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ANTIOXIDANT ACTIVITY OF POTATO JUICE*

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ABSTRACT

Background. The interest in potato juice as a therapeutic agent goes back to the 19th century but its application was not supported by any knowledge about biological activity of this raw material. Factors restricting the medical application of potato juice include its inattractive sensory and functional properties. The aim of the presented investigations was preliminary evaluation of the biological activity of potato juice and the impact on it of some technological operations such as: cryoconcentration and hydrolysis in a membrane reactor.
Material and methods. Experiments comprised investigations of antioxidative potentials of fresh potato juice, products of its processing as well as fractions separated because of the size of their molecules using, for this purpose, Folin-Ciocalteu methods and reactions with the ABTS cation radical.
Results. The value of the antioxidative potential of fresh potato juice determined by means of the ABTS reagent corresponded to approximately 330 μmol/100 g which is in keeping with literature data. As a result of the cryoconcentration process, the value determined by the Folin-Ciocalteu method was found to increase only slightly whereas the value determined by means of the ABTS reagent almost tripled. The antioxidative potential was found to grow even more strongly in the case of the application of both methods when the process of enzymatic hydrolysis was employed. The total of 5 protein fractions of molecular masses ranging from 11 000 Da to over 600 000 Da, as well as an organic non-protein fraction of the molecular mass of 600 Da, were obtained as a result of the performed separation. All the examined fractions exhibited antioxidative activities. The highest values determined by the Folin-Ciocalteu method were recorded for the protein fraction of 80 000 Da mean molecular mass, while using the ABTS reagent – for the organic, non-protein fraction.
Conclusions. Potato juice possesses antioxidative activity which can be enhanced by means of processing, especially, in the course of enzymatic hydrolysis. In addition, it was demonstrated that the organic non-protein fraction of 600 Da mean molecular mass was characterised by the highest antioxidative activity.

Key words: potato juice, antioxidant activity, enzymatic hydrolysis

INTRODUCTION

Potato juice is a waste product which is obtained in the course of manufacture of potato starch. The juice is made up of both minerals (about 1%), as well as organic (4%) substances, primarily, of protein character (2%). The non-protein organic substances comprise mainly vitamins (B_1, B_2, B_6, PP, C and E), as well as antinutritional (phytates) or even toxic (glycoalkaloids) substances [Gumul et al. 2011, Lachman et al.

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2006]. Concentration levels of individual potato juice constituents can vary even by five orders of magnitude depending on the potato variety and cultivation conditions [Lachman et al. 2009, Hamouz et al. 2008] as exemplified by literature data in which the content of solanine is reported to range from 0.001-47.2 mg·100 g^{-1} [Burlingame et al. 2009]. Although the usefulness of potato juice in the treatment of gastric ulcer has been recognised since the end of the 19th century, nevertheless first scientific investigations assessing its biological activity were initiated only during the last decade [Vlachojannis et al. 2010]. Presently, it is believed that the observed physiological action of potato juice is associated mainly with substances of protein nature, especially, with protease inhibitors [Pouvreau et al. 2001, Ruseler-van Embden et al. 2004]. Major concerns, however, arise from the presence in potato juice of compounds of glycoalkaloid nature, primarily, solanine and chaconine [Korpan et al. 2004]. That is why, in industrial practice, potato juice is processed by way of acid-thermal coagulation into protein feed concentrate and the primary objective of such a treatment is to reduce the load on sewage [Lubiewski et al. 2006].

Enzymatic hydrolysis of proteins is a process employed mainly to improve their functional properties. The degree of protein hydrolysis exerts a significant influence not only on their organoleptic features but, equally importantly, on their salubrious value (e.g. antioxidative potential, ability to bind bioelements) [Bilska et al. 2009, Je et al. 2007]. An attempt to employ a membrane reactor for protein hydrolysis (a standard procedure used for saccharification of initially hydrolysed starch, for example) was made only recently in order to reduce its allergenicity and increase its solubility [Prevot-D'Alvise 2004].

The objective of the research project was to perform preliminary assessment of the biological activity of potato juice and the impact exerted on this juice by some technological operations bearing in mind the fact that its food utilisation is hindered not only by ambiguities associated with its biological activity but also by its uninviting sensory and functional properties. The scope of the performed experiments comprised investigations of the antioxidative activities (using for this purpose Folin-Ciocalteu methods as well as reactions with the ABTS cation radical of both fresh juice as well as juice subjected to processes of cryoconcentration and hydrolysis in a membrane reactor.

