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DESIGN OF NEW STRUCTURED BIOEMULSIONS, BASED ON VEGETABLE EXTRACTS AND SURFACTANTS, USING INNOVATIVE BIOTECHNOLOGIES

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New bioemulsions were created using innovative biotechnologies based on: sage, geranium and lemongrass extracts and 3 surfactants mixture, Lauryl glucoside/Tween 20/Tween 80, for different concentrations of extract plants, to improve surface properties. A comparison was made for the way to solubilize and encapsulate the three vegetable extracts in emulsions. The order of introduction the components into the developed biotechnologies, the working conditions and especially the choice of the concentration of surfactants >CMC, is essential in the solubilization of vegetable extracts and obtaining the desired bioemulsions. The bioemulsions were characterized by optical microscopy with or without three types of vegetable extracts at 23-50°C. The changes in the aggregation process were observed for each type of emulsion, the influence of temperature and the solubilization of vegetable extracts. By dynamic light scattering (DLS) the stability, concentration, particle size, polydispersity, and zeta potential of bioemulsions were observed. FTIR measurements highlighted the interaction mechanism of surfactants with vegetable extracts from the created bioemulsions. A mechanism of solubilization of vegetable extracts in surfactant micelles was proposed in this research. Vegetable extracts are hydrophilic and attach to the hydrophilic groups of the chains. For Tween 80, the amount of solubilized vegetable extract is higher than in the case of Tween 20, because it has more hydrophilic groups. Van der Waals interaction forces are responsible. The modeling of release speed in water for each vegetable extract solubilized and encapsulated in bioemulsions can be done based on the first-order profile.

Keywords: structured bioemulsions, innovative biotechnologies, vegetable extracts

INTRODUCTION

Three oil vegetable extracts from geranium, lemongrass, and sage were selected to be solubilized and encapsulated in bioemulsions based on mixture of Lauryl glucoside/Tween 20/Tween 80. We compared the way to solubilize and encapsulate the three vegetable extracts in emulsions. Lauryl glucoside is a surfactant used in cosmetics and laundry detergents. It is a glycoside produced from glucose and lauryl alcohol. Lauryl glucoside is a non-ionic surfactant and member of the alkyl glucoside family which are substances formed by mixing alohols and sugar and/or glucose. This ingredient is usually sustainably sourced from palm kernel oil, corn sugar or coconut. It improves the cleansing process without stripping necessary moisture (Das *et al*., 2022).

Tween 80 is a polyethylene sorbitol ester, also known as Polysorbate 80, PEG (80) sorbitan monooleate, polyoxyethylenesorbitan monooleate. Tween 80 is widely used in biochemical applications including: solubilizing proteins, emulsifying and dispersing substances in medicinal and food products (Hughes *et al*., 2021; Nuraje *et al*., 2013). Tween 80 is an antibacterial agent, schematic representation in Fig. 1.

Tween 20 is a polyoxyethylene sorbitol esteris, a frequently used member of the polysorbate family. These have been used as emulsifying agents for the preparation of stable oil-in-water emulsions. Tween 20 has been used in pre-extraction of membranes to remove

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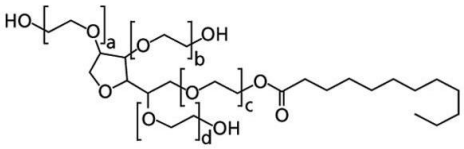
peripheral proteins (used at 2% for extraction of membrane-bound proteins). It has been used as a blocking agent for membrane-based immunoassays at a typical concentration of 0.05% (Vărășteanu, 2014). A schematic representation of Tween 20 is shown in Fig. 2.



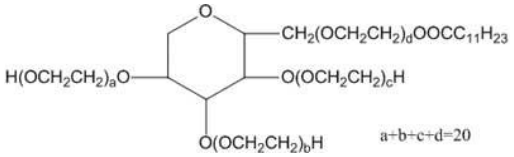


Figure 1. Schematic representation of Tween 80 Figure 2. Schematic representation of Tween 20

Tween is a group of non-volatile surfactant derivatives derived from glycerol esters (Baker and Sanders, 1986). Tween 20 and Tween 80 vary in chemical and physical properties and are usually solubilized or suspended in water. Tween 20 is mainly used as an effective binding agent in the production of foam and other polymers by means of its high solubility and low boiling point. Tween also has other important uses as a thermosetting agent in the process of manufacturing thermosetting plastics and as an adhesive for repairing paper materials. The most important usage of Tween is its application as an oil absorber and emulsifier. It is also used in the manufacturing of water-based and oil-based goods like shampoos, facial masks, hair gels, ointments, soaps, and cleansers. These goods are usually produced using emulsifying waxes. These three surfactants selected have been used as emulsifying agents for the preparation of stable water/in water emulsions.

