Katarzyna Ławińska[1,*], Magdalena Lasoń-Rydel[1], Dorota Gendaszewska[1], Edyta Grzesiak[1], Katarzyna Sieczyńska[1], Carmen Gaidau[2], Doru-Gabriel Epure[3], Andrzej Obraniak[4]

[1] **ŁUKASIEWICZ Research Network, Institute of Leather Industry,**
Zgierska 73, 91-462 Lodz, Poland
* e-mail: k.lawinska@ips.lodz.pl

[2] **INCDTP Leather and Footwear Research Institute Division, Leather Research Department,**
93, Ion Minulescu St, Bucharest, 3, 031215, Romania

[3] **Probstdorfer Saatzucht Romania SR.,**
20, Siriului Str., Bucharest, 1, 014354, Romania

[4] **Lodz University of Technology, Faculty of Process and Environmental Engineering,**
Wolczanska 213, 90-001 Lodz, Poland

Coating of Seeds with Collagen Hydrolysates from Leather Waste

DOI: 10.5604/01.3001.0013.1819

Abstract
The subject of this paper is improvement in the growth and yield of three different types of legumes and rape in drought conditions by coating seeds with hydrolysed collagen from tanning waste. In addition, the impact of various additives in the seed shell on the growth of the plant was investigated. The encapsulation process of seeds was conducted on a disc granulator. A centrally placed seed was first coated with a layer of fungicides. The next layer was collagen hydrolysate, collagen hydrolysate with latex or a solution of yellow dextrin and polyvinyl alcohol. The outer layer was a mineral additive e.g. dolomite or kaolin. After the end of the encapsulation process on the disk granulator, all of the seeds tested were sown into soils. Seeds without coating were also sown as control seeds. Seedlings were maintained for 29 days with cultivation without irrigation. The length of the seedlings was analysed for all of the seeds sown. Higher seedling growth values were obtained for seeds coated with collagen hydrolysate in comparison with control seeds (without coating). The use of collagen hydrolysate gave slightly better results than in the case of a solution of dextrin with polyvinyl alcohol.

Key words: *leather, collagen, seeds, coating.*

Introduction

The foremost grain legume species contributors to human nutrition are the soybean (although the species is often classified for statistical purposes as an oil crop), common bean, chickpea, bean and pea [1-3]. Among crop plants, legumes have great potential as a protein production platform because of their naturally high protein content, nutritional value, independence of N-nutrition, pollen containment, availability of processing technology, storage stability, etc. [4]. In addition, proteins from legume seeds have been widely studied as regards their functional and bioactive properties, important for novel food development and for human health [5-8]. Oilseed rape has become one of the most important oil crops within the last decades. The main reason behind this trend is its multifunctional usage [9]. Unfortunately, crop losses due to drought and soil salinity are becoming increasingly severe and commonplace. Drought, one of the environmental stresses, is the most significant factor restricting plant growth and crop productivity in the majority of agricultural fields in the world [10]. Water scarcity, declining quality of water for irrigation, and soil salinity are problems which are becoming more and more acute [11, 12].

The coating technique can be used to cover the seeds of agricultural products with the objective of achieving the adherence of macro and micronutrients, inoculants (bacteria) and fungicides to their surface or modifying their format and size with inert material [13-16]. Seed coating is a mechanism involving the application of necessary materials in such a way that they affect the seed or soil at the seed–soil interface. Coating can protect the seed from many kinds of diseases and pests [17]. Seed-coating agents are widespread and used in crop protection [18, 19]. In the previous paper, we found that it is possible to form a collagen-based seed coating using a disk granulator. Preliminary studies also confirmed that the addition of collagen hydrolysates during the process of pea encapsulation improves the growth of seedlings [20, 21]. A collagen-based product used to coat the surface was first produced and used in the EU and Russia. Importantly, processing collagen-based materials into valuable materials for agriculture will help solve the problem of solid waste generated by the leather industry. The fibre and textile industry is a diverse sector that covers the entire production chain of transforming natural and chemical fibres into end-use products [22]. Tannery waste with the addition of relevant mineral substances may then be used for the production of leathery materials, composition leather, and composite materials based on fragmented collagen fibres [23-26].

The subject of this paper is improvement in the growth and yield of three different types of legumes (peas, soybean, broad bean) and rape in drought conditions by coating seeds with hydrolysed collagen from tanning waste. In addition, the impact of various additives in the seed shell on the growth of the plant was investigated.

