NEW STRUCTURED EMULSIONS BASED ON RENEWABLE RESOURCES GENERATED BY LEATHER AND FUR INDUSTRY, WITH APPLICATION IN AGRICULTURE

DEMETRA SIMION¹, CARMEN GAIDĂU¹, CORINA CHIRILĂ¹, MARIANA DANIELA BERECHET¹, MIHAELA NICULESCU¹, DORU GABRIEL EPURE²

¹INCDTP - Division: Leather and Footwear Research Institute, 93 Ion Minulescu St., 031215, Bucharest, Romania, icpi@icpi.ro

²SC Probstdorfer Saatzucht Romania SRL, 20 Siriului St., 1, Bucharest, Romania

The paper is focused to development of new structured emulsions, based on bolaform and gemini surfactants and collagen, keratin additives, with applications in agriculture as foliar fertilizers. Collagen and keratin are renewable resources generated by leather and fur industry. The preparation of new emulsions was based on optimisation of main parameters system: composition, emulsifiers, shearing speed and temperature in a two-stage process. The used lipophilic nonionic emulsifier is a long chain fatty acid ester-isopropyl oleate, the hydrophilic emulsifier is a diester of sucrose and a vegetable oil in order to obtain a multiple water-oil-water emulsion due to the ability of surfactants to orient and make honeycomb formations. The saturation of an aqueous solution of collagen and keratin hydrolysates with microelements was done up to 40% by using 2% of diester of sucrose. Due to biodegradability, nontoxicity, adherence to surfaces, surfactants based on sugar may used as fertilizers in agriculture. We have elaborated a new method for including microelements and collagen or keratin hydrolysates in emulsions, with application as a new class of foliar fertilizer. Experiments regarding the nutritional and biostimulation capacity on plant growth, were performed with dilutions of these new structured emulsions on wheat seeds (Tamino and Mirastar type) and plants. The germination of wheat seeds was monitored and a stimulation effect was recorded against the control samples. The use of new emulsions in foliar fertilization of wheat plants indicated an increase in plant length as compared to the water-treated control samples, which demonstrates a stimulating growth effect.

Key words: bolaform and gemini surfactants, new structured emulsions, foliar fertilizers for agriculture

INTRODUCTION

The aim is to create new structured emulsions based on surfactants (bolaform and gemini) and collagen, keratin additives with potential applications in agriculture as foliar fertilizers.

Collagen and keratin additives are renewable resources generated by leather and fur industry. Bolaamphiphiles and Gemini are new classes of amphiphilic surfactants with large potential of applications due to the high ability to deliver active substances (Niculescu and Gaidau, 2014). In this study the nonionic lipophilic emulsifier is a long-chain fatty acid ester-isopropyl oleate, the hydrophilic emulsifier - sucrose diester, and thyme oil.

The aim is saturation of an aqueous solution of microelements or collagen/keratin hydrolysate (<40%) in an aqueous solution of sucrose diester (2%). The hydrophilic properties of non-ionic surfactants are provided by hydroxyl groups (OH), ether linkages (-O-), amide groups (-CONH-), etc. Such groups are found in sugar, polyethers and similar combinations. Sugar esters and ethers with tenside properties are known as surfactants based on carbohydrates.

Surfactants used in this study, figure 1, are sucrose esters - *R alkyl radicals* C₈-C₁₈.

Figure 1. Representation of sucrose ethers and esters (Gaidau *et al.*, 2013)

Experiments regarding the nutritional and biostimulation capacity on plant growth were performed with dilutions of new structured emulsions obtained on wheat seeds (Tamino and Mirastar type) and plants (Gaidau *et al.*, 2009). The germination and growth of the plants were monitored and a stimulation was recorded against the control samples. Other formulations aimed at obtaining foliar blends containing microelements, collagen and keratin hydrolysates that have been applied to wheat plants. The results indicated an increase in plant length by 20% as compared to the water-treated control samples, which demonstrates a stimulating growth effect. The good results obtained in applications of keratin and collagen hydrolysates introduced in the new structured emulsions dilutions, in the agriculture show that can be used as foliar fertilizers with ecological effects.