MATERIAL AND METHODS

The experimental material was potato juice collected from the production line of the Wielkopolska Potato Industry Enterprise in Luboń (Production Plant in Staw) in the course of 2010 potato processing campaign. Fresh potato juice of 6°Brix general extract was concentrated by the cryoconcentration method using for this purpose a Freeze Tec Company Pilot20L device (the Netherlands) and as a result obtaining, from the total of 200 l of fresh juice, 40 l of concentrate extract of 30.5°Brix concentration.

The hydrolysis of fresh potato juice, as well as the product of its concentration was conducted in a membrane reactor equipped with a ceramic ultra filtration pipe with a cut-off point of 5 kDa and 0.2 m^2 surface. The installation consisted of a reaction container of 20 dm^3 volume (New Brunswick Scientific Co., Inc. USA) manufactured from acid-resistant steel, heat exchanger, thermostat (Haake, Germany), lobar pump (Johnoson, USA) and a ceramic ultra filtration membrane, cut-off point: 50 kDa. The process of hydrolysis was catalysed with the assistance of an Alcalase 2.4L FG (Novozymes) preparation which is an endopeptidase manufactured by a strain of *Bacillus licheniformis* and was carried out in conditions optimal for the activity of the enzymatic preparation applied in the experiment (pH = 8.0 ±0.7, T = 30°C). Different doses of the enzymatic preparation were applied and transmembrane pressure was changed during

Table 1. Enzymatic hydrolyses parameters

Sample	Dry matter content of the raw material %	Transmembrane pressure MPa	Enzyme doses µl·g^{-1}
S1	5.5	2	4
S2	5.5	1.5	4
S3	5.5	2	8
K1	24.2	2	8
K2	24.2	3	8

the performed experiment. Parameters of experimental hydrolyses are collated in Table 1.

Determination of the dry matter content was carried out in three replications at the temperature of 105°C using the gravimetric method in accordance with the PN-75/C-04616.01 standard. Determination of hydrolisate dry matter content was conducted for samples collected on the 210th minute of the hydrolysis process.

Protein content was determined by Kjeldahl's method in accordance with the ISO 5378 standard by burning the examined samples at the temperature of 420°C in a Digeston furnace (Tecator, Canada) and then distilling the obtained sample in a Kjelteck apparatus (Tecator, Canada).

The antioxidative activity was assessed against the ABTS radical (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) in accordance with the method described by Re et al. [1999] and expressed in Trolox µmols per 1 g of dry matter of the examined material.

The content of compounds was determined by the Folin-Ciocalteu method [Fang et al. 2006] and expressed as an equivalent of the chlorogenic acid (CAE) per 1 g of dry matter. The determination of the hydrolisate antioxidative activity was performed for samples collected on the 210th minute of the process. The collected data has been shown as arithmetic means ±SD (n = 9).

RESULTS AND DISCUSSION

Proper functioning of living organisms is preconditioned, among others, by ensuring a delicate balance between the creation and elimination of oxygen reactive forms (indispensible in certain metabolic processes). In other words, it is essential to maintain a pro-oxidative – antioxidative balance [Alamdari et al. 2007]. Violation of this balance in favour of pro-oxidative factors is known as an oxidation stress and is believed to be a cause of a number of diseases. They can be prevented thanks to the consumption of food articles characterised by high antioxidative potentials [Przeciwutleniacze... 2007]. However, such measures should be preceded both by the assessment of the content of bioactive substances and by precise evaluation of individual requirements of a given organism for these types of substances [Alamdari et al. 2007]. Due to their different structure, mode of action as well as mutual interactions, investigations of general antioxidative potentials are considered to be the most advantageous but no single binding standard has been agreed upon and several different ones are employed each of which has its own limitations [Prior and Cao 1999]. The following two methods were employed in the presented investigations: application of the ABTS reagent [2,2'-azobis(3-etylobenzotiazolino-6-sulfoniane)] and Folin-Ciocalteu reagent (CAE). The determined values for fresh juice (Figs 1 and 2) amounted to: 26.56 ±0.42 mg·g^{-1} DM (CAE) and 0.0663 ±0.001 mmol·g^{-1} DM (ABTS). Bearing in mind the fact that the dry matter of potato juice constitutes about 5% g/g, the value

