THE INFLUENCE OF SURFACTANTS IN OBTAINING NEW BYPRODUCTS, FOR AGRICULTURE APPLICATIONS

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The aim of the paper is to obtain new byproducts based on surfactants (gemini – polymethylene-α, ω-bis (N, N-dialkyl-N-deoxy-d-glucitolammonium iodides or bolaform – demecarium bromide) and protein hydrolysates (keratin and collagen) with micro and macro nutrients for applications in agriculture. A method was developed to include micro and macronutrients in keratin and collagen hydrolysates, in order to obtain new byproducts-bioemulsions (stable because of surfactants), with final goal of application as a new class of root fertilizers for cereals (e.g., corn). The newly obtained byproducts (bioemulsions based on surfactants) were characterized by: dynamic light scattering measurements, contact angle, optical microscopy and microbiological tests against fungal attack of *Fusarium* spp. and *Botrytis cinerea*. Better results were obtained for gemini surfactant based on sugar – polymethylene-α, ω-bis (N, N-dialkyl-N-deoxy-d-glucitolammonium iodides) due to the properties such as: biodegradability, nontoxicity and adherence to surfaces. The new fertilizer created in this research – bioemulsions based on surfactants, can support the general structure of the grains as well as the chlorophyll content, increasing the growth yield. The fertilizer is indicated for any type of crops and soils, with recommended use as additional fertilizer for plants (cereals) in the vegetation and growth phases, with a maximum need for nutrients.

Keywords: hydrolysates (keratin and collagen) with micro and macro nutrients; byproducts-bioemulsions based on surfactants; new class of root fertilizers in cereals

INTRODUCTION

In this research new byproducts based on surfactants (gemini – polymethylene-α, ω-bis (N, N-dialkyl-N-deoxy-d-glucitolammonium iodides or bolaform – demecarium bromide) and protein hydrolysates (keratin and collagen) with micro and macro nutrients were created and their applications in agriculture were studied, as a new class of root fertilizers for cereals (e.g., corn) (Tadros, 2005; Hughes *et al.*, 2021; Nuraje *et al.*, 2013; Varasteanu, 2014). The keratin and collagen hydrolysates were obtained by acid hydrolysis at 80°C for four hours. These keratin and collagen hydrolysates contain peptides and free amino acids with micro (Cu, Mg, Mo, B, Zn, Fe) and macro (N, P, K) nutrients, respectively. The new byproducts-bioemulsions are original due to the successful inclusion of surfactants/mezzo and microelements/ hydrolysates of keratin and collagen.

Bolaamphiphiles and gemini are new classes of amphiphilic surfactants, with different applications due to their high capacity for emulsification (e.g., micro and macro nutrients). Due to properties of the surfactants such as: biodegradability, nontoxicity, adherence to surfaces, they may be successfully used in processing of byproducts destined for agriculture, in improvement of surface properties. In this research a new method was elaborated for including micro and macronutrients in hydrolysates of keratin and collagen, in order to obtain new byproducts-bioemulsions, with application in agriculture. These bioemulsions were applied as a new root fertilizer to 20 corn kernels compared to a control and the evolution of the plants was followed by measuring the stems and roots 10 days before planting and 10 days after they were

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planted by applying the treatment, i.e., spraying the plants with 10 ml every 3 days. Due to the content of protein hydrolysates with a complex composition of peptides and free amino acids, with pelliculogenic, chelating and buffering properties, the new bioemulsions provide the complete macro and micronutrient requirements for biostimulation and nutrition in the growth of cereals (e.g., corn).

EXPERIMENTAL

Materials and Methods

The following materials were used: sugar based gemini surfactant (polymethylene-α, ω-bis(N,N-dialkyl-N-deoxy-d-glucitolammoniumiodides) – Gemini, from SERVA Feinbiochemica GmbH & Co; demecarium bromide – Bola, from Sigma-Aldrich; ethanol of AnalaR grade. Phosphate buffer solution (PBS) of pH 6 is prepared in the laboratory by dissolving potassium dihydrogen phosphate in water, adjusting the pH with 1.0 M potassium hydroxide and diluting to 1.0 L with water. Triple distilled deionized water for sample preparation and all-PyrexTM glass apparatus was always used. The experimental techniques used consist in: BS-2082 Research Biological Microscope, magnification: 40x-1000x for optical microscopy; "MALVERN" zetasizer-nano equipment, with measuring range between 0.3 nm-60.0 microns and zeta potential determination with an accuracy of +/-2%; DataPhysics OCA 25 contact angle system.

RESULTS AND DISCUSSIONS

Obtaining Bioemulsions Based on Surfactants (Gemini or Bolaform) and Protein Hydrolysates (Keratin and Collagen) with Micro and Macro Nutrients

The hydrolysates (keratin and collagen) were obtained by acid hydrolysis at 80°C for four hours. Dried hydrolysates (keratin and collagen) were mixed with a solution containing micro and macro nutrients. Physico-chemical characterizations of protein hydrolysates (keratin and collagen) with micro and macro nutrients were performed according to the standard in force or literature methods for: dry substances, ash, total nitrogen and protein content, aminic nitrogen, pH and viscosity (Table 1).

