leading to products with retained biological activity.

The enzymatic hydrolyzation progress of patatin with pepsin was examined by SDS-PAGE (Fig.6) and RP-HPLC (Fig. 7). The pepsin hydrolysates provide only slight bands at 40 kDa (patatin), 15 kDa and below 10 kDa.

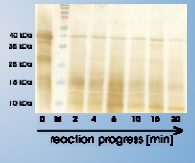


Figure 6: SDS-PAGE of patatin hydrolyzates

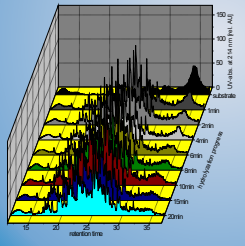


Figure 7: RP-HPLC analysis of progressing patatin digestion

Summary

This work shows the possibility to isolate and purify two native potato protein fractions, patatin and protease inhibitors, from potato fruit juice. Applying the membrane adsorber technology as downstreaming processing unit, proteins can be separated fast and effectively in a four step process. Under these gentle conditions patatin and protease inhibitors retain their biological activity.

An analytical RP-HPLC method has been established to characterize the quality of the protein fractions in regard to non-accumulation of toxic glycoalcaloids. Furthermore, an enzyme assay has been performed for monitoring activity of undesired polyphenol oxidases.

In contrast to potato protein, the protein fraction patatin undergoes a fast enzymatic reaction with pepsin. This suggests an efficient gastric digestion in human alimentary system.

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