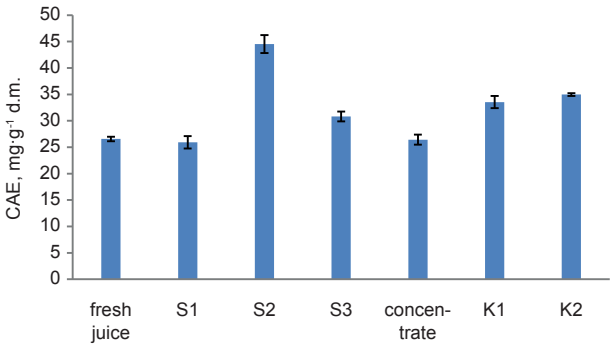

Fig. 1. Values of the antioxidative potential of fresh juice and of processed products determined using Folin-Ciocalteu method

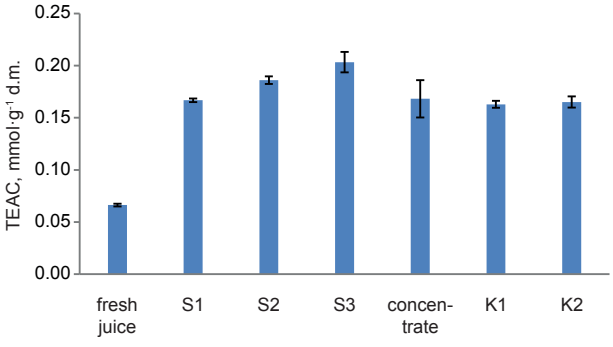

Fig. 2. Values of the antioxidative potential of fresh juice and of processed products determined using the method utilising the ABTS reagent

of the antioxidative potential determined by means of the ABTS reagent corresponded to approximately 330 μmol·100 g^{-1} which is in keeping with literature data. According to Burlingame et al. [2009], fresh potatoes are characterised by antioxidative potential ranging from 43-892 μg TEAC·g^{-1} of fresh matter which corresponds to 17-356 μmol·100 g^{-1} fresh matter. These values are considerably lower in comparison with berry fruits or cabbage but of similar order as some vegetables, e.g. cucumbers or carrots.

As a result of the cryoconcentration process, the value determined by the Folin-Ciocalteu method was found to increase only slightly to the level of 26.42 ±0.96 mg·ml^{-1} (CAE), whereas the value determined by means of the ABTS reagent almost tripled reaching the level of 0.1681 ±0.018 mmol·ml^{-1} (ABTS). Since the determinations refer to the dry matter of the examined material, the recorded enhanced values of the antioxidative potential should be attributed to a change in the concentrate composition in relation to the raw material. This implies that, in the course of the cryoconcentration process, compounds exhibiting antioxidative activity in the experiment with the ABTS reagent were selectively seized. This, however, was not associated with a change in the content of protein substances because both in fresh juice, as well as in the concentrate, the proportion of the protein fraction amounted to about 50% (Table 2).

Table 2. Physicochemical characteristics of raw materials subjected to hydrolysis and of filtrates collected on the 210th minute of the process

Sample	Dry matter content of the raw material %	Protein content in the fest matter %	Protein content in the dry matter %
Fresh juice	5.5	2.8	51
Concentrate	24.7	12.3	50
S1	3.7	0.8	22
S2	2.6	0.8	31
S3	2.75	0.8	29
K1	14.9	4.3	29
K2	14.3	7.1	50

The antioxidative potential was found to grow even more strongly in the case of the application of both methods when the process of enzymatic hydrolysis was employed (Figs 1 and 2). These results are in keeping with literature data indicating a possibility of increased antioxidative activity with the assistance of controlled enzymatic hydrolysis [Mendis et al. 2005, Je et al. 2007]. However, it is worth stressing that the percentage proportion of protein in the hydrolysis products was lower than in fresh juice (Table 2). In the case of the results obtained using the Folin-Ciocalteu method, the highest activity was recorded for products of the fresh juice hydrolysis carried out at the lowest transmembrane pressure (S2). On the other hand, in the case of the results obtained using the ABTS reagent, the highest activity was recorded for the hydrolysis products of fresh juice performed at increased enzyme dose (S3). Both fresh juice hydrolysis products of enhanced antioxidative activity contained more protein than S1 products (Table 2).