Table 1. Physical-chemical characterization of protein hydrolysates (keratin and collagen) with micro and macro nutrients

Characterization	Protein hydrolysates (keratin and collagen) with micro and macro nutrients
Dry substance, %	30
Total ash, %	7
Total nitrogen, %	9
Protein substance, %	49
pH, pH units	6-7
Viscosity, cPs	2500

The samples (bioemulsions with gemini or bola) were prepared by dropping a 5-7% solution of surfactant (in water/ethanol, ratio 1:1) under continuous stirring into a aqueous solution of protein hydrolysates (keratin and collagen) with micro and macro nutrients of 10-20% at 70°C for five hours.

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Several experiments were performed with different concentrations of protein hydrolysates (keratin and collagen) with micro and macro nutrients. The sample containing 20% protein hydrolysates (keratin and collagen) with micro and macro nutrients proved to be the most stable. The samples were labeled as presented in Figure 1. A number of three samples: protein hydrolysates (keratin and collagen) with micro and macro nutrients/ with or without surfactant (gemini or bola)/water/ethanol compared with a control sample (water), were prepared in the following working conditions: water-ethanol solvents at 1:1 ratio, temperature=70°C for five hours; surfactant concentration-c=5-7%, at pH=6 adjusting with a phosphate buffer solution (PBS), speed at 100 rpm.

1 **2** **3**

Figure 1. Photographic image of: sample 1 – protein hydrolysates (keratin and collagen) with micro and macro nutrients; sample 2 – protein hydrolysates (keratin and collagen) with micro and macro nutrients + Gemini surfactant (polymethylene-α, ω-bis(N,N-dialkyl-N-deoxy-d-glucitolammoniumiodides); sample 3 – protein hydrolysates (keratin and collagen) with micro and macro nutrients + Bola surfactant (demecarium bromide)

Characteristics of Bioemulsions Based on Surfactants (Gemini or Bolaform) and Protein Hydrolysates (Keratin and Collagen) with Micro and Macro Nutrients

Dynamic Light Scattering (DLS)

The average particle sizes of the two bioemulsions (with gemini or bola surfactant) showed increased dimensions as compared to sample 1 – protein hydrolysates (keratin and collagen) with micro and macro nutrients mixture (803 nm for sample 2; 620 nm for sample 3; 250 nm for sample 1), confirming the formation of the complex aggregates. The highest average particle size of bioemulsion with Gemini surfactant – sample 2 (Fig. 2) showed also the highest Zeta potential absolute value and improved stability as compared to bioemulsion with Bola surfactant – sample 3, and protein hydrolysates (keratin and collagen) with micro and macro nutrients – sample 1.

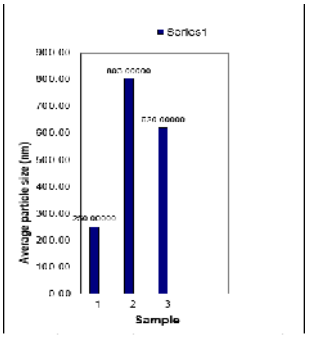

Figure 2. The average particle size for samples 1, 2 and 3

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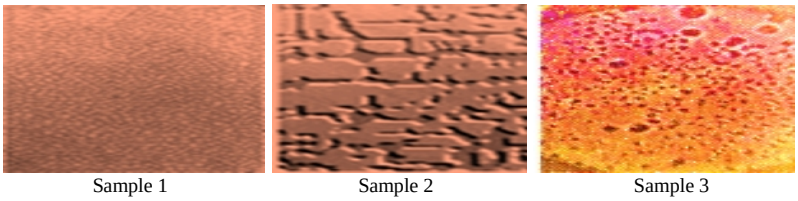
Zeta potential without stirring for 5 minutes showed a tendency towards agglomeration. The use of surfactants leads to obtaining stable bioemulsions.

Optical Microscopy Tests

The optical microscopy images in Fig. 3 show the agglomerated structures for samples (2 and 3) with surfactant (gemini or bola). The results are in agreement with literature data (Tadros, 2005; Hughes *et al.*, 2021; Nuraje *et al.*, 2013; Varasteanu, 2014), related to the formation of aggregate structures in bioemulsions.



Sample 1 Sample 2 Sample 3

Figure 3. Optical microscopy images (1000x) for samples 1-3

Contact Angle Measurements

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 3 obtained with this surfactant is more hydrophilic than the sample 1 without surfactant and the sample 2 obtained with Gemini surfactant.