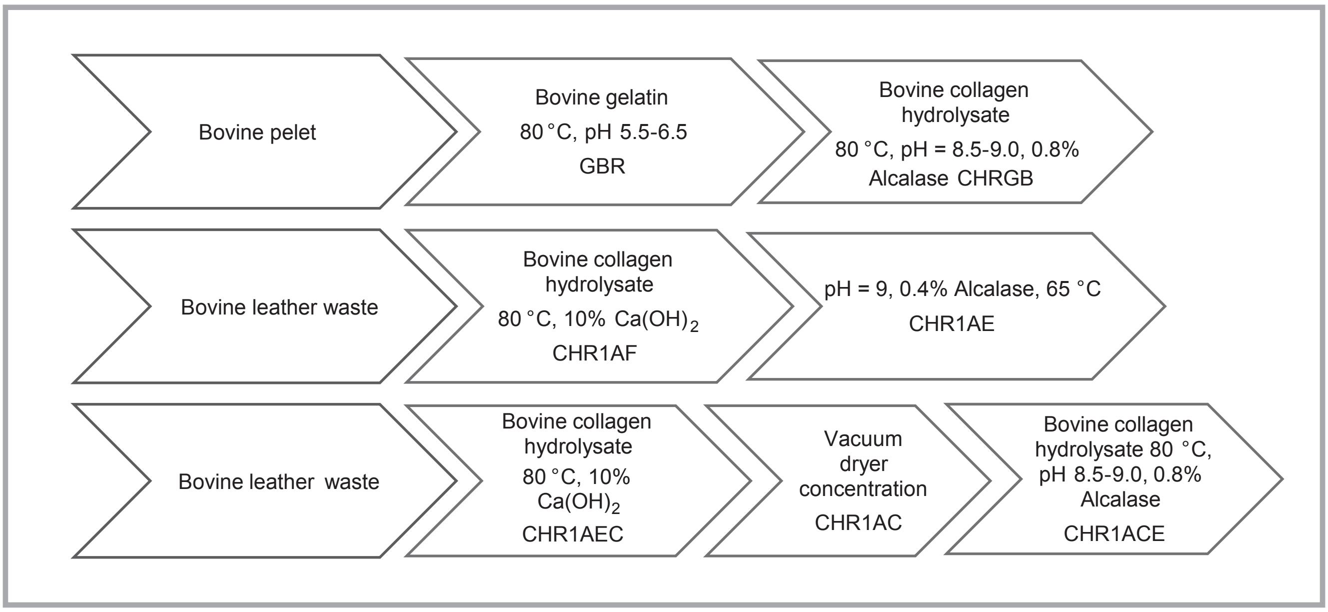

Figure 1. *Diagram of the production of the individual collagen preparations.*

Materials and methods

The research was conducted with three legumes cultivars (peas, soybean and broad bean) and rape grown in laboratory conditions at the Łukasiewicz Research Network – Institute of Leather Industry. These grains were purchased from Plant Breeding Strzelce Sp. z o.o. (Poland) and Plant Breeding Smolice Sp. z o.o. (Poland) IHAR Group. The germination capacity of the seeds of pea, soybean, broad bean and rape was 97%, 83%, 98% and 93%, respectively. Seedlings were maintained for 29 days with cultivation without irrigation.

The bovine shavings used as raw material showed the typical characteristics of semi-finished leather: moisture 51%, total ash 8.6%, total nitrogen 16.5%, chromium oxide 4.4%, and pH of aqueous extract 4.2. Collagen hydrolysates were obtained for the tests in various process conditions (***Figure 1***). On the basis of the results obtained, one preparation was selected that was characterised by the best properties in terms of its use in agriculture.

Characterisation of the collagen hydrolysates was performed by means of classical analyses, e.g. dry substance, total ash, total nitrogen, dermic substance, aminic nitrogen and pH. An HPLC (Prominence-i LC-2030C 3D, Shimadzu, Japan) with DAD detector (Diode Array Detector) was used for qualitative and quantitative determination of amino-acids. An AccQ·Tag amino acid column Nova-Pak C18, 4 μm (150 × 3.9 mm) and AccQ · Tag Reagent Kit from Waters were used. The column was thermostated at 37 °C, and 10 μl was the injection volume. The best gradient separation programme was as follows: 0 min, A 100%, B 0%; 0.5 min, A 98%, B 2%; 15 min, A 93% B 7%; 19 min, A 70%, B 30%; 32 min, A 67%, B 33%; 33 min, A 67%, B 33%; 34 min, A 0%, B 100%; 37 min, A 0%, B 100%; 38 min, A 100%, B 0%; 64 min, A 100%, B 0% (A-prepared from Waters AccQ·Tag Eluent A, B-prepared with 60% acetonitrile with water). The chromatography of the amino-acid composition was performed for the collagen preparation and for encapsulated seeds, selected at random.

