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Formulation and Characterization of Oil-in-Water Emulsions Stabilized by Saponins Extracted from Hedera Helix Algeriensis Using Response Surface Method

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Abstract: Triterpene saponins extracted from *Hedera helix Algeriensis* plants were evaluated in terms of surface characteristics and capacity to be utilized as surfactants for the formulation of oil-in water emulsions. Surface tension and emulsifying properties were used for the identification of the surfactant characters, while emulsions were characterized by rheological methods and their stability was estimated by the control of the creaming index. The design of emulsions was conducted by employing a response surface method (RSM). The factors affecting the rheological parameters and emulsion stability were carefully evaluated by the polynomial models. Triterpene saponins were found as effective biosurfactants; they contribute strongly to the stability of emulsions by interacting with other excipients. Emulsions exhibited a shear-thinning behavior and low apparent viscosities which depend on the amount of xanthan used. They were considered as weak gels with a viscoelastic behavior. In addition, it was found that the presence of a sufficient quantity of saponins improves the stability of emulsions.

Keywords: Saponins; Surface tension; Emulsion; Rheology; Stability; RSM.

1. Introduction

Saponins are secondary heterosidic metabolites found in more than 500 plant species and some marine organisms [1,2]. The original name of saponin is extracted from the Latin word 'sapo' meaning soap for their properties to form foaming solutions [3].

Saponins are constituted of two different parts, lipophilic part called 'genin' or 'aglycone' and a hydrophilic part 'osidic'. On the basis of the nature of their genin and the number of carbohydrate chains linked to the aglycone they are classified as triterpenic or steroidal [4].

The saponins present in the leaves of *Hedera helix* (*Hh*) plant are triterpenic, their properties are determined by the plant origin and extraction procedure [5,6]. They are natural products exhibiting significant surface features and interesting biological characteristics, including antimicrobial, anti-inflammatory, insecticidal and anti-burn [7,8].

Strong consumer demands for biocommodity with biological properties have made saponins as desirable commercially compounds with broad applications, mainly in the medical, food and officinal manufactures [7-10].

The emulsifying agents usually employed in the formulation of emulsions are chemical products which are expensive, irritant and harmful to both human health and environment, it would, therefore, be interesting to substitute them with biodegradable natural surfactants,

renewable, available and no toxic [11]. Therefore, extraction, characterization and investigation of new emulsifiers became a centre of interest of investigators to respond to the increasing need for bio-products. Such natural surfactants include the group of saponins that can be extracted from various types of plants.

Many studies evoked the use of saponins as surfactants in the preparation of oil-in-water emulsions. According to Mitra et al. [12], the major ingredient capable of forming micelles and stabilizing oil/water emulsions is saponin, which was extracted from the *Quillaja saponaria* tree. Other investigations on the emulsifying characteristics of *Quillaja* saponins were reported [13-15]. They were found to form polydispersed emulsions with good particle size distributions of droplets.

Also, Benahmed-Djilali et al. [16] investigated the antibacterial properties of an ointment based on saponins extracts of walnut leaves and found that the formulated systems are of good sensory, physicochemical and rheological qualities. Chung et al. [17] used the mixture of soy lecitin and *Quillaja* saponin to formulate a food emulsion. Kaur et al. [18] also used saponins to stabilize a nanoemulsion and studied its role in protecting damage of quercitin beside UV rays. Doodt et al. [19] reported in their work that thymol nanoemulsions were produced by using *Quillaja* saponin as biosurfactants.

Saponins were also employed in the formulation of drug delivery systems. Cibulski et al. [20] utilized saponins of *Quillaja Brasiliensis* in the preparation of a newly developed vaccine. Recently, Yang el al. [21] developed a collagen microspheres-based steroidal saponin formulation with high encapsulation efficiency.

In this work, we investigated the surfactant potential of triterpene saponins extracted from *Hedera helix* (*Hh*) *Algeriensis* plant in the formulation of an emulsion (o/w) stabilized by xanthan gum. This anionic polysaccharide is considered as a hydrophilic biopolymer of low surface activity [22]; it is generally used for its gelling or stabilizing properties [23].