In this research new structured bioemulsions based on oil vegetable extracts and surfactants were created and analyzed by: optical microscopy, FTIR-ATR spectroscopy and dynamic light scattering (DLS) and microbiological tests.

MATERIALS AND METHODS

In order to obtain new strucured bioemulsions, the following materials have been used: Lauryl glucoside, Tween 20 and Tween 80, ethanol as co-solvent from Sigma-Aldrich; oil vegetable extracts of: geranium, lemongrass, sage from "VIORICA" company. The experimental techniques used in this paper consist in: optical microscopy with an ELTA 90 Medical Research S.R.L. equipment; DLS with a Zetasizer-nano "MALVERN" equipment, with measuring range between 0.3 nm- 60.0 μm and zeta potential determination with an accuracy of +/-2%; FTIR-ATR spectroscopy with a JASCO spectrophotometer. The resistance to the action of bacteria of the 1-4 samples of bioemulsions was determined according to SR EN ISO 20645/2005.

The bioemulsions obtained in this research are presented in Fig. 3: **sample 1** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside; **sample 2** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and geranium extract; **sample 3** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and lemongrass extract; **sample 4** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and sage extract.

RESULTS AND DISCUSSIONS

Obtaining Bioemulsions with Solubilized and Encapsulated Vegetable Extracts

The encapsulation of vegetable extracts in bioemulsions is a two-step emulsification process. The result are multiple emulsions with vegetable extracts encapsulated due to the properties of the 3 surfactants (Tween 20, Tween 80, Lauryl Glucoside) used, to orient and form emulsions at the nano and micro levels.

Multiple bioemulsions are complex systems, also called 'emulsions of emulsions', in which the dispersed phase droplets contain a continuous phase with other dispersed droplets. In the first step (I) – introduction in water/ethanol at 1:1 ratio of the surfactants: Lauryl Glucoside (c=5%), Tween 20 (c=5%), and Tween 80 (c=5%), with HLB from 7 to 10, by homogenizing and stirring at room temperature for 1 hour; speed at 1000 rpm; in the second step (II) – vegetable extract (c=6%), (from geranium, sage or lemongrass) is added or not (for multiple emulsion without extracts) at pH=6 adjusting with a phosphate buffer solution (PBS), and homogenized by stirring at 50°C, for 30 minutes, speed at 1000 rpm and returned to room temperature, stirring for another hour. Multiple structured bioemulsions are obtained with solubilized vegetable extracts, Fig. 4.

Multiple bioemulsions are fragile systems, so the choice of emulsification methods is of particular importance in the success of obtaining vegetable extracts solubilized and encapsulated in emulsions with the desired properties.

Figure 3. a) Photographic image of **sample 1** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside; b) Photographic images of: **sample 2** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and geranium extract; **sample 3** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and lemongrass extract; **sample 4** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and sage extract

Characteristics of Bioemulsions with Solubilized and Encapsulated Vegetable Extracts

Dynamic Light Scattering (DLS)

The four samples obtained and presented in the Fig. 3 were characterized by dynamic light scattering. Dynamic light scattering tests showed that all 4 bioemulsions with or without vegetable extracts are nano and microstructured. The size, percentage of the particles and Zeta potential were determined (indicating their stability), Table 1.

The experimental results of dynamic light scattering showed that the size of the particles and their distribution differ depending on the way of encapsulation of the plant extract in bioemulsions and the type of plant extract used. Zeta potential shows a tendency towards agglomeration. The influence of the three surfactants selected (Lauryl glucoside/Tween 20/Tween 80) was to obtain stable bioemulsions with encapsulated vegetable extract. For the bioemulsions with sage extract, the particles with the smallest dimensions of 55.7 nm were obtained.