The encapsulation process took place at the laboratory of the Lodz University of Technology using a disk granulator [27-29] at optimum process conditions. Basic elements may be distinguished in the design of the granulator used for the granulation tests described: the granulator disk, the electric motor with rotational speed regulation and the granulated deposit moistening system. A binding liquid is fed directly onto the granulated material using spraying nozzles. A precise description of the process parameters and the test station are provided in [30, 31]. The individual layers of the coating were formed in a specific order. A centrally placed seed was first coated with a layer of fungicides. The process of seed coating usually involves the use of adhesives to bind materials to the surface of seeds. Such procedures are adequate for about 90% of small seed species [32]. Therefore, the next layer was collagen hydrolysate (clear, yellow liquid, density 1.1125 g/ml, water content 67.19%, pH 7.5) or a solution of yellow dextrin and polyvinyl alcohol. Dextrin was used as a classical encapsulation reference liquid [33]. The outer layer of the coating was a mineral additive fostering germination (dolomite and kaolin, size of grains < 250 μm).

Dolomite flour (magnesium – calcium carbonate) and kaolin were chosen as typical fine grained materials [34-36]. What is more, encapsulation using latex (CAS: 25085-39-6) was attempted, as latex is considered a material improving the stability of a collagen preparation [37, 38]. The formulation was developed in collaboration with Pestila II sp. z o.o. S.k. After the end of the encapsulation process in the disk granulator, all of the seeds tested were sown into soils (sowing in stable climatic conditions, without access to water). Seeds without coating were also sown as control seeds. The growth was observed for 29 days. The length of the seedlings was analysed for all of the seeds sown and compared in terms of the impact of having a coating and using additives in the coating on the plants' growth.

Results and discussion

Seeds are usually coated for ease of handling, singulation, precise placement, and the incorporation of beneficial chemicals or microbials. Quality demands for seeds suitable for coating have improved knowledge of physiological seed quality. The incorporation of nutrients into seed coatings provides a unique opportunity to supply each seedling sown with an accurately controlled quantity of nutrients that may be preferentially available to the species sown [39]. The solution proposed is to increase the yield of various legume species using by-products of the leather industry.

The parameters of collagen hydrolysates obtained from tanning waste are listed in ***Table 1***. A CHR1AEC collagen preparation was used for coating, since it has optimum parameters in terms of the properties of the binding liquid in encapsulation processes.

Table 2 contains chromatography results of the amino-acid composition of the collagen preparation. By comparing the retention times of chromatography peaks of the sample tested with those of amino-acids present in the standard, 7 amino-acids were identified, i.e. glutamic acid, arginine with NH_3, alanine, proline, leucine, lysine and hydroxyproline.

Thus, the presence of small, free amino-acids in the collagen hydrolysate was confirmed. This is beneficial as coating containing collagen will constitute a valuable source of nutrients necessary for plant growth. Significantly, the specific amino-acids of collagen, such as proline and hydroxyproline, act mainly as a hydric balance of the plant, strengthening the cellular walls in such a way that they increase resistance to unfavourable climatic conditions [40]. Collagen hydrolysates with an average molecular weight below 13000 Dalton, with large polydispersity, contain peptides, olygopeptides and a mixture of free amino-acids (glutamic acid, arginine, alanine, proline, leucine, lysine and hydroxyproline). Free amino-acids, organic nitrogen and film forming properties will protect and nurture the seeds in the long term.

After the end of the encapsulation process, we obtained a full, closed coating. Granules were of a homogeneous spherical shape and did not form agglomerates. The coatings were characterised by high durability. Stabilisation of an average mass of 1 coated seed was observed after 3 days from the coating process (saturation with moisture from the environment). As a result of the sprout growth, the shell naturally cracks in the ground (in the case of peas and broad beans) or above the ground (in the case of soybeans). It was also observed that the coating does not dissolve in contact with wet soil. Despite cracking, the collagen coating continues to adhere to the seed for a long time, which is why the seed is kept protected and supplied with nutrients (***Figure 2***).

