ANNALS OF THE UNIVERSITY OF ORADEA
FASCICLE OF TEXTILES, LEATHERWORK

THE INFLUENCE OF NOVEL TENSIDIC "ARCHETYPES" ON COLLAGEN BYPRODUCT FOR AGRICULTURE APPLICATIONS

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Abstract: *The European Biostimulants Industry Council defines biostimulators as a class of compounds that include substances or microorganisms which have a positive impact on plant growth, yield and chemical composition, as well as on the growth of effects on tolerance to biotic and abiotic stress. Gelatin was proven to enhance plant growth due to better transport of amino acids in plants when is used as a root biostimulant. Tomato plants treated with protein hydrosysates had a higher content of ascorbic acid, lycopene, total polyphenols and higher lipophilic antioxidant activity than the control samples treated with water. Bolaamphiphiles and gemini are new classes of amphiphilic surfactants, with different applications due to their high ability to deliver active substances and capacity to emulsify the mezzo nutrients and microelements in gelatin.*
The aim of the paper is to develop new structured emulsions and study the influence of novel tensidic "archetypes" like layered networks, based surfactants (bolaform, gemini) on collagen gelatin with mezzo and micronutrients, for agriculture applications. In our research we have elaborated a method for including mezzo and microelements in collagen gelatin, obtaining novel stable structured emulsions, with the final purpose of application as a new class of root fertilizers in agriculture. The new multiple structured emulsions are original due to the successful inclusion of surfactants/mezzo and microelements/collagen gelatin, with high potential for biostimulation and nutrition of tomato plants.

Key words: *"archetypes" like layered networks, collagen byproduct, tomato plants biostimulation and nutrition in agriculture*

1. INTRODUCTION

The European Biostimulants Industry Council defines biostimulators as a class of compounds that include substances or microorganisms which have a positive impact on plant growth, yield and chemical composition, as well as on the growth of effects on tolerance to biotic and abiotic stress [1].

Gelatin was proven to enhance plant growth due to better transport of amino acids in plants when is used as a root biostimulant [2]. Tomato plants treated with protein hydrosysates had a higher

content of acid acorbic, lycopene, total polyphenols and higher lipophilic antioxidant activity than the control samples treated with water [3,4].

Due to properties of the surfactants such as biodegradability, nontoxicity, and adherence to surfaces, they may be successfully used in the processing of collagen byproducts destined for agriculture, in the improvement of surface properties.

The aim of the paper is to create new structured emulsions and study the influence of novel *tensidic "archetypes" like layered networks*, based on surfactants (bolaform, gemini) on collagen gelatin and mezzo and micronutrients [5-7]. The preparation of new structured emulsions was based on optimization of the main parameters system: composition, emulsifiers, and temperature, in a two-stage process. This research elaborated a new method for including mezzo and microelements in collagen gelatin obtaining novel stable structured emulsions, with application in agriculture for biostimulation and nutrition tomato plants.

2. METHOD FOR OBTAINING STRUCTURED EMULSIONS

The collagen gelatin was obtained from bovine delimed hide by acid hydrolysis at 80°C for four hours. Dried bovine gelatin was mixed with a solution containing mezzo and microelements. Physico-chemical characterizations of bovine gelatin were performed according to the standard in force or literature methods for dry substances, ash, total nitrogen and protein content, aminic nitrogen, pH, contact angle, dynamic light scattering measurements, bloom test, and viscosity.

The samples with gemini and bola were prepared by dropping a 4% solution of surfactants under continuous stirring into a solution containing 2% nonylphenol ethoxylate and 10% bovine gelatine at 60°C. Several experiments were performed with different concentrations of gelatin. The sample containing 10% gelatin proved to be the most stable. Physical-chemical characterization of bovine gelatine is presented in Table 1.

Table 1. *Physical- chemical characterization of bovine gelatine with mezzo and microelements*

Characterization	Bovine gelatine with mezzo and microelements	
Dry substance, %	23.60	
Total ash, %	7.78	
Total nitrogen, %	2.15	
Protein substance, %	12.08	
pH, pH units	6.77	
Bloom, g	248.9	
Viscosity, cPs	3450	

The samples were labeled as follows: **1**-collagen gelatin with mezzo and microelements*;* **4**-collagen gelatin with mezzo and microelements+surfactant Gemini*;* **5**-collagen gelatin with mezzo and microelements +surfactant Bola (fig.1) and **2**-surfactant Gemini; **3**-tenside Bola.