RESULTS AND DISCUSSION

Technological Process of Obtaining New Structured Emulsions

The two-step emulsification process is used in this paper, where the result is a multiple nanostructured emulsion due to the properties of surfactants used to orient and form honeycomb formations at the nano and micro levels. The main types of multiple emulsions are water-oil-water and oil-water-oil. Two types of emulsifiers are used: a hydrophobic I one, isopropyl oleate (for W/O emulsion) and a hydrophilic II one-diester of sucrose based on sugar (for O/W emulsion). In the first step, water, vegetable oil (3%), lipophilic-isopropyl oleate surfactant (2%) are added and homogenized by stirring at 60°C to obtain a water-oil emulsion. In step II a solution of microelements saturated up to 40% (or collagen/keratin hydrolysate) and a sugar-based surfactant-hydrophilic-diester (2%) are added to the water-oil emulsion, and homogenized by stirring at 60°C, obtaining a multiple water-oil-water emulsion (W/O/W).

Figure 2. Representation of the two-step technological process of obtaining nano- and micro- structured emulsions (Gaidau *et al.*, 2013)

Finally, a multiple W/O/W nano and microstructured emulsion is obtained by mixing/stirring at 60°C. The thermal introduction of phase inversion for oil-water concentrated emulsions is preceded by obtaining a W/O/W emulsion. The technological process is analogous to obtaining emulsions by replacing microelements with collagen hydrolysate or a 1:1 hydrolysed collagen-keratin blend. In order to obtain new structured nano and microemulsions an important part is to select the characteristics of the working parameters: system composition, emulsifiers, shear rate, tension, stirring speed, temperature. Long-chain fatty acid esters, including isopropyl oleate, vegetable oils, have been used to vary the physico-chemical properties of multiple emulsions as well as to attempt to control the transfer rate of the solute through the oil phase. Structured and stable micro and nanoemulsions are formed, able to incorporate microelements or collagen/keratin hydrolysates with a 40% saturation, and the properties derive from the surfactants used, as well as the conditions and working parameters. In our research we have elaborated a new method for including microelements and collagen or keratin hydrolysates in stable emulsions with the final purpose of application as a new class of foliar fertilizer.

Characterisation of New Structured Emulsions

The three types of emulsions with: microelements (MC), collagen hydrolysates (HC) and collagen/keratin hydrolysate (HK) mixture were characterized by dynamic light diffusion and optical microscopy. Dynamic light diffusion test showed that all three types of emulsions are nano and microstructured. The size, percentage of the particles and Zeta potential were determined (indicating their stability). MC emulsions have sizes ranging between: 42 nm, 225 nm, 5269 nm and Zeta potential was: -20.7 mV. HK emulsions have particle sizes ranging between: 109.7 nm, 621.8 nm, 3371 nm with value of -13.5 mV for Zeta potential. For the third sample, HC, with collagen hydrolysate, experimental results are given emulsion particles of 44.8 nm and 776.3 nm and Zeta potential was -2.74 mV. The influence of high molecular weights of collagen and keratin were revealed through higher particle size of emulsions and lower value for Zeta potential. Optical microscopy images (Figure 3) show that emulsions with collagen/keratin hydrolysate (HK) and emulsions with microelements (MC) are structured in honeycomb formations and only the collagen hydrolysate (HC) emulsions are oriented and agglomerated in a chain. The results are in agreement with literature data (Nuraje et al., 2013) related to the formation of honeycomb and chain structures in multiple water-oil-water emulsions.

Figure 3. Optical microscopy images (1000x) of emulsions structures: a) honeycomb of HK emulsion; b) honeycomb of MC of; c) chain structure of HC emulsion

Dilutions of the four samples made using the presented technology, as follows: 1) emulsion with microelements only (MC), 2) emulsion with collagen hydrolysates (HC) and 3) emulsion with collagen/keratin hydrolysate (HK) in 1:1 ratio and 4) emulsion with keratin hydrolysate (HK). Dilutions of these four samples are presented in Table 1.

Table 1. Dilutions of obtained and tested samples

Sample	Dilution 1
1	10 ml of sample 2) + 990 ml distilled water (solution I)
2	14 ml of sample 4) + 986 ml distilled water (solution II)
3	10 g of sample 1) + 990 ml distilled water (solution III)
4	6 g of sample 3) + 994 ml distilled water (solution IV)
Dilution 2	
5	42 ml of solution I + 956 ml distilled water
6	255 ml of solution II + 745 ml distilled water
7	231 g of solution III + 769 ml distilled water
8	442 ml of solution IV + 558 ml distilled water
Dilution 3	
9	2,3 ml of sample 3) + 997,7 ml distilled water
10	Control

The four solutions, I-IV, from Table 1 were also microbiologically tested, to determine behaviour to fungal attack of *Fusarium* spp, carrying out analysis seven days from inoculations, with images presented in figure 4.

