Collagen Hydrolysate as Sustainable Additive for Cereal Seed Treatment

CARMEN GAIDAU[1], MIHAELA DOINA NICULESCU[1]*, DORU GABRIEL EPURE[2], MADALINA TUDORACHE[4], ELENA RADU[3], RUSANDICA STOICA[3], MADALINA IGNAT[1], MIHAI GIDEA[5]

[1]Leather Research Department, INCDTP-Leather and Footwear Research Institute Division, 93 Ion Minulescu Str, 031215, Bucharest, Romania,

[2]Probstdorfer Saatzucht Romania SRL, 20 Siriului Str., 014354, Bucharest, Romania

[3]National Institute for Research &Development in Chemistry and Petrochemistry- ICECHIM, Bioresources Department, 202 Splaiul Independentei, 060021, Bucharest, Romania

[4] University of Bucharest, Department of Organic Chemistry, Biochemistry and Catalysis, 4-12 Regina Elisabeta Blvd., Romania

[5]University of Agronomic Science and Veterinary Medicine-USAMV, 59Marasti Str., 01146, Bucharest, Romania

The paper presents new results on the application of collagen hydrolysates characterised in terms of molecular weight, polydispersity (GPC/ SEC), chemical composition and free amino acid composition (GC-MS) for the treatment of cereal seeds. Surface tension and contact angle determinations (VGA Optima XE) have highlighted an improvement in the hydrophilicity of collagen hydrolysates in pesticide mixtures as premises for the adherence of wheat seeds onto the surface. The identification of a 39% higher gibberellic acid concentration (RP-HPLC) in germinated seeds in the collagen hydrolysate, and the presence of free amino acids and low molecular weight fractions were correlated with the biofertilizing and biostimulating effects registered in field experiments on wheat crops.

Keywords: collagen hydrolysate, wheat seed treatment, gibberellic acid, biostimulant

The interest for leather industry by-product reclaiming has recently increased due to the environmental constraints, reduction of conventional sources of energy and raw materials scarcity [1]. Agriculture is also facing an increased pressure against pesticides toxicity, soil quality, climate change and increased demand for organic products. Already on the market, fertilizers with vegetable or animal protein hydrolysates and amino acids content are presented as systemic products with nutrition, growth stimulation and beneficial properties for plant health.

The main actions of this class of products, recommended for organic agriculture, are the increase of stress resistance (i.e. chill, drought etc.) and an unspecific hormone-like activity on plant metabolism. The compatibility of protein hydrolysate with many other chemical materials used in seed and plant treatment due to the high miscibility in different solvents, adhesion, increased absorption at leaf level, biodegradability opens a way for toxicity reduction and plant health stimulation. The specific amino acids of collagen, proline and hydroxyproline act mainly as hydric balance of the plant, strengthening the cellular walls in such a way that they increase resistance to unfavorable climatic conditions.

Many papers were devoted to the chemical-enzymatic hydrolyses of shavings and separation of protein from chromium [2-5]. There are some reports regarding the use of collagen hydrolysates as soil fertilizer with effect on reduced nitrate content of lettuce [6], or as foliar fertilizer for maize and sunflower crops [7], but without information on seed treatment and the influence on seed physiological modifications.

The paper presents the research progresses [8, 9] on cereal seed treatment with collagen hydrolysate and mixtures with specific pesticides and the influence of the collagen hydrolysate concentration on wheat crops experimented at field level in different conditions of soil *p*H and climate.

* email: mihaelaniculescu59@yahoo.com; Phone: (+40)213235060

Experimental part

Materials

The bovine shavings used as raw material showed the typical characteristics for wet blue leathers: moisture-51%, total ash-8.6%, total nitrogen-16.5%, chromium oxide -4.4%, *p*H of aqueous extract-4.2.

Collagen hydrolysate (CH) was extracted from bovine shavings according to chemical enzymatic technology reported in our previous papers [10, 11], at pilot level.