Table 1. Experimental results of dynamic light scattering for the four bioemulsions obtained

No. sample	Sample at room temperature	Average diameter (nm)	% Intensity	Zeta Potential (mV)
1	Multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside	1209.6	76.3	-33.5
		32.33	19.1	
		13.20	4.6	
2	Multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and geranium extract	412.1	91.7	-52.3
		58.13	7.39	
		1000	1.0	

No. sample	Sample at room temperature	Average diameter (nm)	% Intensity	Zeta Potential (mV)
3	Multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and lemongrass extract	163.7	100	-38.2
4	Multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and sage extract	341.3 55.7	93.6 6.4	-47.2

Optical Microscopy Tests

The optical microscopy images from Fig. 4 showed the four bioemulsions with or without vegetable extract encapsulated at room temperature and 50°C. All four bioemulsions presented in Fig. 4 had a good encapsulation process of vegetable extract. Fig. 4 shows oriented and agglomerated structures. The results are in agreement with literature data (Das *et al*., 2022; Hughes *et al*., 2021; Nuraje *et al*., 2013; Vărășteanu, 2014) related to the formation of structures in multiple bioemulsions.

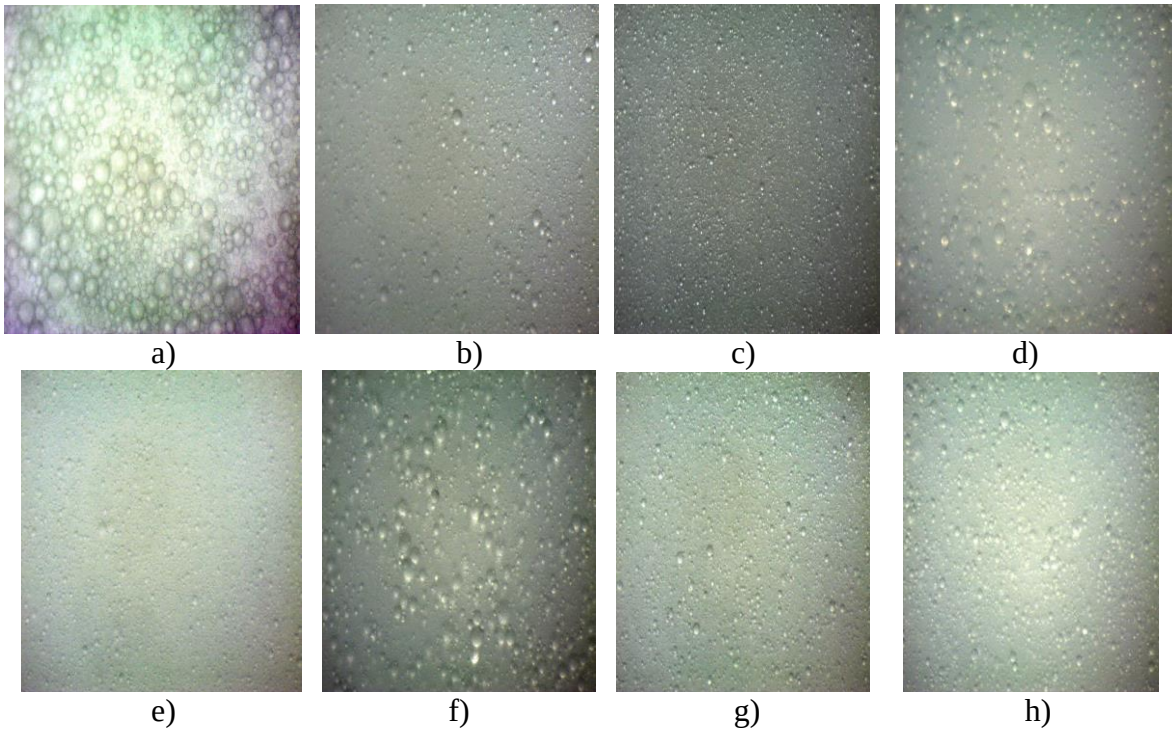


Figure 4. Optical microscopy images (1000x) for samples 1-4 at room temperature and 50°C: a) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside at room temperature; b) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside at 50°C; c) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside with geranium extract at room temperature; d) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside with geranium extract at 50°C; e) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside with lemongrass extract at room temperature; f) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside with lemongrass extract at 50°C; g) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside with sage extract at room temperature; h) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside with sage extract at 50°C

It is observed that the introduction of a vegetable extract into the bioemulsion leads to a decrease in the size of the structures formed both at room temperature and at 50°C (Fig 4.a reported to c, e, g).

FTIR-ATR Spectroscopy Tests

An FTIR-ATR spectrophotometer was used to analyze bioemulsion samples **1-4** (Fig. 3) and each extract selected or surfactants Tween 20, Tween 80 (Fig. 5).