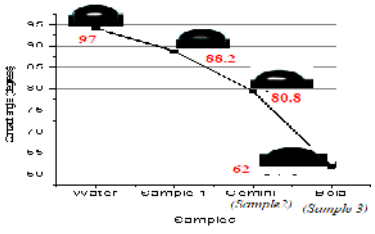


Figure 4. The contact angle measurements results for three samples and water, on a teflon surface: sample 1 – protein hydrolysates (keratin and collagen) with micro and macro nutrients; sample 2 – protein hydrolysates (keratin and collagen) with micro and macro nutrients + Gemini surfactant (polymethylene-α,ω-bis(N,N-dialkyl-N-deoxy-d-glucitolammoniumiodides); sample 3 – protein hydrolysates (keratin and collagen) with micro and macro nutrients + Bola surfactant (demecarium bromide)

Microbiological Tests

The stock cultures of microbian inoculums of *Fusarium spp ATCC 36031* and *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 8.92×10^3 CFU / mL for *Fusarium* spp. and 9.3×10^3 CFU / mL for *Botrytis cinerea*. The experiments were performed in Eppendorf tubes, previously sterilized at 121ºC for 15 minutes. The microbial inoculum was mixed

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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). Table 2 shows that bioemulsions based on Gemini (sample 2) or Bola surfactants (sample 3) had excellent antifungal resistance as well as sample 1 – protein hydrolysates (keratin and collagen) with micro and macro nutrients that showed antifungal properties between 98.70% and 100%.

Bioemulsion based on gemini surfactant – sample 2 had a positive influence on antifungal resistance of protein hydrolysates (keratin and collagen) with micro and macro nutrients mixture for all tested strains, meanwhile bioemulsion based on Bola surfactant – sample 3 showed slightly decreased antimicrobial resistance.

The new byproducts-bioemulsions based on surfactants and protein hydrolysates (keratin and collagen) are original due to the successful inclusion of micro and macro nutrients with high potential for cereals (e.g., corn) biostimulation and nutrition. The final goal of bioemulsions is their application as a new class of root fertilizers for cereals (e.g., corn).

Table 2. Antimicrobial resistance of new bioemulsions against fungus species

Sample	Result, UFC/mL	R%	Log_{10} red.
Botrytis cinerea		-	-
Inoculum concentration	$T_0=9,5x10^3$		
sample 1- protein hydrolysates (keratin and collagen) with micro and macro nutrients	$T_{24}= 6,2x10^1$	98,70	3,38
sample 2- protein hydrolysates (keratin and collagen) with micro and macro nutrients + Gemini surfactant (polymethylene-α,ω-bis(N,N-dialkyl-N-deoxy-d-glucitolammoniumiodides)	$T_{24}= 1,0x10^1$	99,99	4,10
sample 3- protein hydrolysates (keratin and collagen) with micro and macro nutrients + Bola surfactant (demecarium bromide)	$T_{24}= 2,12x10^2$	99,88	2,80
Fusarium spp. Inoculum concentration	$T_0=8,92x10^3$	-	-
sample 1 – protein hydrolysates (keratin and collagen) with micro and macro nutrients	$T_{24}= 5,0x10^1$	98,88	2,10
sample 2 – protein hydrolysates (keratin and collagen) with micro and macro nutrients + Gemini surfactant (polymethylene-α,ω-bis(N,N-dialkyl-N-deoxy-d-glucitolammoniumiodides)	$T_{24}= 0$	100	4
sample 3- protein hydrolysates (keratin and collagen) with micro and macro nutrients + Bola surfactant (demecarium bromide)	$T_{24}= 3,0x10^1$	99,80	1,44

The Evolution of Plant Growth

The bioemulsions created in this research, were applied as a new root fertilizer to 20 corn kernels compared to a control sample (water) and the evolution of the plants was followed by measuring the stems and roots 10 days before planting (Fig. 5a) and 10 days after they were planted (Fig. 5b) by applying the treatment, i.e., spraying the plants with 10 ml every 3 days.

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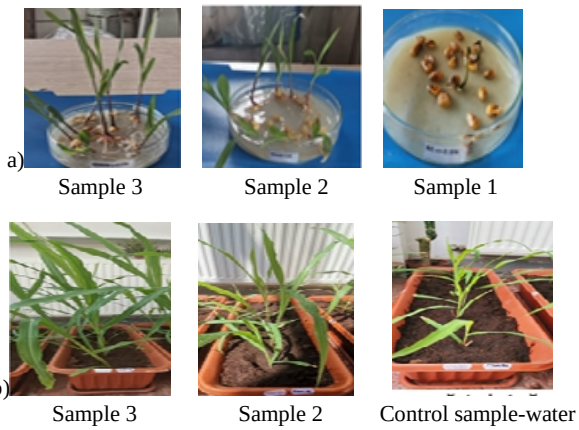

Figure 5. a), b). Images with the evolution of the plants, by measuring the treated stems and roots 10 days before and 10 days after planting, for samples 1, 2 and 3

The best results were obtained for sample 3 with Gemini surfactant.

CONCLUSIONS

New byproducts-bioemulsions were successfully made and can be used as a new class of root fertilizers for cereals (e.g., corn). The structure of new bioemulsions was demonstrated by optical microscopy.

The bioemulsions had particle sizes of: 803 nm for sample 2; 620 nm for sample 3 and 250 nm for sample 1. The new bioemulsions 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.

Bioemulsions created with Gemini surfactants had the best results, showing an improved influence on antifungal resistance and emulsion stability.

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