The main characteristics of collagen hydrolysate were: dry substance-11%, total nitrogen-16%, total ash-5.2%, amino nitrogen-1.6%, organic substance - 94.8%, calcium oxide - 4.6%, *p*H-8.9, chromium oxide-not detectable.

Analytical methods

The physical-chemical methods for leather shaving and collagen hydrolysate analyses where: EN ISO 4684 (leather moisture and dry substance of CH), ISO 5397 (total nitrogen content), ICPI methods for aminic nitrogen and calcium oxide content, EN ISO 4047 (total ash content), ISO 5397 (chromium oxide), EN ISO 4045 and STAS 8619/3 for p*H*.

The molecular weight and polydispersity of collagen hydrolysate were evaluated by gel permeation chromatography system GPC / SEC (Agilent Technologies 1260 Infinity).

The identification of amino-acids, peptide, polypeptide sequences was performed by GC-MS Triple Quad (Agilent 7000).

Surface tension of selected materials based on collagen hydrolysate as well as the static contact angle on wheat seeds were measured with VGA Optima XE system (AST Products SUA) in order to estimate the compatibility with seed surface in industrial treatment.

The gibberellic acid content of wheat seeds germinated in water and in collagen hydrolysate medium for 6, 18 and 24 h was assessed [9] by RP-HPLC (Agilent HPLC 1100)

Table 1
COMBINATIONS OF COLLAGEN HYDROLYSATE, FUNGICIDES AND INSECTO-FUNGICIDES FOR COMPATIBILITY AND PHYTOTOXICITY EVALUATION

CH+ fungicide 1+insecticide 1, L/t				CH, L/t				CH+ fungicide 2, L/t			
V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
3/0.5/0.6	5/0.5/0.6	7/0.5/0.6	9/0.5/0.6	3	5	7	10	3/1	5/1	5/0.5	5/0.3

CH+ insecticide 2, L/t		CH+ insecticide 3, L/t		CH+ fungicide 4, L/t				CH+ fungicide 3, L/t			
V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24
5/1	3/1	7/0.6	9/0.6	3/0.5	5/0.5	7/1	9/1.5	3/1	5/1	7/0.3	8/0.5

as an indicator for growth stimulation properties of collagen hydrolysate.

Different mixtures of fungicide and insecto-fungicide commercial products based on imidacloprid and tebuconazole with collagen hydrolysates were evaluated for compatibility and phytotoxicity with the aim of pesticide reduction. The germination indexes were evaluated according to SR 1634.

The compatibility and phytotoxicity of collagen hydrolysate in combination with specific fungicide and insecto-fungicide commercial products were assessed for different dosages for wheat seeds preservation and are presented in table 1.

Wheat seeds treated in installation for seed treatment with collagen hydrolysate or mixture of collagen hydrolysate and specific pesticide were germinated in laboratory conditions and planted in different experimental fields with different climate and soil conditions. The range of soils *p*H was: 6; 6.3; 7.6; 8.2 and 8.9.

Results and discussions

Collagen hydrolysate analysis by gel permeation chromatography GPC/SEC (fig. 1) has led to a dispersion factor DP = 16.75, indicating a heterogeneous system associated with a broad polydispersity with complex functions such as nutrition, growth stimulation and protection (due to the composition of amino-acids, oligopeptides with amphoteric, buffer, complexing or film forming properties).

Three oligomers were identified, with average molecular weights of 6351 Da (53%), 1293 Da (40%) and under 100 Da (7%) that are presented in table 2.