Hydrolysis products of the cryo-concentrate exhibited a slightly elevated antioxidative activity in comparison with the concentrate itself but the differences were not so spectacular as in the case of the hydrolysis products of the juice alone. In addition, the difference between antioxidative properties of K1 and K2 products was, practically, negligible (Figs 1 and 2) despite the fact that they were obtained at different transmembrane pressure (Table 1) and, in addition, contained significantly different quantity of protein fraction (Table 2).

In order to identify substances responsible for potato juice biological activity, the experimental raw material was separated using the method of gel permeation chromatography and its results are presented in Figure 3. Investigations were conducted on potato juice previously concentrated during a cryoconcentration process due to the fact that fractions obtained in the result of separation of fresh juice contained insufficient quantities of dry matter to allow successive analyses.

The total of 5 protein fractions of molecular masses ranging from 11 000 Da to over 600 000 Da, as well as an organic non-protein fraction of the molecular mass of 600 Da were obtained in the result of the performed separation.

All the examined fractions exhibited antioxidative activities. The highest values determined by the Folin-Ciocalteu method were recorded for the protein fraction of 80 000 Da mean molecular mass, while using the ABTS reagent – for the organic, non-protein fraction (Figs 4 and 5). It should be emphasised that the antioxidative activity of the latter fraction exceeded majority of protein fractions also when it was analysed with the assistance of the Folin-Ciocalteu method (Fig. 4).

The observed differences in values of the antioxidative potential determined using different methods should be attributed to the limitations of each of these methods. The Folin-Ciocalteu method was developed to determine the content of polyphenolic compounds utilising the colour reaction taking place between polyphenols contained in a given sample and the F-C reagent. However, this reagent also reacts with other organic substances such as: organic acids, sugars, aromatic and inorganic amines, e.g. ammonium sulphate, sodium phosphate [Zhang et al. 2006, Yu and Dahlgren 2000]. Also the method which utilises the ABTS reagent, although in theory should make it possible to establish the total antioxidative activity, in practice is not free of making interpretational errors. The limitation of this method is the fact that the ABTS reagent, in combination with flavonoids, develops strong antioxidative complexes leading to the determination of overestimated values of the antioxidative potential [Arts et al. 2004].

Irrespective of the limitations of both analytical methods, it should be emphasised that the non-protein fraction is a key fraction for the biological activity of potato juice. This confirmed the need to continue investigations on this raw material employing more differentiating methods, for example using cell cultures.

CONCLUSIONS

Fresh potato juice exhibits antioxidative activities at levels similar to some vegetable, e.g. cucumbers or carrots, although:

- potato juice antioxidative activity can be enhanced by processing, especially in the course of enzymatic hydrolysis
- six main fractions can be distinguished in potato juice differing with respect to their molecular mass, five of which are proteins

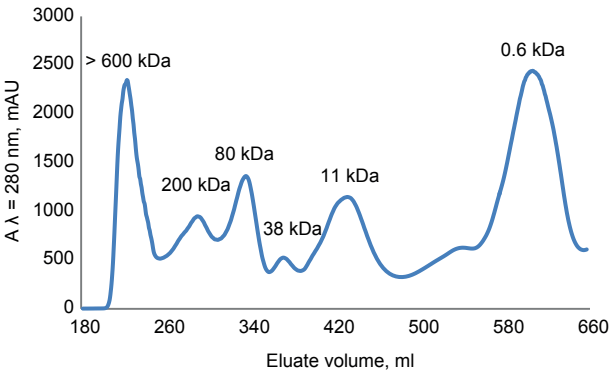

Fig. 3. GPC chromatogram of the concentrate of potato juice

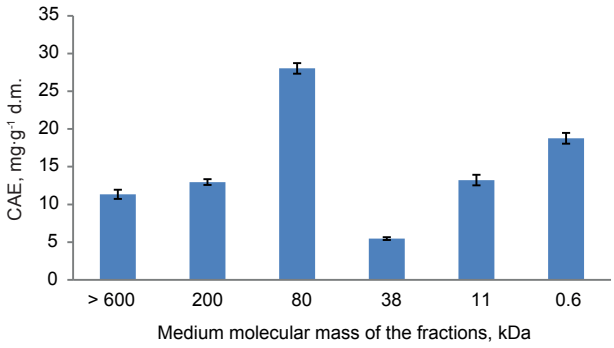

Fig. 4. Values of the antioxidative potential of potato juice fractions determined using Folin-Ciocalteu method