Figure 4. Images of the 4 solutions I-IV (from Table 1) with fungal attack of *Fusarium* spp for 7 days: a) after 2 days; b) after 3 days; c) after 6 days; d) after 7 days

After 7 days the appearance of the 4 solutions attacked by *Fusarium* spp is shown in figure 5.

Figure 5. Images of the 4 solutions I-IV (from Table 1) with fungal attack of *Fusarium* spp for 7 days

The best results were registered for solution II which contains only keratin hydrolysate. The evolution of solution II upon *Fusarium* spp attack for 7 days was presented in figure 6.

Figure 6. Images of solution II (from table 1) with fungal attack of *Fusarium spp* for 7 days: a) after 2 days; b) after 3 days; c) after 6 days; d) after 7 days

Experiments regarding the nutritional and plant growth biostimulation capacity, were performed with dilutions 1-10 (table 1) of these new structured emulsions on wheat seeds (Tamino and Mirastar type) and plants. The germination of wheat seeds was monitored and a stimulation effect was recorded against the control samples. Experiments on wheat seeds indicated a biostimulatory effect in the first 5 days of germination in the presence of samples 1-10 from table 1. For Mirastar wheat type sample 6 from table 1, containing keratin hydrolysate, showed the highest germination rate and the shortest germination time, figure 7.

Figure 7. Germination rate evolution of sample 6 Mirastar wheat

Samples 2 and 3 from table 1 had a strong phytotoxic effect on both types of wheat. Samples 6 and 7 from table 1 stimulated germination in both Tamino and Mirastar types. Germination rate for the two types, Tamino and Mirastar, are comparatively presented for the 10 samples from table 1, in figure 8.

Figure 8. Germination rates for the two types of wheat, Tamino and Mirastar for the 10 samples from table 1

The experimental on obtaining foliar blends containing microelements, collagen and keratin hydrolysates that have been applied to wheat plants showed an increase in plant length by 20% as compared to the water-treated control samples, which demonstrates a stimulating growth effect. The good results obtained in applications of keratin and collagen hydrolysates in agriculture show that they can be the basis for obtaining new biomaterials with various ecological applications.

Plants were sprayed with the 10 samples from table 1, and shown in figure 9.

Figure 9. a) Method of spraying plants with the 10 samples from table 1; b) Growth of plant stimulated with the 10 samples, after 10 days

The use of new emulsions in foliar fertilization of wheat plants indicated an increase in plant length as compared to the water-treated control samples, which demonstrates a stimulating growth effect.

CONCLUSIONS

Studies from this research report led to the following conclusions:

- The most effective method of obtaining multiple emulsions (e.g. phase inversion, single step emulsification, etc.) is the two-step emulsification process
- Inversion of multiple emulsion phases occurs when the dispersed droplets are packed tightly into the fluid in which they are suspended
- The thermal induction of phase inversion for O/W concentrated emulsions is preceded by obtaining an W/O/W emulsion. When an aqueous solution of a hydrophilic emulsifier is introduced into an oil containing a lipophilic surfactant, the W/O/W emulsion is obtained due to the inversion of the phases by modifying the HLB of the surfactant mixture.

Acknowledgement

The present work was supported by Romanian National Authority for Scientific Research, CNDI-UEFISCDI, project number 55PTE_BIOFOL_CER and by Ministry for Research and Innovation, project number 16 Nucleu/2018, PN 18 23 01 04_SMART_PIEL.

REFERENCES

- Gaidau, C. *et al.* (2009), "Additives and advanced biomaterials obtained from leather industry by-products", *Revista de Chimie*, 60, 5, 501-507.
- Gaidau, C. *et al.* (2013), "New Mixes Based on Collagen Extracts with Bioactive Properties, for Treatment of Seeds in Sustainable Agriculture", *Current Pharmaceutical Biotechnology Journal*, 14, 9, 792-801, <https://doi.org/10.2174/1389201014666131227112020>.
- Niculescu, M.D. and Gaidau, C. (2014), "New collagen extracts conditioned for applications in crop protection against pests", *Revista de Chimie*, 65, 12, 1457-1461.
- Nuraje, N. *et al.* (2013), "Bolaamphiphilic molecules: Assembly and applications, Progress", *Polymer Science*, 38, 302- 343.
- Tran, H.D. *et al.* (2011), "The oxidation of aniline to produce "polyaniline": a process yielding many different nanoscale structures", *J Mater Chem*, 21, 3534-3550, <https://doi.org/10.1039/C0JM02699A>.
- Varasteanu, D.S. (2014), "Surfactants based on proteins - Obtaining and applications" (in Romanian), PhD thesis, "Politehnica" University of Bucharest.