The main objective was the evaluation of the surface properties of saponins and their capacity to be employed as biosurfactants. A response surface modeling (RSM) was employed in order to determine the influence of saponin/xanthan interactions on the rheological properties and stability of formulated emulsions.

2. Materials and Methods

2.1. Materials.

Triterpene saponins were extracted by maceration from *Hh* leaves collected in the region of Blida (Algeria). A quantity of 100 g of crushed and defatted dried leaves was macerated in ethanol (80%, w/v) with a proportion of 1/7 (m/v). Pure ethanol (99%) was supplied by Merck (Germany). Xanthan gum was purchased from Rhodia-Solvay (France). Paraffin oil of pharmaceutical grade was provided graciously by Isopharm (Algeria).

2.2. Methods.

2.2.1. Surface tension measurements.

The critical micelle concentration (CMC) was determined by tensiometry. For each test, three measurements of the surface tension were realized at 20 °C using a Wilhelmy blade tensiometer (Gibertini TSD, Italia).

2.2.2. Determination of emulsifying properties.

The capacity of emulsification after 24 h was determined according to the method described by Burgoz-Diaz et al. [24]. The emulsions were elaborated by stirring a volume of paraffin oil (2.5 mL) with an equivalent volume of the aqueous phase containing different amounts of saponins (0.05, 0.5, 1 and 2%) for about 3 min. The mixtures were stirred and left standing for 1 h. The capacity of emulsification (CE_{24}) and the stability of emulsion (SE) are calculated according to Eq.1 and Eq.2:

$$CE_{24} = (H_E/H_T).100 \quad (1)$$

$$SE = (CE_{24}/CE_0).100 \quad (2)$$

where H_E is the height of emulsion, H_T is the total height of solution, CE_0 is the ratio (H_E/H_T) after 1 h and CE_{24} is the ratio (H_E/H_T) after 24 h.

2.2.3. Preliminary formation of emulsions.

To determine the efficiency and the appropriate aqueous phase/oil phase ratio to form emulsions, solutions of saponins were elaborated at different concentrations in the interval of 0.05 to 1%; then the aqueous phases were stirred at 1400 rpm for 2 min with different ratios of paraffin oil (80/20, 50/50 and 20/80). After a whole day, the emulsion height formed in each tube was measured. For the formulation of emulsions, first, the aqueous phases were prepared by dissolving during 24 h an appropriate amount of xanthan and saponin extract in milli Q using a magnetic agitator, and then the appropriate amounts of paraffin oil were added. Emulsification was realized by using a specific homogenizer (IKA, Germany) for 10 min at 24,000 rpm. The constituents of emulsions and their intervals of variation are grouped in Table 1.

Table 1. Emulsion composition

Constituents	Concentration (%, w/w)	Function
Triterpene saponin	0.05-0.25	Biosurfactant
Xanthan gum	0.2 - 0.8	Thickening
Parafin oil	10 - 20	Organic phase

2.2.4. Microscopic analysis and droplet size distribution.

Morphology of freshly prepared emulsions was examined using a Zeiss optical microscope (B3 Professional Series), which allows magnification up to 100 times. The size of droplets was evaluated by the treatment of images using the ImageJ.Ink software.

2.2.5. Determination of the index of creaming.

The index of creaming (I-C) is a distinctive parameter to evaluate the stability. Just after the preparation of emulsions, they were placed in test tubes and the heights of the separated phases were measured. I-C was then deduced using Eq.3:

$$I\text{-}C = (H_{aq}/H_T).100 \quad (3)$$

where H_{aq} is the height of the aqueous phase (lower phase) and, H_T is the total height of the emulsion.

2.2.6. Rheological characterization.

Measurements of the rheological parameters were realized by using an Anton Paar oscillating rheometer (MCR 302, Germany), using a parallel-plate geometry (diameter of 25

mm and gape of 1 mm). The rheometer was controlled by a microcomputer for the control and data processing using Rheoplus US200 software.

The flow test was achieved at 20 °C under a deformation rate ranging between 10^{-3} and 10^{3} s^{-1}. The flow curves representing the apparent viscosity in terms of the shear rate were adjusted by the model of Carreau [25]:

$$\frac{\eta-\eta\infty}{\eta0-\eta\infty} = \frac{1}{(1+(K\dot{\gamma})\hat{}2)\hat{}n} \quad (4)$$

where η is the solution viscosity, η_0 is the zero shear viscosity, η_∞ is the infinite shear viscosity, $\dot{\gamma}$ is the shear rate, K is characteristic of the relaxation time and, n is the exponent of the Cross model.