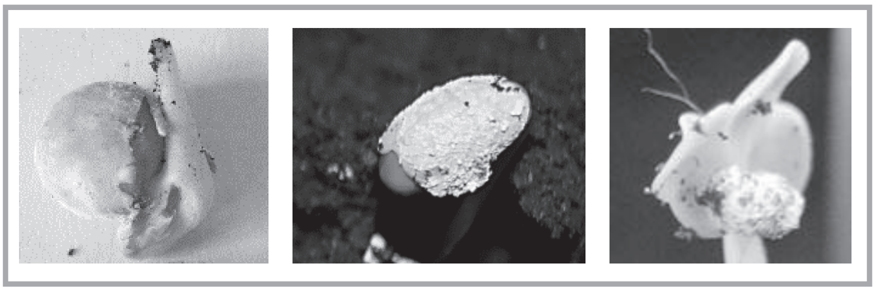

Figure 2. *Disintegration of coatings with collagen hydrolysate.*

In order to determine the impact of a collagen preparation on plant growth, a comparative analysis of the average length of seedlings in relation to another reference binding liquid used in seed encapsulation processes was performed [33]. In this series of tests, seeds were coated only with a binding liquid, i.e. a collagen preparation, as well as yellow dextrin and polyvinyl alcohol (without a mineral additive). The coated seeds were sown in stable climatic conditions. After 7 days following sowing, the length of the seedlings was measured and compared in terms of the binding liquid applied. When analysing the average seedling length for collagen preparations, slightly longer seedlings (in the case of broad beans, peas and rape) or seedlings comparable (in the case of soybeans) with the control seeds were observed (***Figure 3***). Coating containing a collagen preparation thus provides a plant with favourable conditions at the early stage of sprout growth, which translates into an effective growth of the plant.

Legume breeding is difficult considering the scarce and complex nature of resistance in legumes in general [41]. Their cultivation is strongly hampered in the Mediterranean and Middle East farming systems by the occurrence of broomrape, causing significant yield losses [42, 43]. Considering the above, the average seedling length after 29 days following sowing into the soil without access to water in socalled laboratory drought conditions was determined. Seedling length for seeds coated with a collagen preparation and addition of dolomite, as well as seeds without coating, i.e. control seeds were analysed (***Figure 4***). Higher seedling growth values were obtained for seeds coated with collagen hydrolysate in comparison with control seeds. That is why collagen coating rich in micronutrients necessary for seed growth improves the condition of plants in laboratory drought

Table 1. *Characteristics of collagen hydrolysates.*

Parameter	GBR	CHRGB	CHR1AF	CHR1AE	CHR1AEC	CHR1AC	CHR1ACE
Dry substance, %	8.46	10.85	8.46	9.22	28.43	28.22	35.35
Total ash, %	0.47	5.53	6.50	11.61	5.38	6.59	6.49
Total nitrogen, %	15.60	15.48	16.19	14.86	15.97	15.84	14.91
Dermic substance, %	87.67	87.00	91.02	83.51	89.75	89.01	83.79
Aminic nitrogen, %	0.02	2.10	1.35	3.12	0.88	0.99	1.77
pH	3.70	8.31	11.12	8.05	7.91	11.12	8.76

Table 2. *Concentration of amino-acids in collagen hydrolysate.*

No.	Amino-acid	Abbreviation	Concentration, mg/l	Concentration, pmol/ul
1	Glutamic acid	GLU	82.02	557.44
2	NH3/Arginine	NH3/ARG	22.29	116.59
3	Alanine	ALA	41.13	461.69
4	Proline	PRO	39.15	340.03
5	Leucine	LEU	45.94	350.27
6	Lysine	LYS	48.42	324.53
7	Hydroxyproline	HYP	57.60	439.27