The GC-MS (fig. 2) confirmed the composition of collagen hydrolysate with small peptides and free amino-

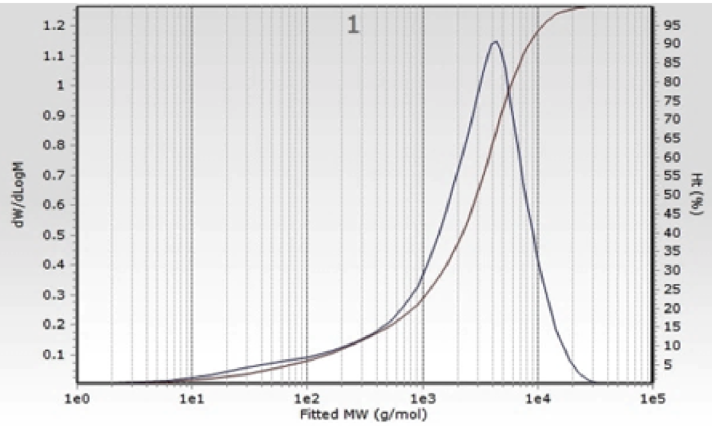

Fig. 1. GPC / SEC analysis of collagen hydrolysate

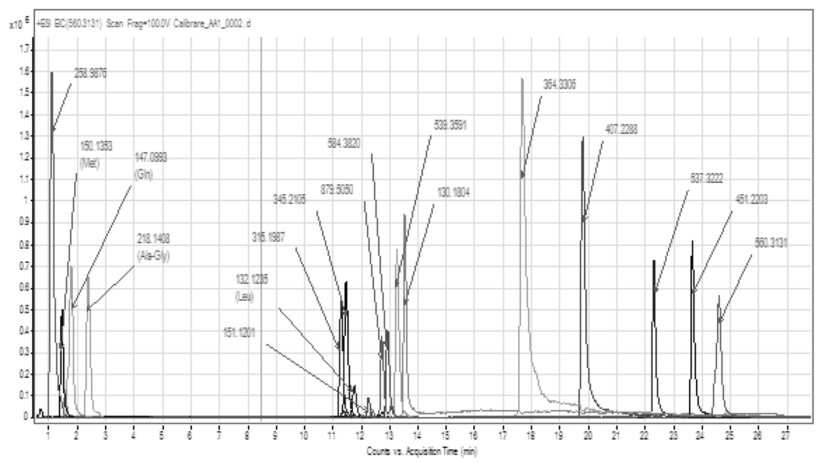

Fig. 2. GC-MS chromatogram of collagen hydrolysate

Table 2
THE AVERAGE MOLECULAR WEIGHTS OF COLLAGEN HYDROLYSATE

Oligomer	O1	O2	O3
Average molecular weights (Mw), Dalton	6351	1293	<100
Share oligomers in the mixture,%	53	40	7

Composite	V1	V2	V3	V4	V5	V6	V7	V8
Compatibility	1	1	1	1	-	-	-	-
Phytotoxicity	0	0	1	1	0	0	0	1

Composite	V9	V10	V11	V12	V13	V14	V15	V16
Compatibility	1	1	1	1	1	1	0	1
Phytotoxicity	0	0	0	0	1	1	1	1

Composite	V17	V18	V19	V20	V21	V22	V23	V24
Compatibility	1	0	1	1	1	1	1	1
Phytotoxicity	0	1	0	1	0	0	0	0

Table 3
THE COMPATIBILITY AND PHYTOTOXICITY OF COLLAGEN HYDROLYSATE IN PESTICIDE COMPOSITE

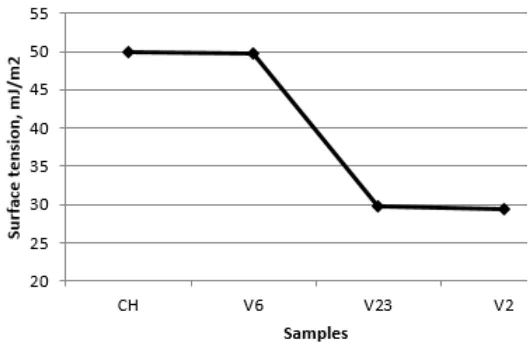

Fig. 3. Surface tension of collagen hydrolysate mixtures with insecticide and fungicide

acids (methionine, glutamine, alanine, glycine, leucine) ensuring a fast absorption of nutrients and easy metabolism and with higher particles with film forming properties for seed surface covering.