The viscoelastic properties were determined in dynamic mode using oscillatory tests. The deformation was varied from 0.001 to 100% at a frequency of 1 Hz. Then, the evolution of the conservation modulus (G') and loss modulus (G'') was recorded as a function of the deformation. .

2.3. Design of experiments.

The adopted approach is based on the method of the design of experiments; this method is utilized to obtain according to the formulation factors the predictive models of the responses. The most appropriate experimental strategy is based on the application of a response surface method (RSM) to account all the interactions between factors by a second-order model. The design that meets our objectives is a central composite face-centered (CCF) design. The selected responses represent the index of creaming (I-C) and the rheological parameters. The retained factors (X_1, X_2 and X_3) are between -1 and 1, and are associated with concentrations of triterpenic saponin (0.05-0.25%), xanthan (0.2- 0.8%) and oil (10-20%). The matrix of experiments that meets these objectives contains 14 tests.

The quality of the statistical results obtained from the adjustments is conditioned by the coefficient explaining the variance (R^2), which indicates how the model is of good quality. In addition, the statistical significance of the models was checked by using the analysis of variance (software Modde, version 6, Umetrics AB, Umeå, Sweden).

3. Results and Discussion

3.1. Surface and emulsifying properties of saponins.

Figure 1 illustrates the variation of the surface tension versus the triterpenic saponin concentration. It decreases up for a value of about 0.05% of saponin; from this concentration, the molecules of surfactant saturate the surface and the surface tension is stabilized at a minimum value which is in the order of 40 N/m. At this value (0.05%, in wt.), the surfactant molecules start to form micelles; this concentration represents the critical micelle concentration (CMC). These results agree well with those obtained by Mitra et al. [12] which determined the CMC of triterpenic saponins from different extract sources; they found that the CMC values vary between 0.013 and 0.074%. Moreover, Mironenko et al. [26] also found that the CMC of saponins from sugar beetroot extracts is between 0.06 and 0.08%. Stanimirova et al. [27] have shown that the CMC of saponin solutions from quillaja root extracts is approximately equal to 0.025%. According to these results, we note that the CMC of saponins is variable and depends on the source and extraction methods.

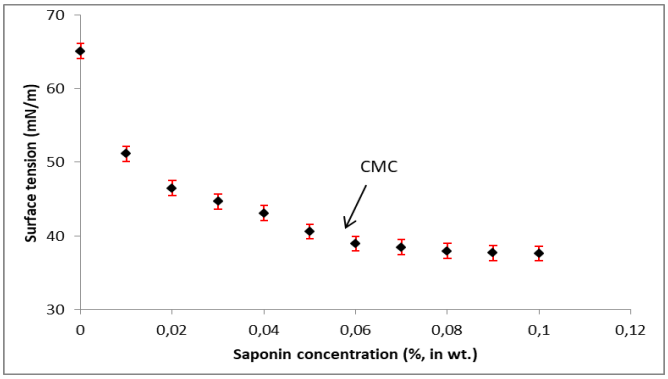

Figure 1. Variation in surface tension as a function of saponin concentration.

For the best characterization of surface properties, emulsification capacity and emulsion stability are two important parameters to demonstrate the possibility to use extracted saponins as emulsifying agents. Table 2 illustrates the variation of CE_{24} (capacity of emulsification) and SE (stability of emulsion) depending on saponin concentration. The results showed that CE_{24} and SE raise with saponin concentration increasing. This demonstrates that extracted triterpenic saponins are effective bioemulsifiers for forming oil-in-water emulsions.

Table 2. Emulsification capacity and stability of emulsions.