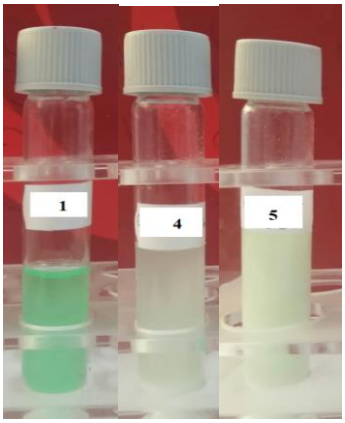

Fig. 1*: The images of* ***1****-collagen gelatin with mezzo and microelements;* ***4****-collagen gelatin with mezzo and microelements +surfactant Gemini;* ***5****- collagen gelatin with mezzo and microelements +surfactant Bola*

3. RESULTS AND DISSCUTION

Characteristics of nano-structured emulsions were obtained by: optical microscopy analyses, DLS, contact angle and microbiologically tests.

Optical microscopy analyses

The optical microscopy images from Figure 2 (e) show that the emulsion **sample 5- with Bola** *is structured like a layered network* due to the influence of *novel tensidic "archetypes".*

All emulsions made with Gemini and Bola surfactants are oriented and agglomerated in new archetypes. The results are in agreement with literature data [5-7] related to the formation of chain structures in multiple water-oil-water emulsions.

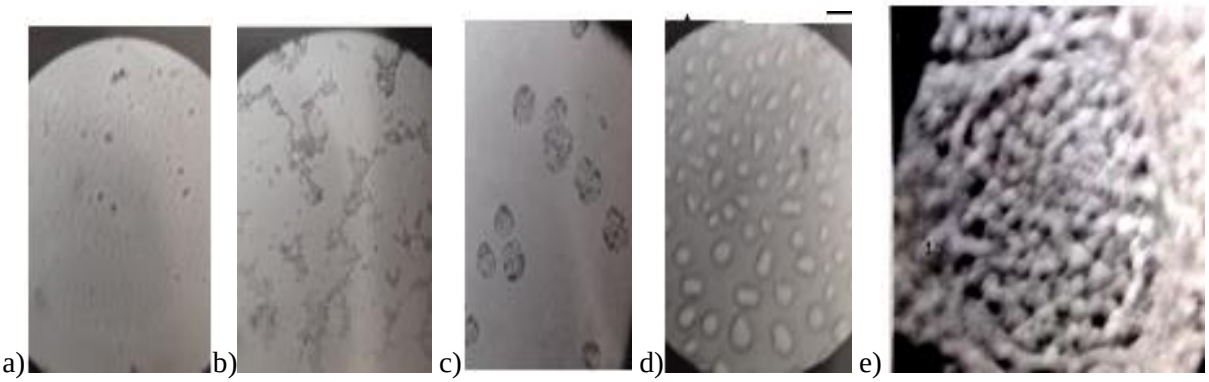

Fig.2 *: Optical microscopy images (1000x) for samples: a)****4****- mezzo and microelements; b)****2****-Gemini surfactant; c)****3****-Bola surfactant; d)****4****-collagen gelatin with mezzo and microelements +surfactant Gemini; e)****5****- collagen gelatin with mezzo and microelements +surfactant Bola*

Dynamic light scattering (DLS)

The average particle sizes of new network emulsions showed increased dimensions as compared to the collagen-mezzo-microelements mixture (697.9-613.9 nm and 304.3 nm, respectively), confirming the formation of the complex aggregates.

The highest average particle size of Gemini emulsion (Fig.3a) showed also the highest Zeta potential absolute value and improved stability as compared to bola emulsion and collagen-mezzo-microelements (Fig.3b).