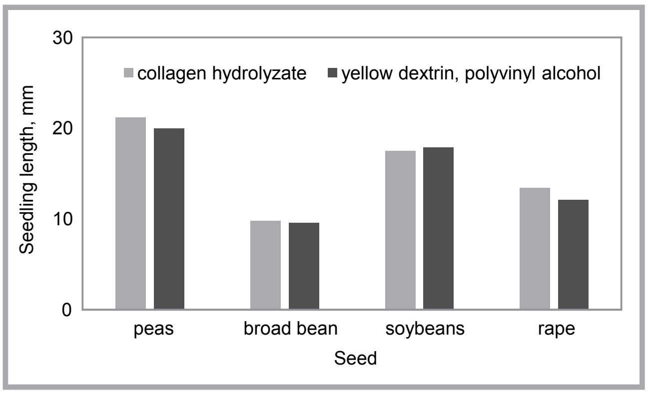

Figure 3. *Average seedling length for seeds coated with a different binding liquid (collagen hydrolysate, yellow dextrin, and polyvinyl alcohol).*

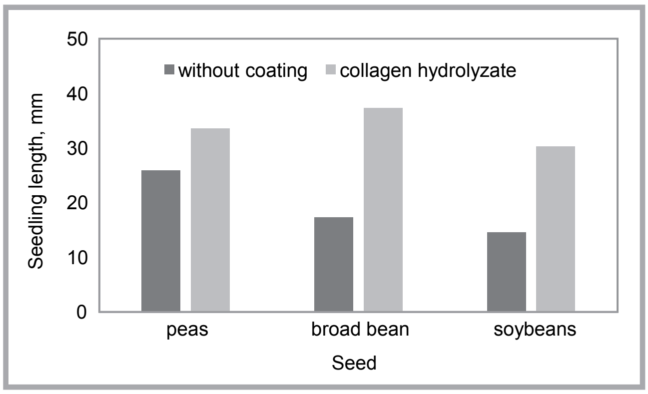

Figure 4. *Average seedling length after 29 days following sowing, without access to water, for seeds with and without coating.*

conditions as well. These conclusions were confirmed with an organoleptic inspection of the plants' condition.

After 29 days following sowing, all of the legume species tested originating from coated seeds were characterised by greater flexibility and elasticity than those originating from noncoated seeds. Therefore it may be concluded that the use of collagen hydrolysate for seed coating improves the growth and condition of legumes in difficult cultivation conditions (drought conditions). This coincides with the results provided by Gaidau *et al*., who confirmed that the use of collagen hydrolysate for coating wheat seeds has a significant impact on the density and growth of a plant's biomass at extreme soil pH conditions [40].

Type I collagen is an important biopolymer and has been widely used in biomaterials due to its excellent biocompatibility and biodegradable properties [44]. Inherent chemical or physical differences in the properties of collagen vary depending on the species of collagen. In order to enhance the durability of collagen-based biomaterials, a cross-linking procedure becomes necessary to improve their biological stability [45]. In order to provide proper durability and stability of a collagen preparation made from tanning waste, an optimum composition of a seed coating formulation was determined that contained latex (20%) and collagen hydrolysate (80%). Seeds coated with collagen hydrolysate and dolomite, as well as coated using the latex and dolomite formulation tested were sown into the ground in laboratory drought conditions. The seedling length was measured at regular intervals and compared in terms of the coating additives applied. Having analysed the average seedling length for seeds coated with a collagen preparation and the formulation developed, it was found that the addition of latex to the collagen preparation improves the growth of plants in the days following sowing in the case of all of the types of seeds tested, i.e. for peas, broad beans and soybeans (***Figure 5***). What is more, the average length of seedlings originating from non-coated seeds was marked on diagrams on the 29th day. In this way it was confirmed yet again that the addition of coating containing a collagen preparation significantly improves plant growth, which coincides with observations found in references [20, 21, 40].

Conclusions

Collagen hydrolysate contains specific amino-acids, such as proline, hydroxyproline and glutamic acid, which are nutrients and irrigation agents for plants. Therefore, increased resistance to unfavourable climatic conditions will be provided. Also, the compatibility of protein hydrolysate with many other chemical materials (dolomite, kaolin, latex) used in seed and plant treatment due to the high