Saponin concentration (%, in wt.)	Emulsification capacity CE_0 (%)	Emulsification capacity after 24 h CE_{24} (%)	Emulsion stability SE (%)
0.05	56.66	6.67	11.75
0.50	60.06	7.87	12.98
1.00	22.86	17.14	74.97
2.00	28.57	22.85	80.00

3.2. Properties and morphology of emulsions.

The prepared emulsions are easily diluted in milli Q so the nature of the prepared emulsions is oil-in-water. These observations were confirmed by the measurement of the conductivity at 20 °C which was found to be greater than distilled water. The microscopic analysis results (Figure 2) of the freshly emulsions showed the presence of improved dispersion of oily droplets in the aqueous phase. This demonstrates the emulsification capacity of extracted saponins.

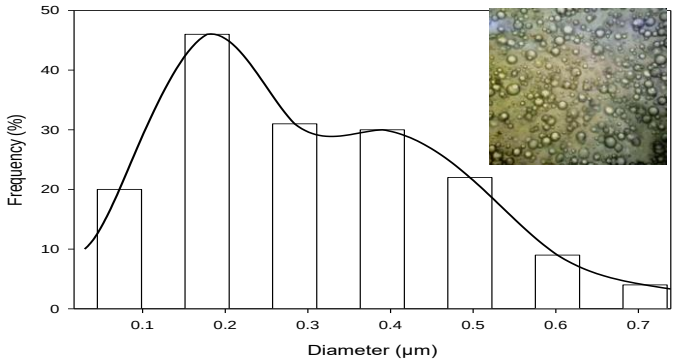

Figure 2. Microscopic image and distribution of mean diameter of droplets.

In Figure 2, the distribution of the mean diameter of a model of prepared emulsions (Test 3) is also presented. It was observed that the profile of distribution is characterized by two populations, large and small droplets. The mean diameter varies between 0.1 and 0.7 µm. So, the profile of this distribution seems to be of bimodal type, with the first mode around 0.2

μm and a second around 0.45 μm. However, it seems that 50% of the population has a diameter greater than 0.5 μm, which infers that the grade of this system is micronized but not colloidal.

3.3. Rheological characterization of emulsions.

Associated rheogrammes for prepared emulsions are characterized by the appearance of two regions, a first Newtonian region in the interval of weak shearing (< 0.01 s^{-1}) and a second region presenting a shear-thinning behaviour (Figure 3).

The flow curves of all samples were adjusted by the Carreau model (Eq.4) using Statistica software (version 8.0, StatSoftInc, France), which offers a multitude of nonlinear optimization methods based on an iterative calculation. For all emulsions, the coefficient R^2 was found greater than 0.97, which confirms the adequacy of this rheological model.

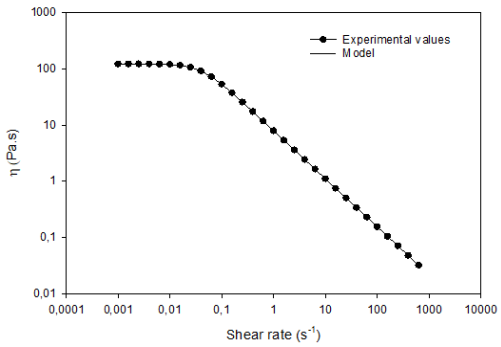

Figure 3. Flow Curve of formulated sample (Test 5).

Figure 4 represents the evolution of the conservation modulus G' and that of loss modulus G" as a function of the deformation for the test 5 (chosen as model). G' represents the elastic behavior corresponding to the deformation energy stored in the sample during shear, while G" represents the viscous behavior.

From the curves of viscoelasticity obtained, it was noticed that G' is greater than G". This indicates that the emulsions behave like a viscoelastic solid where the elastic behavior dominates the viscous one. This measurement made it possible to deduce the values of G'_0, and G''_0, in the linear viscoelastic range (Figure 4).

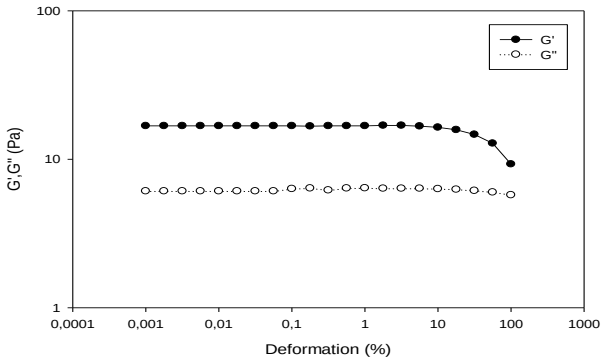

Figure 4. Viscoelasticity curve of formulated sample.

