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Antioxidant and Antiproliferative Activities of a Nonprotein Potato Juice Fraction Against Gastrointestinal Cancer cells: A Preliminary Study

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Section
Health Sciences

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Abstract

Research results that have appeared in recent years indicate the broad biological activity of potato juice (PJ). The activities that are described include anti-inflammatory, antioxidant and cytotoxic effects. This paper is a report on the antiproliferative and antioxidant activity of the nonprotein fraction (NPF) of PJ. NPF was obtained using a novel method of enzymatic protein hydrolysis combined with multistage membrane separation, characterized chemically and compared with raw PJ. Antioxidant as well as antiproliferative activities against human cancer cells isolated from the colon (Caco-2 and HT-29 cell lines), and normal cells isolated from the human normal colon (CCD 841 CoN cell line) were investigated. NPF was shown to have a higher antiproliferative activity than PJ, and it was more effective in cancer than in normal cells.

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Introduction

Potato juice (PJ) is one of the by-products of the production of potato starch. At present, acidic thermal coagulation is the most commonly used PJ management process. Unfortunately, protein obtained in this way is insoluble and enzymatically inactive (Kot et al. 2020). The poor emulsifying, foaming and water-binding properties of potato protein significantly limit its potential use in food production (Jeżowski et al. 2020). In starch production factories, acid-thermal coagulation of PJ proteins is commonly used, and the protein mass is then separated, dried and used as an animal feed ingredient (Tuśnio et al. 2011). Therefore, new methods of PJ management are sought, and the potential uses of PJ described in the literature include microbiological mediums (Bzducha-Wróbel, Błażejak et al. 2018; Bzducha-Wróbel et al. 2015; Bzducha-Wróbel, Pobiega et al. 2018), but also health-promoting food (Kowalczewski, Olejnik, Białas, Kubiak et al. 2019; Kowalczewski et al. 2022). PJ is increasingly gaining interest as a source of nutritional and bioactive compounds. On the one hand, the proteins present in PJ are characterized by high nutritional value and interesting technological properties (Jeżowski et al. 2020; Kowalczewski, Olejnik, Białas, Rybicka et al. 2019; Miedzianka et al. 2014), and on the other hand, nonprotein compounds with anti-inflammatory, cytotoxic and antioxidant activities are also present (Burlingame et al. 2009; Chrubasik et al. 2006). The compounds that contribute to the antiproliferative properties of PJ against cancer cells of human skin (Cham, Meares 1987), the liver (Kuo et al. 2000), the prostate, and the breast (Hu et al. 1999), include glycoalkaloids (GAs), principally α-chaconine and α-solanine. The published data indicate that the thermal treatment of PJ allows for the improvement of biological activity (Kowalczewski, Olejnik, Białas, Kubiak et al. 2019), so it seems correct to believe that thermostable nonprotein compounds are responsible for this activity. Isolating the biologically active ingredients of PJ may allow them to be used in pharmacy and medicine.

This article is a report on the antiproliferative activity of the nonprotein fraction (NPF) of PJ, obtained using a novel membrane separation process (micro-, ultra- and nanofiltration) of previously hydrolyzed PJ (using the Savinase® proteolytic enzyme). In this study cancer (Caco-2 and HT-29) and normal (CCD 841 CoN) cells of the gastrointestinal tract were used.

Materials and Methods

Preparation of NPF via enzymatic hydrolysis and membrane separation of PJ

The experimental material, PJ, was collected during the starch production season from the production line of Potato Industry Company Trzemeszno sp. z o. o.