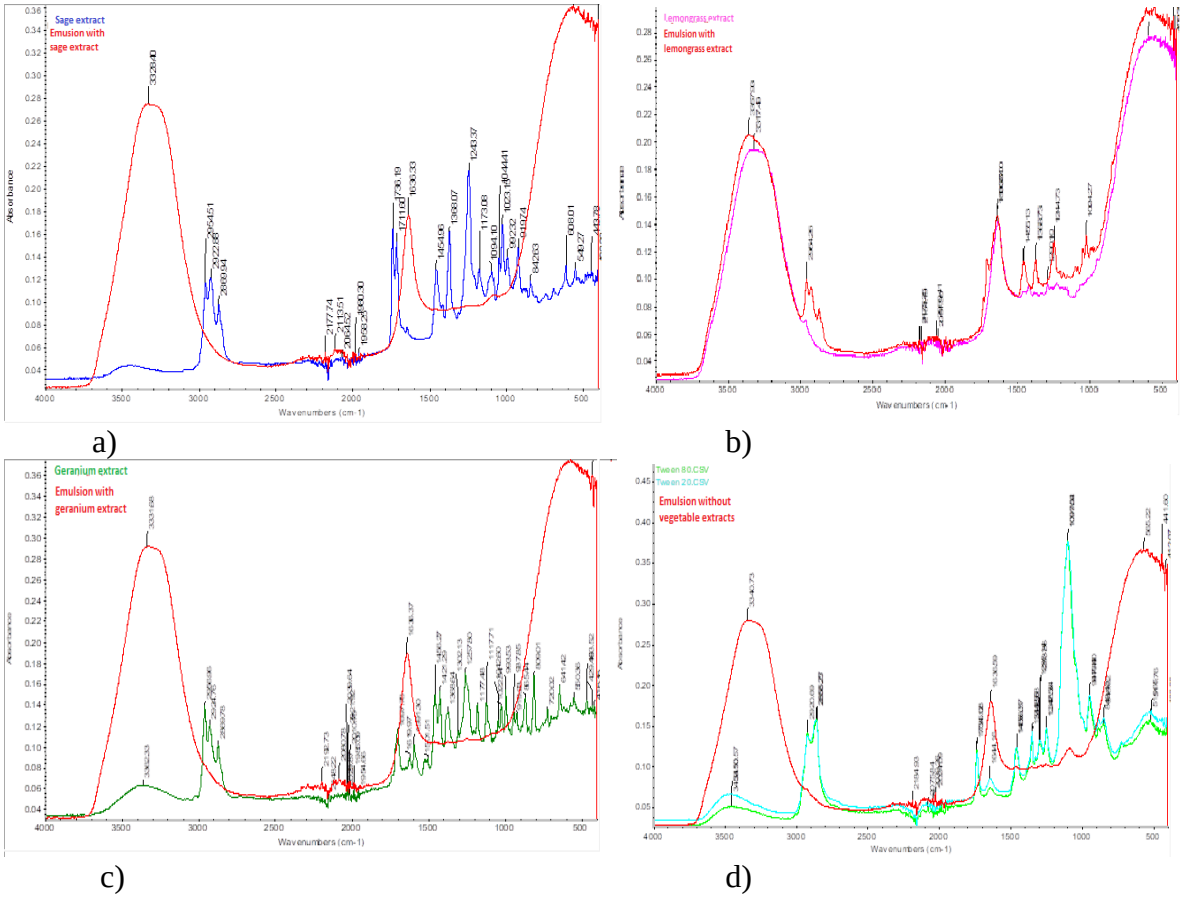


Figure 5. Overlapping FTIR-ATR fingerprint spectra of: a) sage extract -- and bioemulsion with sage extract--; b) lemongrass extract -- and bioemulsion with lemongrass extract--; c) geranium extract -- and bioemulsion with geranium extract--; d) emulsion without vegetable extracts – and Tween 80—Tween 20--

All analyzed bioemulsions (samples **1-4**) have a maximum absorption at the wave number 3343 cm^{-1} due to the presence of Tween 20 and Tween 80 surfactants in emulsions (Fig. 5-d).

Microbiological Tests

The four samples were also microbiologically analysed, to determine behaviour to bacterial attack of *Staphylococcus aureus* and *Escherichia coli*, carrying out analysis three days from inoculations. The best results in order, were obtained both for *Staphylococcus aureus* and *Escherichia coli* for samples **4> 2>3>1.**

Specimens of the material to be tested are placed on two-layer agar plates. The lower layer consists of a culture medium without bacteria while the upper layer is seeded with the selected bacteria. The level of antibacterial activity is assessed by examining the area of bacterial growth in the area of contact between the agar and the test tube, and if applicable the area of the zone of inhibition around the test tube.