3.4. CCF modeling.

In order to select the dependent responses used in the experimental design (Table 3), we explored the correlations between all the responses. Thus, a strong link was observed

between G'_0, G''_0 and η_0. It is, therefore, unnecessary to keep the three responses and, we propose to maintain only G'_0 and K as rheological responses.

Table 3. Experimental matrix of the CCF design.

Test	X_1 (%)	X_2 (%)	X_3 (%)	K	η_0 (Pa.s)	G'_0 (Pa)	G''_0 (Pa)	I-C (%)
9	0.25	0.2	10	1.51	0.68	0.82	0.93	90
5	0.05	0.8	10	41.73	187.35	16.3	6.28	10
12	0.25	0.8	10	41.63	161.35	14.78	6.28	0
14	0.05	0.2	20	623.6	9.62	1.49	1.18	90
2	0.05	0.8	20	69.54	256.61	21.80	8.78	64
10	0.25	0.8	20	52.89	178.93	16.92	7.05	0
7	0.05	0.5	15	5.25	6.39	4.47	3.18	70
3	0.25	0.5	15	36.01	73.35	8.50	3.42	60
1	0.15	0.8	15	36.36	82.64	13.30	7.15	0
6	0.15	0.5	10	44.41	152.97	13.00	5.50	4
8	0.15	0.5	20	34.52	37.37	7.15	3.67	70
4	0.15	0.5	15	17.90	21.59	5.40	2.80	80
13	0.15	0.5	15	17.01	21.06	4.95	2.83	80
11	0.15	0.5	15	17.50	20.76	5.00	2.80	80

Based on the values of the coefficients of regression (0.931, 0.940 and 0.889 for K, G'_0 and I-C, respectively), in terms of fit and prediction the quality of the models is considered satisfactory.

The polynomial models expressing the responses (K, G'_0 and I-C) as functions of formulation factors are respectively given by the following equations (Eq.6, Eq.7 and Eq.8):

$$K = 22.31 - 67.35X_1 - 127.57X_2 + 61.78X_3 + 10.68X_1^2 + 134.35X_2^2 + 13.51X_3^2 + 79.81X_1X_2 - 4.11X_1X_3 - 68.72X_2X_3 \quad (6)$$

$$G'_0 = 5.52 - 0.33X_1 + 7.64X_2 - 0.58X_3 + 0.66X_1^2 - 0.47X_2^2 + 4.25X_3^2 - 0.68X_1X_2 - 0.84X_1X_3 + 1.90X_2X_3 \quad (7)$$

$$I\text{-}C = 65.76 + 3.80X_1 - 31.66X_2 + 24.20X_3 - 9.90X_1^2 - 12.75X_2^2 - 18.09X_3^2 - 24.50X_1X_2 - 13.50X_1X_3 - 8.50X_2X_3 \quad (8)$$

The ratio F deduced from the test of variance (ANOVA) was utilized to define the statistical significance of the responses; it is a proportion of two independent estimates of the experimental error. In addition the probability (*p)* quantifying the risk of error was also employed. The obtained results revealed that the three models are statistically significant based on the values of F for K, G'_0 and I-C (6.0082, 6.9443 and 3.5617) and the low values of *p* (< 0.05) for K and G'_0. Nevertheless, the value of *p* for I-C is relatively high (> 0.05), which explains that the values of I-C are statistically insignificant considering the value of *p* (0.117). This can be interpreted by the fact that I-C is rather a qualitative response.

3.5. Effects of factors on the rheological properties.

From Figure 5, it was shown that xanthan has a strong effect on G'_0 (Elastic modulus) irrespective of the used quantities of oil or saponins. Indeed, the polysaccharide with an important molecular weight behaves as a thickener, forming a polymeric network within the emulsion that enhances the cohesion of the structure. The increase in xanthan concentration increases the rigidity by raising the number of macromolecules.