The analyses of surface tension of collagen hydrolysate and combinations with pesticides (V6, V23 and V2) showed that the surface tensions of collagen hydrolysate mixtures with fungicide (V23) and fungicide with insecticide (V2) are lower as compared to collagen hydrolysate (CH and V6), as can be seen in figure 3. The surface tension dropped from 50 mJ/m^2 (CH and V6) to 29 mJ/m^2 in the case of collagen hydrolysate in mixtures with fungicides and insecticides (V23 and V2).

The surface tension values were in agreement with the contact angle values measured on wheat seeds surfaces and presented in figure 4. The hydrophilic properties of the mixtures of collagen hydrolysate with fungicides and insecticides (V2 and V23) are the premises for good quality treatments of cereal seeds.

The identification of the influence of collagen hydrolysate on seed metabolism was evaluated by analyzing the gibberellic acid, an unspecific hormone-like substance involved in seed metabolism in germination stage. The analysis results are presented in figure 5 and show that the collagen hydrolysate has stimulated the wheat seed metabolism after 24 h of germination. Increasing of gibberellic acid by 34-39% after 18 and 24 h of sprouting represents a significant influence [12] of collagen hydrolysate on seed germination and an original result.

The compatibility and phytotoxicity tests for different concentrations of collagen hydrolysate in specific pesticides composites allowed setting the best concentration of collagen hydrolysate in pesticide composition. From the results of table 3 we can conclude that the concentration of 7L collagen hydrolysate per 1 ton of wheat seeds does not display phytotoxicity and the combinations of collagen hydrolysate and pesticides are compatible and without phytotoxicity in different proportions depending on the type of pesticides.

The treated seeds were germinated in laboratory conditions and were seeded in experimental fields. The experimental fields of 10 sqm were seeded with wheat treated with collagen hydrolysate with or without pesticides (table 3) and with untreated seeds in double repetitions.

The influence of collagen hydrolysate (3L/t) on crop density was evaluated on different *p*H of soil, at values of 6, 6.3; 7.6; 8.2 and 8.9 and the highest increased crop densities were registered for *p*H 6 and 8.9 as compared to the untreated seeds. The results presented in figure 6 showed that the collagen hydrolysate has the capacity to act favorably in extreme conditions of soil *p*H supporting the literature related to good influence of protein based fertilizers in climate change conditions.

The average increase of crop density was by 9.5% as compared to untreated seeds.

The highest increase of germination (fig. 7) was recorded for the seeds treated with collagen hydrolysate in concentration of 7L/t in combination with 0.3L/t fungicide (V23).

In this case the reduction of fungicide was from 1L/t (recommended by producer) to 0.3L/t.

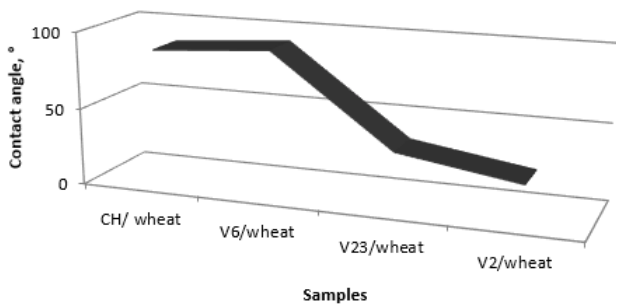

Fig. 4. Contact angle of collagen hydrolysate on seed surface

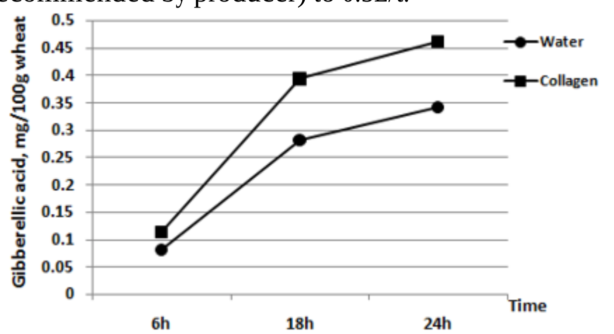

Fig. 5. Gibberellic acid content of germinated wheat seeds in water and in collagen hydrolysate