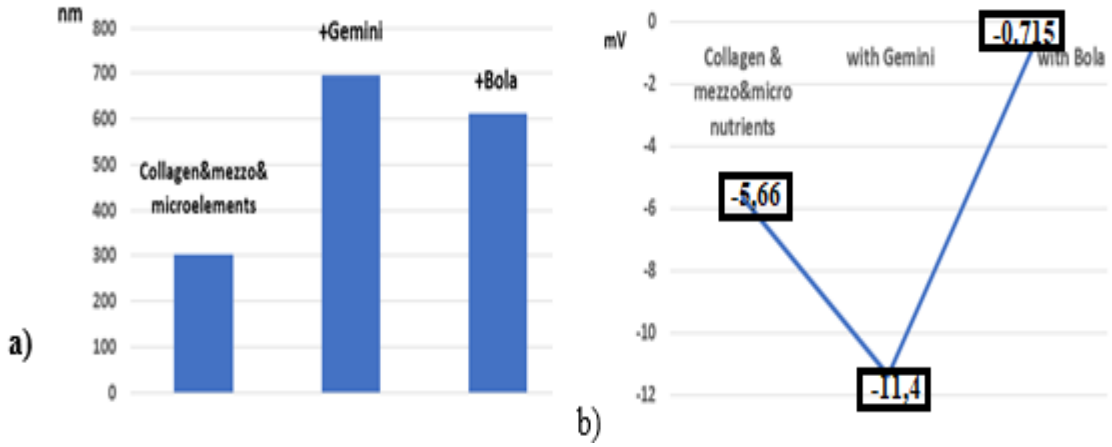

Fig. 3 *a) The average particle size and b) Zeta potential of new emulsions as compared to collagen gelatin with mezzo and micronutrients*

Contact angle

The contact angle measurements were made with a DataPhysics OCA 25 contact angle system. In Figure 4 it can be observed that Bola surfactant is the most hydrophilic and the sample obtained with this surfactant is more hydrophilic than the sample without surfactant and the sample obtained with Gemini surfactant.

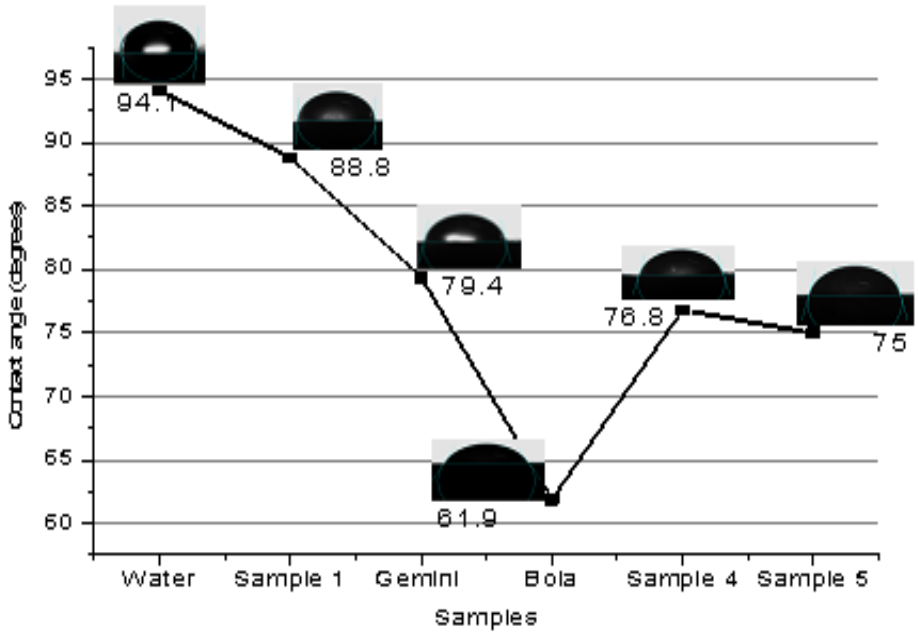

Fig. 4*: The contact angle measurements results for samples* ***1****- collagen gelatin with mezzo and microelements,* ***4****- collagen with mezzo and microelements +surfactant Gemini,* ***5****- collagen with mezzo and microelements +surfactant Bola as compared to water,* ***2****-Gemini and* ***3****-Bola surfactants on a teflon surface*

Microbiologically tests

The stock cultures of microbian inoculums of *Fusarium spp ATCC 36031, Penicilium spp, Aspergillus niger ATCC 16404, Botrytis cinerea* were grown in Czapek-Dox nutritive medium, at 28ºC for 14 days. Two decimal dilutions of paraffin oil (10:2) were made from each culture and the cell concentration in the inoculum used was $9.8x10^3$ CFU / mL (Colony Forming Units) for *Aspergillus niger*, $8.92x10^3$ CFU / mL for *Fusarium spp*., 9.5 x 10^3 CFU / mL for *Penicilium spp*., and $9.3x10^3$ CFU / mL for *Botrytis cinerea*. The experiments were performed in Eppendorf tubes, previously sterilized at 121ºC for 15 minutes. The microbial inoculum was mixed with the sample, both the microbial inoculum and the sample having constant volumes of 500 µL. All samples were tested in duplicate, and the results were expressed as a mean percentage and logarithmic reduction between the readings on the two Petri dishes corresponding to each sample (Table 2).