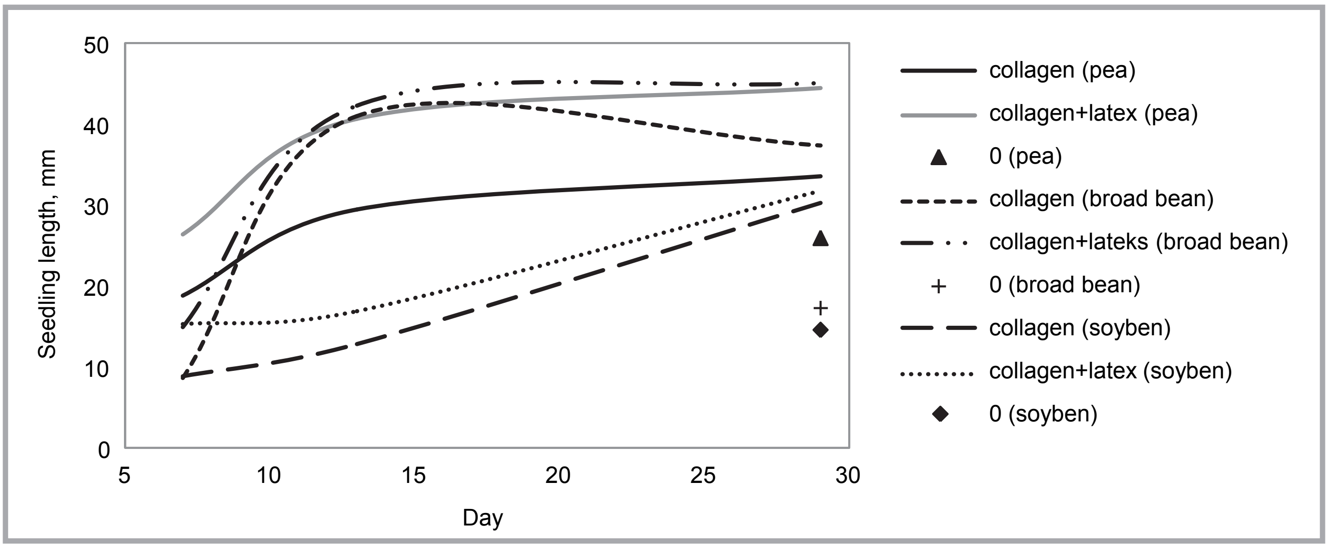

Figure 5. *Average length of peas, broad beans and soybean seedlings in the days following sowing (without access to water) for seeds coated with collagen hydrolysate and the latex formulation and for seeds without coating.*

miscibility in different solvents, adhesivity increased absorption at leaf level, and biodegradability opens the way for toxicity reduction and plant health stimulation. The higher and better-defined quality standards in the seed and coating industry, combined with additional quality demands for enhanced seeds, will continue to improve stand establishment potential for growers. Due to interdisciplinary approaches through research and technological innovations in biosciences, biotechnology and engineering, it is possible to design eco-friendly specialty chemicals from nature's abundant renewable resources. With increasing public awareness of the potential environmental and health hazards of both agrochemicals and fertilisers, as well as advances in biotechnology to improve the performance of agricultural products, the application of collagen to seeds is likely to increase in the future.

Acknowledgements

This paper was written as part of the "New treatment based on collagen hydrolysates for increasing the drought resistance of Leguminosarum seedlings" (Agreement No. 6/ RUSPLUS-INNO/2016) and "New treatment for rape seeds based on collagen hydrolysates in order to increase the drought resistance of rape seedlings" (Agreement No. EUREKA/COLL-RAPE/5/2017) projects, financed by the National Centre for Research and Development.