This character is explained by the entanglement of rigid xanthan rods forming a network by ionic and hydrogen interactions imposing a high stiffness of the medium. Also, we note that saponins have no effect on the rigidity until a concentration of about 0.15%; above this concentration, it seems that saponins have a relatively significant effect depending on the quantity of the involved oil. In fact, for an oil concentration of 10%, the increase in saponins

beyond 0.15% decreases G'_0 while for oil concentrations of 15 or 20%, G'_0 increases beyond this critical concentration of saponins.

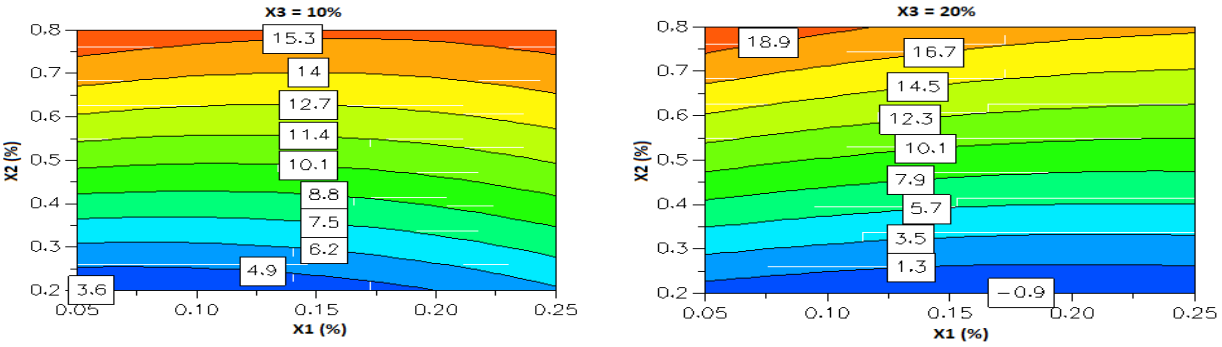

Figure 5. Effects of factors on the conservation modulus (G'_0).

According to Holmoberg [28], surfactants beyond a certain concentration form large micelles that become entangled in the appearance of polymers and make it possible to viscosify the middle and increase the rigidity of the emulsion. This result is in accord with those of Wojciechowski [29] who found that the hydrophobic phase has a remarkable effect on the rheological properties. Thus, the increase in G'_0 may be caused by the entrapment of the oil droplets within the polymeric xanthan network thus preventing the droplets from flocculating.

Otherwise, xanthan seems to reduce K (relaxation time) as shown in Figure 6. In addition, K which characterizes the interval of the Newtonian region decreases with the increase of xanthan concentration; indeed, when the quantity of xanthan increases the hydrophobic interactions set up between the entangled chains. Below the effect of shearing, there are reorganisations of the liaisons to form intra-chains hydrophobic ones that explain the shear-thinning phenomenon.

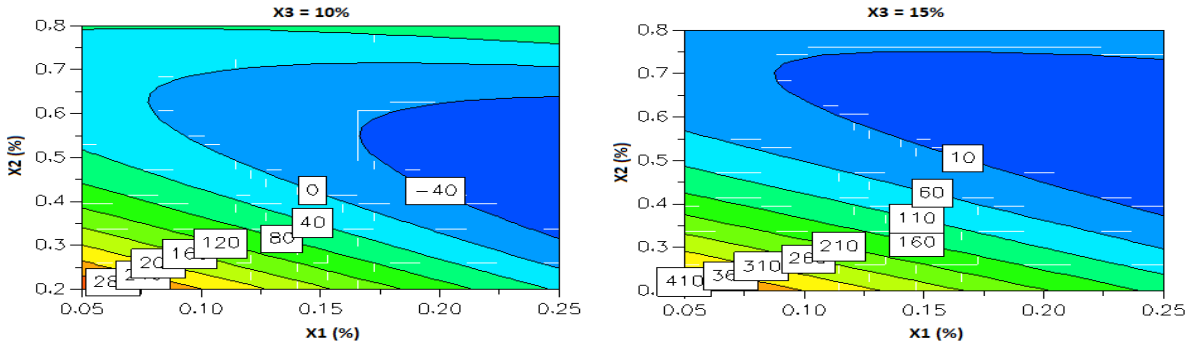

Figure 6. Influence of factors on the consistency index (K).

This behavior is typical for polymers observed particularly for the semi flexible polysaccharides [30]. This result is in agreement with those achieved by Rodd et al. [31] which indicate that xanthan is a polysaccharide with strong shear-thinning properties.