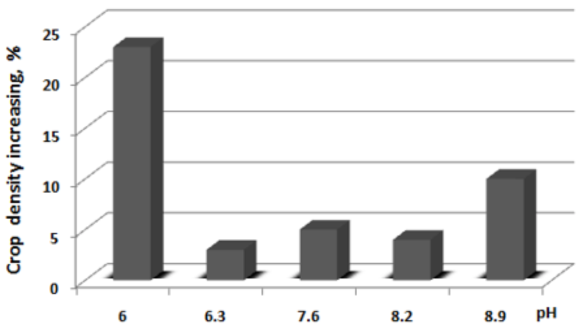

Fig. 6. The wheat crop density increasing in different soils

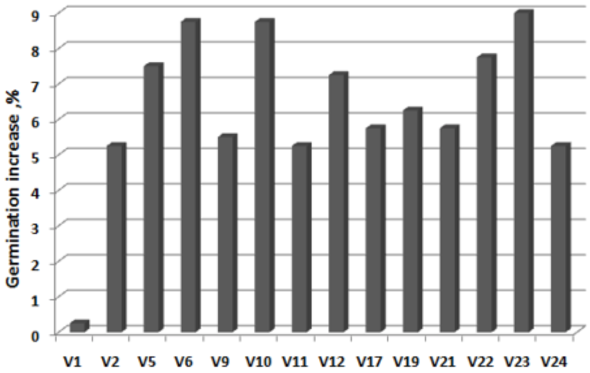

Fig. 7. The germination increase of treated wheat seeds

The biomass increased for all treated seeds (fig. 8) and the highest increase (8.7%) was for the samples treated with collagen hydrolysate in concentration of 3 L/t (V5) and 5L/t (V6) with (V23) and without pesticides.

The other important index in seed treatment is the number of abnormal seeds with influence on crop yield and quality. Figure 9 shows that the number of abnormal seeds decreased as compared to untreated seeds up to 6.7%, due to the influence of collagen hydrolysate on seed metabolism and health.

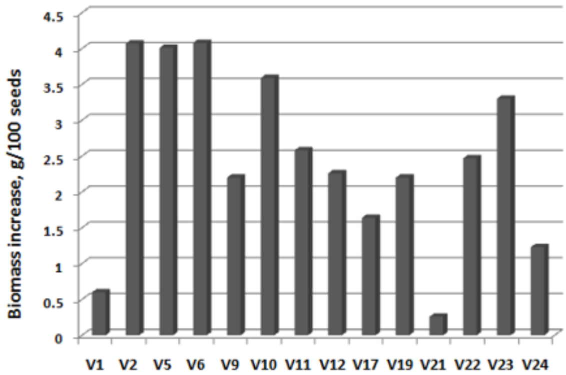

Fig. 8. The wheat crop biomass increase

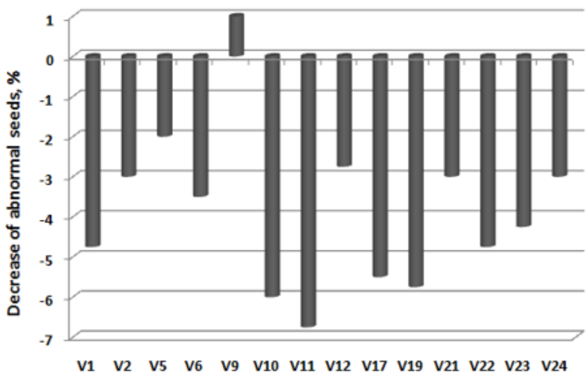

Fig. 9. The decrease of abnormal treated wheat seeds as compared to the untreated seeds