References

1. Kuchlan MK, Dadlani M, Samuel DVK. Seed coat properties and longevity of soybean seeds. *Journal of New Seeds* 2010; 11(3): 239-249.
2. Laskowski J, Skonecki S. Influence of moisture on the physical properties and parameters of the compression process of legumes' seeds. *International Agrophysics* 1997**;** 11(45): 245-256.
3. Talebi R, Naji AM, Fayaz F. Geographical patterns of genetic diversity in cultivated chickpea (Cicer arietinum L.) characterized by amplified fragment length polymorphism. *Plant Soil Environment 2008;* 54(10): 447-452.
4. Borowska M, Prusinski J, Kaszkowiak E. Production results of intensification of cultivation technologies in three lupin (Lupinus L.) species. *Plant, Soil and Environment 2015;* 61 (9): 426-431.
5. Adebiyi AP, Aluko RE. Functional properties of protein fractions obtained from commercial yellow field pea (Pisum sativum L.) seed protein isolate. *Food Chemistry* 2011; 128: 902-908.
6. Can Karaca A, Low N, Nickerson M. Encapsulation of Flaxseed Oil Using a Benchtop Spray Dryer for Legume Protein–Maltodextrin Microcapsule Preparation. *Journal of Agricultural and Food Chemistry* 2013; 61(21): 5148-5155.
7. Oakes JL, Bost KL, Piller KJ. Stability of a soybean seed-derived vaccine antigen following long-term storage, processing and transport in the absence of a cold chain. *Journal of the Science of Food and Agriculture* 2009; 89: 2191-2199.
8. Pereira HVR, Saraiva KP, Carvalho LMJ, Andrade LR, Pedrosa C, Pierucci APTR. Legumes seeds protein isolates in the production of ascorbic acid microparticles. *Food Research International* 2009; 42(1): 115-121.
9. Szczepaniak W, Grzebisz W, Potarzycki J, Łukowiak R, Przygocka-Cyna K. Nutritional status of winter oilseed rape in cardinal stages of growth as the yield indicator. *Plant, Soil and Environment* 2015; 61(7): 291-296.
10. Tas S, Tas B. Some physiological responses of drought stress in wheat genotypes with different ploidity in Turkiye. *World Journal of Agricultural Sciences* 2007; 3: 178-183.
11. Flowers TJ. Improving crop salt tolerance. *Journal of Experimental Botany* 2004; 55: 307-319.
12. Yazar A, Gökçel F, Sezen MS. Corn yield response to partial rootzone drying and deficit irrigation strategies applied with drip system. *Plant Soil and Environment* 2009; 55(11): 494-503.
13. Duarte CR, Neto JLV, Lisboa MH, Santana RC, Barrozo MAS, Murata VV. Experimental study and simulation of mass distribution of the covering layer of soybean seeds coated in a spouted bed. *Brazilian Journal of Chemical Engineering* 2004; 21(1), 59-67.
14. Mazibuko TG, Modi AT. Comparison of osmopriming and seed coating with calcium salts for green bean performance under field conditions. I. Cotyledonal cracking. *South African Journal of Plant and Soil* 2005; 22(1): 9-15.
15. Tapan A, Kundu S, Rao AS. Zinc delivery to plants through seed coating with nano-zinc oxide particles. J*ournal of Plant Nutrition* 2016; 39(1): 136-146.
16. Tripathi B, Pandey A, Bhatia R, Walia S, Yadav A K. Improving soybean seed performance with natural colorant-based novel seed-coats. *Journal of Crop Improvement* 2015; 29(3): 301-318.
17. Qiu J, Wang R, Yan J, Hu J. Seed film coating with uniconazole improves rape seedling growth in relation to physiological changes under waterlogging stress. *Plant Growth Regulation* 2005; 47(1): 75-81.
18. Rehman A, Farooq M. Boron application through seed coating improves the water relations, panicle fertility, kernel yield, and biofortification of fine grain aromatic rice. *Acta Physiologiae Plantarum* 2013; 35(2): 411-418.
19. Zeng D, Wang F, Wang Z. Preparation and study of a novel, environmentally friendly seed-coating agent for wheat. *Communications in Soil Science and Plant Analysis* 2012; 43(10): 1490-1497.
20. Ławińska K, Gendaszewska D, Grzesiak E, Jagiełło J, *Obraniak A*. Use of tanning waste in seed production. *Przemysl Chemiczny* 2017: 97(11), 2344-2347.
21. Ławińska K, Gendaszewska D, Grzesiak E, Lasoń-Rydel M, Obraniak A. Coating of leguminosarum seeds with collagen hydrolyzates from tanning waste. *Przemysł Chemiczny* 2017; 9: 1877-1880.
22. Dziuba R, Jabłońska M, Sulak K, Ławińska K. Textile Sector of the Visegrad Group Countries in Trade with the European Union. *FIBRES & TEXTILES in Eastern Europe* 2018; 26, 6(132): 24-29. DOI: 10.5604/01.3001.0012.5160.
23. Ławińska K, Serweta W, Modrzewski R. Qualitative evaluation of the possible application of collagen fibres: composite materials with mineral fillers as insoles for healthy footwear. *FIBRES & TEXTILES in Eastern Europe* 2018; 26, 5(131): 81-85. DOI: 10.5604/01.3001.0012.2536
24. Sundar VJ, Raghavarao J , Muralidharan C, Mandal AB. Recovery and utilization of chromium-tanned proteinous wastes of leather making: A review. *Critical Reviews in Environmental Science and Technolog*y. 2011; 41(22): 2048-2075.
25. Ławińska K, Obraniak A, Modrzewski R. Granulation process of waste tanning shavings. *FIBRES & TEXTILES in Eastern Europe* 2019; 27, 2(134): 107-110. DOI: 10.5604/01.3001.0012.9994
26. Ławińska K, Serweta W, Modrzewski R. Studies on water absorptivity and desorptivity of tannery shavings-based composites with mineral additives. *Przemysł Chemiczny 2019;* 98(1): 106-109.
27. Gluba T, Obraniak A. The kinetics of agglomeration of particulate material in the disc granulator. Chemical Engineering and Equipment 2009; 48(4): 46-47.
28. Obidziński S, Joka M, Fijoł O. Two-stage agglomeration of fine-grained herbal nettle waste. *International Agrophysics* 2017; 31: 515-523.
29. Obraniak A, Lawinska K. Spectrophotometric analysis of disintegration mechanisms (abrasion and crushing) of agglomerates during the disc granulation of dolomite. *Granular Matter* 2018; 20: 7.
30. Obraniak A, Gluba T, Ławińska K, Derbiszewski B**.** Minimisation of environmental effects related with storing fly ash from combustion of hard coal. *Environment Protection Engineering* 2018; 4: 177-189.
31. Heim A, Obraniak A, Gluba T. Change in the properties of beds granulated in disc granulators. *Physicochemical Problems of Mineral Processing* 2010; 44: 53-62.
32. Almeida C, Rocha S C S, Razera L F. Polymer coating, germination and vigor of broccoli seeds. *Scientia Agricola (Piracicaba, Braz.)* 2005; 62(3): 221-226.