Method used:

- replication of the bacteria used in the test: *Escherichia coli* ATCC 11229 (gram-negative), *Staphylococcus aureus* ATCC 6538 (gram-positive). We work with a pure, freshly propagated culture;

- dry sterilization of laboratory glassware in an oven at 180°C;
- preparation of the culture medium, characteristic of the test bacteria used, namely: -Nutrient Agar for the genus *Escherichia coli* and Mannitol Salt Agar for the genus *Staphylococcus aureus*;
- wet sterilization in the autoclave and Erlenmeyer vessels with culture media;
- the samples must be circular, with a diameter of 25±5 mm.

The agar volume is prepared for the bottom layer without bacteria. In each sterilized Petri dish (10 ± 0.1) ml are placed and the agar is allowed to solidify. The amount of agar for the top layer is prepared and cooled to 45°C on a water bath. 150 ml of agar are inoculated with 1 ml of bacterial working solution (1-5 x 10^8 cfu/ml). The container is shaken vigorously to distribute the bacteria evenly. To each Petri dish (5±0.1) ml are added and the agar is allowed to solidify. The samples are placed on the surface of the nutrient medium and then incubated at 37°C.

The Mechanism Proposed for Vegetable Extracts Solubilization (Encapsulation) in Surfactant Micelles

The mechanism proposed for encapsulation of vegetable extracts (sage, geranium, lemongrass) in three surfactant micelles (Tween 20/Tween 80/Lauryl Glucoside) and obtained structured bioemulsions were investigated using FTIR-ATR spectroscopy, DLS and optical microscopy, Fig. 6.

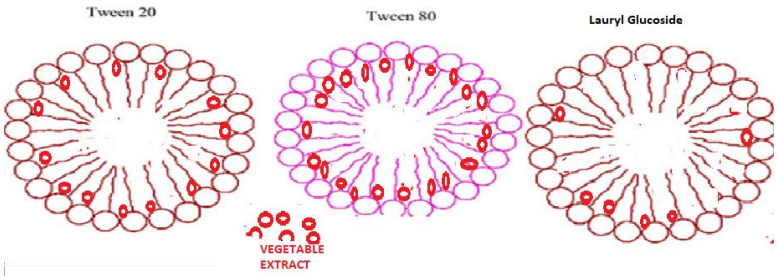


Figure 6. Mechanism proposed for vegetable extracts selected, solubilization (encapsulation) in surfactant micelles of: Tween 20, Tween 80, Lauryl Glucoside

The results have outlined three distinct processes depending on the surfactant concentration. In the pre-micellar range, the variation in absorbance and peak was attributed to the attraction of the initially head group to the β-diketone group of each selected vegetable extract. At surfactant concentration in intermediate/micellar region including CMC, a second type of interaction is observed, corresponding to the attachment of alkyl chains of surfactant to aryl groups of each selected vegetable extract and displacement of head group from β-diketone group of the vegetable extract. Finally at surfactant concentration higher than the CMC, in the postmicellar region, a type of interaction is observed, which corresponds to the encapsulation/solubilization of each selected vegetable extract into micelles, predominantly in monomeric form (Fig. 7).

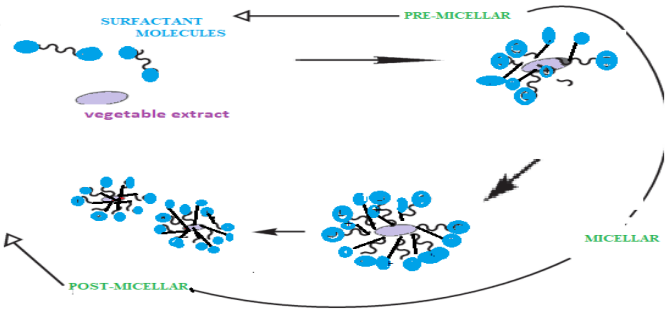


Figure 7. Schematic presentation of a proposed mechanism of interaction between selected vegetable extracts and surfactant molecules of Tween 20, Tween 80, Lauryl Glucoside

Modeling the Encapsulation Process of Selected Vegetable Extracts in the Obtained Structured Bioemulsions

Modeling encapsulation process of vegetable extracts selected in structured bioemulsions obtained was carried out taking into account all the parameters: the concentration and type of surfactants, the ratio between vegetable extracts selected and surfactants, micellar critical concentration of surfactants, speed and time of stirring, temperature, pH, average diameter of particles, zeta potential.