Conclusions

The treatment of wheat seeds with low molecular weight collagen hydrolysate (under 6.5 kDa) in concentrations of 3L/t to 5L/t has a benefic influence on plant density and on biomass increase in extreme conditions of soil *p*H. The increased concentration of gibberellic acid by 39% in germinated seeds in collagen hydrolysate can explain the seed and plant metabolism improvement due to the easy assimilation of low molecular peptides with free amino acid content. The wheat seeds were treated with a mixture of 5L/t collagen hydrolysate with specific pesticides according to conventional technologies and showed that a reduction of pesticides from 1L/t to 0.3L/t can be done with influence on biomass increase and abnormal seeds decrease. The decrease of abnormal seeds number was recorded in almost all experimental wheat crops treated with collagen hydrolysate.

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References

1.*** KET-INDUSTRIAL BIOTECHNOLOGY, 2011 http://www.bio-economy.net/reports

2. ALEXANDER, K. T. W., The conversion of solid tannery waste into saleable by-products, US/RER/94/105, UNIDO, 1995.

3. TAYLOR, M. M., CABEZA, L. F., DIMAIO, G. I., BROWN, E. M., MARMER, W. N., CARRIO, R., CELMA, P. J., COT, J., Processing of Leather Waste: Pilot Scale Studies on chromium shavings. Part I. Isolation and characterization of protein products and separation of chromium cake, JALCA, **93**, 1998, p. 61.

4. KOLOMAZNIK, K., MLADEK, M., LANGMAIER, F., JANACOVA, D., Experience in Industrial Practice of enzymatic dechromation of chromium shavings, JALCA, **94**, 1999, p. 55.

5. CANTERA, C.S., Adding value to chrome shavings: hydrolysates as retanning materials, World Leather, **2-3**, 2003, p. 27.

6. KOLOMAZNIK, K., VISWANATHAN, M., BULJAN, J., Enzymatic digestion of chrome shavings, UNIDO Report US/RAS/92/120, 2001.

7. OPRICA, D. I., CIOROIANU, T. M., LUNGU, M., BAREA, I. A., A new eco-friendly foliar fertilizer with bone glue suitable for crops of maize and sunflower, Rev. Chim.(Bucharest), **65**, no. 1, 2014, p. 1.

8. GAIDAU, C., NICULESCU, M., STEPAN, E., EPURE, D.-G., GIDEA, M., New Mixes Based on Collagen Extracts with Bioactive Properties, for Treatment of Seeds in Sustainable Agriculture**,** Current Pharmaceutical Biotechnology Journal, **14**, no. 9, 2013, p. 792.

9. EPURE D.-G., GAIDAU C., NICULESCU M., STEPAN E., EPURE L.-I., GIDEA M., Use of collagen hydrolysate for cereal seed treatment, e-Proceedings of 10th AICLST, Okayama, Japan, 2014.

10. GAIDAU, C., NICULESCU, M., STEPAN, E., TALOI, D., FILIPESCU, L., Additives and advanced biomaterials obtained from protein and protein by-products, Rev. Chim.(Bucharest), **60**, no. 5, 2009, p. 501.

11. NICULESCU, M., BAJENARU, S., GAIDAU, C., SIMION, D.,FILIPESCU,L., Extraction of the Protein Components as Amino-Acids Hydrolysates from Chrome Leather Wastes through Hydrolytic Processes, Rev. Chim.(Bucharest), **60**, no. 10, 2009, p. 1070.

12. COLLA, G., ROUPHAEL, Y., CANAGUIER, R., SVECOVA, E., CARDARELLI, M., Biostimulant action of plant-derived protein hydrolysate produced through enzymatic hydrolysis, Frontiers in Plant Science, **5**, no. 448, 2014, p. 1.

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