(Trzemeszno, Poland). The enzyme Savinase® (Sigma-Aldrich, Saint Louis, MO, USA), isolated from the Bacillus species, was used as the proteolytic preparation. On the basis of previous preliminary studies (data not shown), an enzyme dose of 4 μL/g of potato protein was adopted. The enzymatic hydrolysis process was carried out at a constant temperature of 27°C, and the pH was strictly maintained at 8.5 via the controlled addition of 2N NaOH. The process was conducted continuously for a total duration of 8 h. The enzyme was added at the start of the hydrolysis process, according to the initial volume of PJ used in the experiment, and further portions were added every 60 min to maintain enzyme activity and stability, as the finished hydrolysis product was continuously removed and a new portion of PJ was added in its place. Intermediate samples of the hydrolysate were collected after 1, 4, and 7 h of the continuous operation. A polyethersulfone spiral-wound ultrafiltration membrane with a molecular weight of 1 kDa cut-off and an area of 3.5 m^2 (type 3838, SUEZ Water Technologies & Solutions, Budapest, Hungary) was used to perform the enzymatic hydrolysis and, consequently, to obtain a PJ protein hydrolysate (PJPH). The non-hydrolyzed PJ was returned to the initial tank of the system (recirculation). The PJPH was then concentrated on a polyamide thin film composite nanofiltration membrane, with a molecular weight of 300–500 Da cut-off and an area of 4.0 m^2 (type 3838, SUEZ Water Technologies & Solutions, Budapest, Hungary), to obtain a concentrated fraction of hydrolyzed, soluble potato proteins (retentate) and a nonprotein low molecular weight fraction (filtrate, denoted as NPF). For further analysis, NPF as well as PJ were lyophilized using an Alpha 2-4 LD plus lyophilizer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) without any lyophilization media. The process was initiated with a freezing stage at –35°C for 20 h, followed by the main drying step on a shelf at 5°C for 12 h, and final drying at 15°C for 4 h. The dried products that had been obtained were then packed in glass vials and stored until use in a frozen state (–24°C).

Chemical analysis

Total nitrogen content was determined using the Kjeldahl method according to ISO 1871 (ISO 2009) and protein content was calculated by multiplying the result by a conversion factor of 6.25. Ash content was determined according to ISO 763 (ISO 2003). The concentrations of minerals Ca, Cu, Fe, K, Mg, Mn, and Zn were determined using atomic absorption spectroscopy (F-AAS) (SpectrAA-800, Varian, Palo Alto, CA, USA), while Cd and Pb were determined using GF-AAS (Agilent 280Z AA, Agilent Technologies, Santa Clara, CA, United States) preceded by mineralization with nitric acid (Rybicka, Gliszczyńska-Świgło 2017). The content of α-chaconine and α-solanine concentrations were analyzed using high-performance liquid chromatography according to the method described by Joanna Miedzianka et al. (2020).

Samples of 0.5 g (NPF and PJ) were extracted with 40 mL of 80% ethanol for 2 hours. After centrifugation at 4000 × g (Rotofix 32 A, Hettich, Germany) for 10 min, supernatants were decanted and filtered (0.22 µm). The antioxidant activity was assessed using the ABTS radical cation decolorization assay (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) (Re et al. 1999). The absorbance of the reaction mixture was measured at 734 nm (Multiskan GO, Thermo Fisher Scientific, Vantaa, Finland) and the results were presented as Trolox equivalent antioxidant capacity (TEAC). All results were presented per g of dry matter of the material examined.

In Vitro Antiproliferative Activity Assay

The human colorectal adenocarcinoma cell line HT-29 (Cat. no: 85061109) was obtained from the European Collection of Authenticated Cell Cultures (ECACC) and supplied by Sigma-Aldrich (Poznań, Poland). The human colon cancer Caco-2 cell line (ATCC® HTB-37™) and human normal colon CCD 841 CoN (ATCC® CRL-1790™) cell line were obtained from the American Type Culture Collection (ATCC) and supplied by LGC Standards (Łomianki, Poland). These specific cell lines were selected because the gastrointestinal tract is the primary site of exposure to orally administered functional food components, and previous literature strongly indicates the susceptibility of intestinal cancer cells to potato-derived bioactive compounds.