With regard to the action of surfactant, according to the iso-responses of Figure 6, it is clear that saponins exhibit a negative impact on the relaxation time; so the shear velocity decreases with increasing of its concentration. This result indicates that saponins have also a robust shear-thinning capacity. However, dilute emulsions show Newtonian flow behavior. Effectively, increasing the particles concentration of dispersed constituents (surfactant, polymer, and other constituents), modifies the rheological parameters of the system due to the increasing potential interactions within the dispersed emulsion particles [32].

3.6. Effects of factors on the stability of emulsions.

The stability of emulsions was evaluated through the creaming index (I-C). Figure 7 represents the iso-IC responses which reveal that xanthan significantly decreases I-C in all cases. This polysaccharide ameliorates the stability of the emulsion by acting as a thickener by preventing the droplets from flocculating. This result is in accordance with those published by other authors who have used this biopolymer for the stabilization of emulsions [22,33]. However, the extracted saponins have an undesirable impact on I-C for concentrations of oil about 10%. At this concentration, the quantity of unabsorbed saponins is important, thus free molecules of surfactant interact negatively by segregation with xanthan which in turn decreases the homogeneity of emulsion by flocculating the oil droplets. In addition, for an oil fraction of 20%, and for xanthan concentrations below 0.35%, saponins have no influence because the quantity of xanthan is insufficient to interact negatively with the little amounts of unsuitable saponins. Beyond this concentration, their effect becomes positive because the surfactants occupy the entire oily interface thus preventing the flocculation of the oily droplets and ameliorate the emulsion stability; the most relevant influence is recorded for a maximum concentration of oil and saponins.

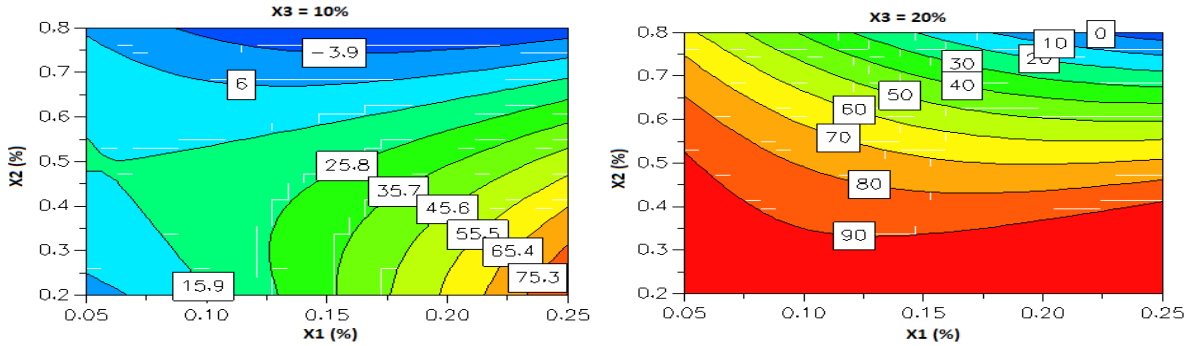

Figure 7. Influence of factors on the index of creaming (I-C).

4. Conclusions

A detailed investigation was conducted on the evaluation of emulsion formation and surface properties of triterpenic saponins. The physicochemical characterization showed that these bioemulsifiers, extracted from *Hedera helix Algeriensis* plan extracts, possess a good surface activity and are effective to form oil-in-water emulsions with low concentrations for an oil/water volume proportion of 80/20. A CCF design was used for better organization of tests and, for determination of the factor effects on the rheological properties and emulsion stability by means of mathematical simulation. It was shown that these natural molecules do not have a specific effect on emulsion's rheological properties, but contribute strongly to their stability by interacting with other excipients of the mixture. Also, it was noted that the hydrophobic phase has a notable impact on the rheological properties. Emulsions containing saponins exhibited shear-thinning and viscoelastic behaviors. Also, saponins improved the emulsion stability for a maximum concentration of oil and surfactant. This study provides useful information about the exploitation of triterpenic saponins as biodegradable and renewable surfactants.

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Conflicts of Interest

The authors declare no conflict of interest.

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