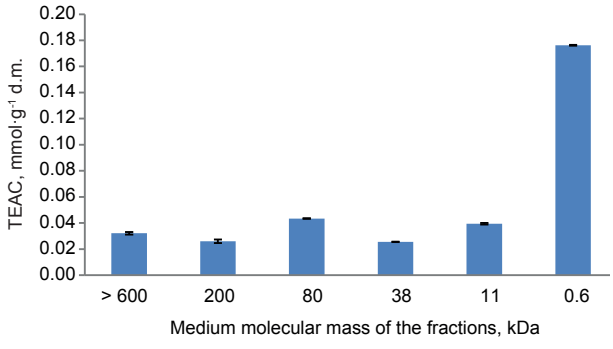

Fig. 5. Values of the antioxidative potential of potato juice fractions determined using the method utilising the ABTS reagent

- organic non-protein fraction of the mean molecular mass of 600 Da is characterised by the highest antioxidative activity.

REFERENCES

Alamdari D.H., Paletas K., Pegiou T., Sarigianni M., Befani C., Koliakos G., 2007. A novel assay for the evaluation of the prooxidant – antioxidant balance, before and after antioxidant vitamin administration in type II diabetes patients. Clin. Biochem. 40, 248-254.

Arts M., Haenen G., Voss H., Bast A., 2004. Antioxidant capacity of reaction products limits the applicability of the Trolox Equivalent Antioxidant Capacity (TEAC) assay. Food Chem. Toxicol. 42, 45-49.

Bilska A., Rudzińska M., Kowalski R., Krysztofiak K., 2009. The effect of soy hydrolysates on changes ion cholestrol content and its oxidation products in fine – ground model sausages. Acta Sci. Pol., Technol. Aliment. 8 (3), 15-22.

Burlingame B., Mouille B., Charrondiere R., 2009. Nutrients, bioactive non-nutrients and anti-nutrients in potatoes. Critical review. J. Food Comp. Anal. 22, 494-502.

Fang Z., Zhang M., Sun Y., Sun J., 2006. How to improve bayberry (*Myrica rubra* Sieb. et Zucc.) juice color quality: Effect of juice processing on bayberry anthocyanins and polyphenolics. J. Agric. Food Chem. 54, 99-106.

Gumul D., Ziobro R., Noga M., Sabat R., 2011. Characterisation of five potato cultivars according to their nutritional and pro-health components. Acta Sci. Pol., Technol. Aliment. 10 (1) 2, 73-81.

Hamouz K., Lachman J., Dvořák P., Hejtmánková K., Čepl J., 2008. Antioxidant activity in yellow and purple-fleshed potatoes cultivated in different climatic conditions. Zesz. Probl. Post. Nauk Roln. 530, 241-247.

Je J.-Y., Qian Z.-J., Byun H.-G., Kim S.-K., 2007. Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis. Process Biochem. 42, 840-846.

Korpan Y.I., Nazarenko E.A., Skryshevskaya, Martelet C., Jaffrezic-Renault N., El'skaya A.V., 2004. Potato glycoalkaloids: true safety or false sense of security. Trends in Biotechn.

Lachman J., Hamouz K., Šulc M., Orsák M., Pivec V., Hejtmánková A., Dvořák P., Čepl J., 2009. Cultivar differences of total anthocyanins and anthocyanidins in red and purple-fleshed potatoes and their relation to antioxidant activity. Food Chem. 114, 836-843.

Lachman J., Hamouz K., Čepl J., Pivec V., Šulc M., Dvořák P., 2006. The effect of selected factors on polyphenol content and antioxidant activity in potato tubers. Chem. Listy 100, 522-527.

Lubiewski Z., Śmigielska H., Lewandowicz G., Balcerek W., 2006. Charakterystyka odcieku po koagulacji białka pozyskiwanego w toku kampanii krochmalniczej [Physiochemical characteristics of supernant resulting after heat-coagulation of potato protein]. Zesz. Probl. Post. Nauk Roln. 511, 617-626 [in Polish].

Mendis E., Rajapakse N., Kim S.-K., 2005. Antioxidant properties of a radical-scavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. J. Agric. Food Chem. 53, 581-587.