In this research is modeling the speed yield of each vegetable extract in water (which is encapsulated in bioemulsions) for the best results of samples: **4** and **2**. The dependence of absorbance to the encapsulated each vegetable extract from samples: **4** and **2**, yielded in water, on the time, was also analysed. A modeling program is created that can calculate the speed of yielding in water, of each vegetable extract encapsulated in emulsions for samples: **4** and **2** (for which the best results were recorded). The calculation modeling program created is in VBA with the Excel Worksheet interface. The modeling program was applied for the two selected samples: **4** and **2**. According to the modeling program, for sample **4**, the speed yield in water of *vegetable extract-sage* encapsulated in emulsion is: speed of yielding in water, of vegetable extract encapsulated in sample 1 is v= 0.00000422 unit. of Abs/min. For sample **2**, the speed yield in water of *vegetable extract-geranium* encapsulated in emulsions is, according to the modeling program: speed of yielding in water, of vegetable extract encapsulated in sample **2** is v= 0.0076597 unit. of Abs/min. It is observed that speed yield of vegetable extract in water (which is encapsulated in emulsions) is different depending on the type of surfactant used and its interaction with each vegetable extract selected (geranium or sage). The modeling of release speed in water, of each vegetable extract encapsulated in bioemulsions can be done based on the study of Baker and Sanders (1986). In Table 2 the theoretical results of three models on a data set (for sample **4**) are presented comparatively, and in Fig. 8, the comparative graphic representation.

Table 2. Comparison of failure profiles (Baker and Sanders, 1986)

	S.10^5	a	σa	b	σb	c	σc
Zero order	4.4	0.496645	5.39E-05	-0.00015	1.97E-05		
Order one	**2.41**	**0.49589**	**0.000156**	**0.000956**	**8.46E-05**	**0.407194**	**0.173009**
Radical order	2.32	0.496968	4.93E-05	-0.00045	3.12E-05		

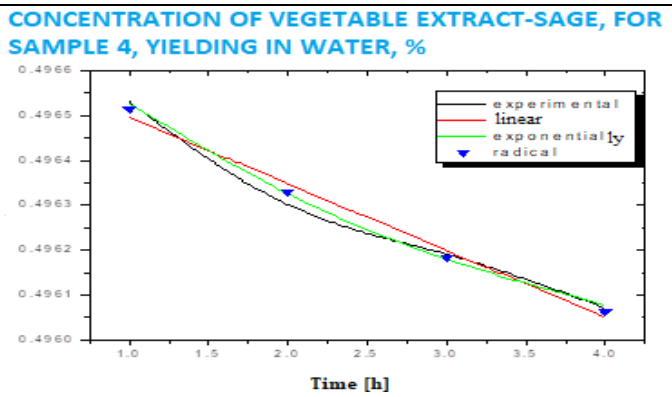


Figure 8. Comparison of yield profiles for sample **4**

The **first order** failure profile was chosen because there are two values of the absorbance (initial and final), and the failure of speeds yield of vegetable extract-sage (sample

4) in water are very low. The modeling of release speed in water, of vegetable extract-sage encapsulated in bioemulsion (sample 4), can be done based on the first-order profile.

CONCLUSIONS

Tween 20, Tween 80, Lauryl glucoside are surfactants that play a critical role in bioemulsion preparations, especially in forming stable bioemulsions and solubilizing vegetable extracts in aqueous solutions.

The paper proposed a mechanism of solubilization (encapsulation) of vegetable extracts (sage, geranium, lemongrass) in surfactant micelles of: Tween20, Tween 80, Lauryl Glucoside. For Tween 80, the amount of solubilized vegetable extract is higher than in the case of Tween 20, because it has a larger hydrophobic chain. Van der Waals interaction forces are responsible for this.

The changes in the aggregation process were observed for each type of bioemulsion, the influence of temperature, the solubilization (encapsulation) of each vegetable extracts by dynamic light scattering and optical microscopy.

The mechanism of interaction for surfactant micelles and each vegetable extract selected was created. Also, the encapsulation of each vegetable extract selected in bioemulsions was modeled taking into account all the parameters.

The versatility, compatibility and stability-enhancing properties of bioemulsions created, make them valuable ingredients in a variety of industries including the leather or textile industries.

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