From Table 2 can be seen that Gemini and Bola surfactants had excellent antifungal resistance as well as collagen-mezzo-microelements that showed antifungal properties between 99.38% and 99.97%. Gemini surfactant had a positive influence on antifungal resistance of collagen-mezzo-microelements mixture for all tested strains, meanwhile, Bola surfactant showed slightly decreased antimicrobial resistance in collagen-mezzo-microelements-bola emulsions.

Table 2. *Antimicrobial resistance of new emulsions against fungus species*

Sample	Result, UFC/mL	R%	Log_{10} red.	Sample	Result, UFC/mL	R%	Log_{10} red.
Aspergillus niger Inoculum concentration	$T_0=9,8x10^3$	-	-	***Botrytis cinerea*** Inoculum concentration	$T_0=9.3x10^3$	-	-
1- Collagen-mezzo-microelements	$T_{24}=3.4x10$	99.65	2.46	1- Collagen-mezzo-microelements	$T_{24}=4.2x10$	99.55	2.35
3 - Bola	$T_{24}=0$	100	4	3 - Bola	$T_{24}=2$	99.98	3.67
5 - collagen-mezzo-microelements +Bola	$T_{24}=2,18x10^2$	97.78	1.44	5 - collagen-mezzo-microelements +Bola	$T_{24}=1.98x10^2$	97.87	1.67
2- Gemini	$T_{24}=0$	100	4	2- Gemini	$T_{24}=1$	99.99	3.97
4 - Collagen-mezzo-microelements + Gemini	$T_{24}=0$	100	**4**	4 - Collagen-mezzo-microelements + Gemini	$T_{24}=2$	99.90	**3.67**
Fusarium spp Inoculum concentration	$T_0=8.92x10^3$			***Penicilium spp.*** Inoculum concentration	$T_0=9.5\ x10^3$	-	-
1- Collagen-mezzo-microelements	$T_{24}=5.5x10$	99.38	2.21	1- Collagen-mezzo-microelements	$T_{24}=3$	99.97	3.50
3 - Bola	$T_{24}=0$	100	4	3 - Bola	$T_{24}=2$	99.98	3.68
5 - Collagen-mezzo-microelements +Bola	$T_{24}=3.22x10^2$	96.39	1.44	5 - Collagen-mezzo-microelements +Bola	$T_{24}=1.32x10^2$	98.61	1.86
2- Gemini	$T_{24}=0$	100	4	2- Gemini	$T_{24}=0$	100	4
4 - Collagen-mezzo-microelements + Gemini	$T_{24}=0$	100	**4**	4 - Collagen-mezzo-microelements + Gemini	$T_{24}=0$	100	**4**

4. CONCLUSIONS

-The aim of this research was fulfilled to develop new structured emulsions and to study the influence of novel tensidic “archetypes” like layered networks, based surfactants (bolaform, gemini) on collagen gelatin with mezzo and micronutrients, for agriculture applications.

-New multiple structured emulsions were successfully made by a two-steps process for mezzo and microelements and collagen gelatin inclusion in a W/O/W system. The archetype structure of new emulsions was demonstrated by optical microscopy.

-The emulsions with particle sizes of 697.9- 613.9 nm as compared to 304.3 nm of collagen gelatin mixture with mezzo and microelements were obtained. The new emulsions showed lower contact angle values which are premises for improved displaying on plant leaves or roots and a higher potential of growth biostimulation and nutrition.

-Gemini surfactants showed an improved influence on antifungal resistance of collagen gelatin-mezzo-microelements mixtures and emulsion stability.

- In our paper it was elaborated a new method for including mezzo and microelements in collagen gelatin, obtaining novel stable structured emulsions, with the final purpose of application *as a new class of root fertilizers in agriculture*.

-The new multiple structured emulsions are original due to the successful inclusion of surfactants/mezzo and microelements/collagen gelatin, with high potential for biostimulation and nutrition of tomato plants.

ACKNOWLEDGEMENTS

The present work was supported by the Romanian Ministry of Research, Innovation and Digitalization, CNDI-UEFISCDI, project number 260/2021, PN-III-P3-3.5-EUK-2019-0249, GEL-TREAT, E!13432

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