Cytotoxic activity was assessed in accordance with the method described previously (Kowalczewski, Olejnik, Białas, Rybicka et al. 2019). The HT-29 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich) supplemented with heat-inactivated fetal bovine serum (FBS; Gibco BRL, Grand Island, NY, USA) to a final concentration of 10% and 1% nonessential amino acids 100X (Sigma-Aldrich). The base medium for CCD 841 CoN cells was ATCC-formulated Eagle's Minimum Essential Medium with 10% FBS addition. Cell cultures were incubated at 37°C in a humidified atmosphere (5% CO_2, 95% air).

Cells were grown in 96-well plates at an initial density of 2.5×10^4 cells/cm^2. The 24 h cultures were treated with NPF and PJ at concentrations ranging from 0.5 to 20 mg/mL and incubated for 48 h under standard culture conditions. Cell viability and metabolic activity were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay (Mosmann 1983). The first cytotoxic dose (IC_{10}), median effective concentration (IC_{50}), and lethal dose (IC_{90}) were calculated on the basis of the MTT results. Cell response to treatment was evaluated relative to control cells treated with neither NPF nor PJ.

Statistical analysis

The data presented in the tables regarding the chemical composition and biological activity of NPF represent the mean ± SD. To ensure the reproducibility and stability of the continuous technological process, samples were collected at 1, 4, and 7 hours of the run. Each of these temporal samples was analyzed in triplicate, and the overall average of these measurements was reported in order to reflect the representative properties of the entire production batch. The data were statistically analyzed using Minitab software version 18.0 (State College, PA, USA). A one-way analysis of variance (ANOVA) followed by Tukey's test, as well as Fisher's least significant difference (LSD) test, was applied at $p < 0.05$.

Results and Discussion

Fresh PJ contains about 1% mineral compounds and 4% organic compounds, mainly proteins (2%), but it is also rich in biologically active compounds, such as β-carotene, polyphenols, ascorbic acid, tocopherol and α-lipoic acid (Camire et al. 2009; McGill et al. 2013; Zwijnenberg et al. 2002). The use of enzymatic hydrolysis and membrane filtration allowed a low molecular fraction of PJ to be obtained (Table 1). The protein content of PJ determined using the Kjeldahl method was 2.5%, while in NPF it was 0.2%. Given the limitations of this method, which potentially overestimates the protein content due to the determination of nitrogen content, which is then converted to protein content (Hayes 2020; Kirk 1950; Sáez-Plaza et al. 2013), it can be assumed that NPF does not contain protein but may still contain some peptides and low molecular organic nitrogen fractions. On the other hand, an accumulation of mineral compounds was observed that were not retained in the nanofiltration process. Although the concentration of Cd increased in the NPF (9.18 μg/g dm), its potential application as a functional food component or pharmaceutical agent must be evaluated very critically in the context of strict dietary exposure limits. Since NPF is a highly concentrated fraction, it would not be consumed directly but rather used as a minor additive or an extract, where the final dosage would ultimately dictate the heavy metal exposure. Nevertheless, this elevated cadmium level raises legitimate safety concerns. Future optimization of the membrane separation process, perhaps involving targeted complexation or selective ion-exchange stages, is strictly required to selectively reduce heavy metal retention without losing the valuable bioactive compounds. Proteins have a broad metal binding capacity (Blundell, Jenkins 1977; Casterline, Barnett 1982; Gonick 2011) and can be removed along with the protein fraction. Removal of mineral compounds was also observed in the case of the membrane separation processes of proteins from other raw materials, mainly dairy (Chen et al. 2009; McDonough et al. 1974; Zulewska et al. 2009). The use of enzymatic

protein hydrolysis of PJ combined with a multi-stage process of membrane filtration resulted in a partial removal of mineral compounds along with proteins. It is worth noting that both PJ and NPF contain significant amounts of potassium and magnesium, important from a nutritional point of view (Champagne 2008; Derom et al. 2013; Weaver 2013; Weaver et al. 2018). Further targeted metabolomic profiling, specifically utilizing advanced techniques such as LC-MS/MS, is absolutely necessary in our follow-up studies to fully uncover the mechanisms and identify all specific compounds responsible for these beneficial effects.