Pouvreau L., Gruppen H., Piersma S.R., van den Broek A.M., van Koningsveld G.A., Voragen A.G.J., 2001. Relative abundance and inhibitory distribution of protease inhibitors in potato juice from cv. Elkana. J. Agric. Food Chem. 49.

Prevot-D'Alvise N., Lesuerur-Lambert C., Fertin-Bazus A., Fertin B., Dhulster P., Guillochon D., 2004. Continuous enzymatic solubilization of alfalfa proteins in an ultrafiltration reactor. Enzym. Microb. Technol. 34 (5), 380-391.

Prior R.L., Cao G., 1999. In vivo total antioxidant capacity: comparison of different analytical methods. Free Radical Biol. Med. 27, 1173-1181.

Przeciwutleniacze w żywności. Aspekty zdrowotne, technologiczne, molekularne i analityczne [Antioxidants in food. Health, technological, and molecular analysis aspects]. 2007. Ed. W. Grajek. WNT Warszawa.

Re R., Pellegrini N., Pannala A., Yang M., Rice-Evans C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231-1237.

Ruseler-van Embden J.G.H., van Lieshout L.M.C., Laman J.D., 2004, Methods and means for preventing or treating inflammation or pruritis. Patent USA 6,723,354.

Vlachojannis J.E., Cameron M., Chrubasik S., 2010. Medicinal use of potato-derived products. A systematic review. Phytother. Res. 24.

Yu Z., Dahlgren R., 2000. Evaluation of methods for measuring polyphenols in conifer foliage. Analyst 26, 98-113.

Zhang Q., Zhang J., Shen J., Silva A., Dennis D., Barrow C., 2006. A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. J. Appl. Physiol. 18, 445-450.

AKTYWNOŚĆ PRZECIWUTLENIAJĄCA SOKU ZIEMNIACZANEGO

STRESZCZENIE

Wstęp. Zainteresowanie sokiem ziemniaka jako środkiem leczniczym sięga XIX wieku, ale jego stosowanie nie było poparte wiedzą na temat biologicznej aktywności tego surowca. Ograniczeniem leczniczego wykorzystania soku ziemniaczanego są również jego nieatrakcyjne właściwości sensoryczne i funkcjonalne. Celem pracy była wstępna ocena aktywności biologicznej soku ziemniaczanego oraz wpływu na nią niektórych operacji technologicznych takich, jak kriokoncentracja i hydroliza w reaktorze membranowym.
Materiał i metody. Badano potencjał antyoksydacyjny świeżego soku, produktów jego przetwórstwa, jak również frakcji rozdzielonych ze względu na wielkość cząsteczek metodami Folina-Ciocalteu oraz z wykorzystaniem reakcji z kationorodnikiem ABTS.
Wyniki. Wartość potencjału antyoksydacyjnego oznaczona za pomocą odczynnika ABTS odpowiada ok. 330 μmol/100 g, co jest zgodne z danymi literaturowymi. W wyniku procesu kriokoncetracji wartość oznaczona metodą Folina-Ciocalteu uległa bardzo niewielkiemu podwyższaniu, natomiast wartość oznaczona za pomocą odczynnika ABTS uległa nieomalże potrojeniu. Jeszcze silniejszy wzrost potencjału antyoksydacyjnego, i to oznaczonego obydwoma metodami, odnotowano w produktach procesu hydrolizy enzymatycznej. W wyniku przeprowadzonej separacji uzyskano pięć frakcji białkowych o masach cząsteczkowych od 11 000 Da do ponad 600 000 Da oraz organiczną frakcję niebiałkową o średniej masie cząsteczkowej 600 Da. Wszystkie badane frakcje wykazywały aktywność antyoksydacyjną, przy czym największe wartości oznaczone metodą Folina-Ciocalteu odnotowano dla frakcji białkowej o średniej masie cząsteczkowej 80 000 Da, natomiast metodą z wykorzystaniem odczynnika ABTS – dla organicznej frakcji niebiałkowej.
Wnioski. Świeży sok z ziemniaka ma aktywność antyoksydacyjną, którą można zwiększać w drodze przetwórstwa, zwłaszcza w toku hydrolizy enzymatycznej. Ponadto wykazano, że największą aktywność antyoksydacyjną ma organiczna frakcja niebiałkowa o średniej masie cząsteczkowej 600 Da.

Słowa kluczowe: sok ziemniaczany, potencjał antyoksydacyjny, hydroliza enzymatyczna

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