33. Domoradzki M, Korpal W. Germination analysis for coated radish seeds, carried out using four selected bed types. *Agricultural Engineering* 2005; 2(111): 27-33.
34. Ławińska K, Modrzewski R, Serweta W. The phenomenon of screen blocking for mixtures of varying blocking grain content. *Gospodarka Surowcami Mineralnymi – Mineral Resources Management* 2018; 34(1): 83-95.
35. Lawinska K, Modrzewski R. Analysis of sieve holes blocking in a vibrating screen and a rotary and drum screen. *Physicochemical Problems of Mineral Processing* 2017; 53(2): 812-828.
36. Lawinska K, Wodzinski P, Modrzewski R. A method for determining sieve holes blocking degree. *Physicochemical Problems of Mineral Processing* 2015; 51(1): 15-22.
37. Grzesik M, Janas R, Górnik K, Romanowska-Duda Z. Biological and physical methods of seed production and processing. *Journal of Research and Applications in Agricultural Engineering* 2012; 57(3): 147-152.
38. Patent PL 205570 Novel fungicide compositions based on pyridylmethylbenzamide and propamocarb derivative (31.05.2010 WUP 05/10).
39. Hirano S, Hayashi M, Okuno S. Soybean seeds surface-coated with depolymerised chitins: chitinase activity as a predictive index for the harvest of beans in field culture. *Journal of the Science of Food and Agriculture* 2000; 81: 205-209.
40. Gaidau C, Epure DG, Niculescu M, Stepan E, Radu E, Gidea M. Application of Collagen Hydrolysate in Cereal Seed Treatment. *XXXIII IULTCS Congress* November 24th – 27th, 2015 Novo Hamburgo/Brazil.
41. Rubiales D, Pérez-De-Luque A, Fernández-Aparicio M, Sillero J C, Román B, Kharrat M, Khalil S, Joel D M, Riches C. Screening techniques and sources of resistance against parasitic weeds in grain legumes. *Euphytica* 2006; 147: 187-199.
42. Parker C. Observations on the current status of Orobanche and Striga problems worldwide. *Pest Management Science* 2009; 65: 453-459.
43. Rubiales D. Legume breeding for broomrape resistance. *Czech Journal of Genetics and Plant Breeding | Agricultural Journals* 2014; 50: 144-150.
44. Lin YK, Liu DC. Comparison of physical–chemical properties of type I collagen from different species. *Food Chemistry* 2006; 99: 244-251.
45. Angele P, Abke J, Kujat R, Faltermerier H, Schumann D, Nerlich M, Kinner B, Englert C, Ruszczak Z, Mehrl R, Mueller R. Influence of different collagen species on physico-chemical properties of crosslinked collagen matrices. *Biomaterials* 2004; 25: 2831-2841.

Received 07.12.2018 ***Reviewed 04.03.2019***