Plants are a source of many bioactive compounds with a broad biological effect. An increasing number of studies indicate that PJ, as a rich source of bioactive compounds such as polyphenols, proteins and GAs, has health-promoting properties, including antioxidant, anti-inflammatory and anticancer properties (Kujawska et al. 2018; Visvanathan et al. 2016). The results of the antioxidant activity, determined using the ABTS method, indicate as a preliminary screening that the low molecular weight fraction has an antioxidant potential 2.5 times higher than that of PJ, while at the same time the content of GAs decreased, both for solanine and chaconine. GAs bind to proteins (Friedman 2006; Friedman et al. 1997), so removal of the protein fraction partially reduces their content.

Table 1. Chemical composition of NPF and PJ

Parameter	NPF	PJ
Protein [%]	0.19 ± 0.07^{b}	2.47 ± 0.13^{a}
Ash [%]	1.14 ± 0.50^{b}	3.48 ± 0.67^{a}
Minerals		
K [mg/100g dm]	926 ± 71^{b}	8173 ± 62^{a}
Mg [mg/100g dm]	212 ± 24^{b}	542 ± 11^{a}
Ca [mg/100g dm]	77 ± 9^{b}	141 ± 6^{a}
Zn [mg/100g dm]	1.12 ± 0.22^{b}	8.18 ± 0.57^{a}
Mn [mg/100g dm]	1.36 ± 0.17^{b}	3.03 ± 0.22^{a}
Cu [mg/100g dm]	0.87 ± 0.20^{b}	16 ± 2^{a}
Fe [mg/100g dm]	0.13 ± 0.04^{b}	164 ± 9^{a}
Cd [µg/g dm]	9.18 ± 0.21^{a}	7.12 ± 0.19^{b}
Pb [µg/g dm]	2.92 ± 0.08^{a}	3.01 ± 0.04^{a}
Glycoalkaloids		
α-chaconine [µg/g dm]	796 ± 12^{b}	993 ± 10^{a}
α-solanine [µg/g dm]	469 ± 13^{b}	597 ± 14^{a}
Antioxidant property		
TEAC [mmol/g dm]	0.79 ± 0.06^{a}	0.26 ± 0.04^{b}

NPF – nonprotein fraction; PJ – potato juice; TEAC – Trolox equivalent antioxidant capacity. Mean values with different letters in the rows are significantly different at $\alpha = 0.05$.

Source: Own study.

Published studies indicate the cytotoxic activity of pure potato GAs against colon (Caco-2, HT-29) and liver (HepG2) cancer cells (Ji et al. 2008; Ji, Gao 2012; Mandimika et al. 2007). GAs have also been shown to reduce cancer metastasis, with α-chaconine being more effective than α-solanine (Visvanathan et al. 2016). Therefore, it should be assumed that NPF containing GAs and other compounds, including antioxidant compounds, will exhibit cytotoxic activity *in vitro*.

Table 2 shows the first cytotoxic dose (IC_{10}), median effective concentration (IC_{50}), and lethal dose (IC_{90}) against human colorectal adenocarcinoma cell line HT-29, human colon cancer Caco-2 cell line and human normal colon CCD 841 CoN. NPF exhibited significantly higher cytotoxic activity against all cell lines analyzed. In the case of the IC_{50} dose for HT-29 cells, an increase in antiproliferative activity of as much as 55% was observed, while a less spectacular increase was noted in the Caco-2 cells (24%). Furthermore, NPF demonstrated a more favourable safety profile compared to raw PJ. Pharmacological anti-neoplastic agents often exhibit highly nonspecific activity, inhibiting the proliferation of both neoplastic and normal cells. However, by calculating the selectivity index (the ratio of the IC_{50} value for normal CCD 841 CoN cells to the IC_{50} value for cancer cells), we observed that NPF is more selective towards tumour cells. For instance, the selectivity index for HT-29 cells increased from approximately 1.67 for raw PJ to 2.25 for NPF, indicating its stronger targeted antiproliferative potential. The pharmacological anti-neoplastic agents used in the treatment of neoplasms exhibit highly nonspecific activity in inhibiting the proliferation of both neoplastic and normal cells (Galluzzi et al. 2015). A similar relationship was demonstrated for PJ, which is consistent with the previously published data (Kowalczewski, Olejnik, Białas, Kubiak, et al. 2019). Despite the decrease in the GA content of NPF (Table 1), its cytotoxic activity increased. This seemingly counterintuitive observation suggests that the antiproliferative effect is not solely dependent on GAs. It is highly probable that other low-molecular-weight bioactive compounds, such as specific phenolic acids, antioxidants, or short bioactive peptides resulting from enzymatic hydrolysis, are concentrated in the NPF. These components may interact with each other strongly, leading to a synergistic effect that significantly enhances the overall biological and cytotoxic activity compared to raw PJ. Further targeted metabolomic profiling is necessary to fully uncover the mechanisms and identify all specific compounds responsible for these beneficial effects.

Table 2. Cytotoxic doses of NPF and PJ [mg dm/mL]

Cell lines	Sample	Cytotoxic doses		
		IC_{10}	IC_{50}	IC_{90}
HT-29	NPF	0.963 ± 0.074^{b}	1.748 ± 0.053^{b}	3.792 ± 0.229^{b}
	PJ	1.801 ± 0.144^{a}	3.892 ± 0.211^{a}	9.381 ± 4.240^{a}
Caco-2	NPF	1.107 ± 0.062^{B}	2.311 ± 0.087^{B}	4.972 ± 0.236^{B}
	PJ	1.642 ± 0.175^{A}	3.076 ± 0.164^{A}	7.192 ± 0.209^{A}
CCD 841 CoN	NPF	$1.561 \pm 0.055^{\underline{B}}$	$3.944 \pm 0.042^{\underline{B}}$	$5.553 \pm 0.066^{\underline{B}}$
	PJ	$4.708 \pm 0.573^{\underline{A}}$	$6.499 \pm 0.198^{\underline{A}}$	$9.062 \pm 1.195^{\underline{A}}$

NPF – nonprotein fraction; PJ – potato juice. Mean values with different letters in the columns are significantly different at $\alpha = 0.05$. Different superscript letters indicate statistically significant differences between samples within a given cell line and cytotoxic dose parameter. Lowercase letters refer to HT-29 cells, uppercase letters to Caco-2 cells, and underlined uppercase letters to CCD 841 CoN cells.

Source: Own study.

Conclusions

In summary, the preliminary results presented here show that the low-molecular-weight fraction of PJ is characterized by higher antiproliferative and antioxidant activity than fresh PJ. Although the study is limited to *in vitro* models, this fraction seems to be an attractive candidate for obtaining biologically active compounds with potential chemopreventive properties against gastrointestinal cancer cells. Furthermore, it may serve as a source of antioxidants for the formulation of functional foods. However, extensive *in vivo* studies, detailed chemical profiling, and rigorous toxicological assessments are necessary before any therapeutic or dietary applications can be fully considered.

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Ethics Approval Statement

This study did not involve human participants or animals.

Informed Consent Statement

Not applicable. The study did not involve human participants, patients, or identifiable personal data.

Data Availability Statement

All raw data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of Interest Statement

Przemysław Łukasz Kowalczewski currently serves as the Editor-in-Chief of this journal. In accordance with the journal's editorial policies and COPE guidelines, Przemysław Łukasz Kowalczewski had no involvement in the peer-review process and excluded from all editorial decisions related to this manuscript. The review process and final decision were managed by an independent Editorial Board member. The other authors declare no conflict of interest. The funding body had no role in the design of the study, data analysis, or the writing of the manuscript.