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

Nadjia Sabri 1, *, Nadji Moulai-Mostefa 1

1 LME, Université de Medea, Ain D’Heb, 26001 Medea, Algeria
* Correspondence: sabrinadja@yahoo.fr; Scopus Author ID 6701700183

Received: 18.04.2020; Revised: 4.05.2020; Accepted: 6.05.2020; Published: 12.05.2020

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 (CE24) and the stability of emulsion (SE) are calculated according to Eq. 1 and Eq. 2:

\[ CE_{24} = \left( \frac{H_E}{H_T} \right) \times 100 \]  
\[ SE = \left( \frac{CE_{24}}{CE_0} \right) \times 100 \]  

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:

\[ I-C = \left( \frac{H_{aq}}{H_T} \right) \times 100 \]  

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]:

$$\frac{\eta - \eta_\infty}{\eta_0 - \eta_\infty} = \frac{1}{(1 + (\dot{\gamma}K)^2)^n}$$

(4)

where $\eta$ is the solution viscosity, $\eta_0$ is the zero shear viscosity, $\eta_\infty$ 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 $\text{CE}_{24}$ (capacity of emulsification) and $\text{SE}$ (stability of emulsion) depending on saponin concentration. The results showed that $\text{CE}_{24}$ and $\text{SE}$ 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 (%) | Emulsification capacity $\text{CE}_0$ (%) | Emulsification capacity after 24 h $\text{CE}_{24}$ (%) | Emulsion stability $\text{SE}$ (%) |
|--------------------------|------------------------------------------|-------------------------------------------------|---------------------------------|
| 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 rheograms for prepared emulsions are characterized by the appearance of two regions, a first Newtonian region in the interval of weak shearing (< 0.01 s<sup>-1</sup>) 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<sup>2</sup> was found greater than 0.97, which confirms the adequacy of this rheological model.

![Figure 3. Flow Curve of formulated sample (Test 5).](image)

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'<sub>0</sub> and G''<sub>0</sub>, in the linear viscoelastic range (Figure 4).

![Figure 4. Viscoelasticity curve of formulated sample.](image)

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 \( \eta_0 \). 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 | \( X_1 \) (%) | \( X_2 \) (%) | \( X_3 \) (%) | \( K \) | \( \eta_0 \) (Pa.s) | \( G'_0 \) (Pa) | \( G''_0 \) (Pa) | I-C (%) |
|------|---------------|---------------|---------------|-------|-----------------|-----------|-----------|--------|
| 1    | 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):

\[
K = 22.31 - 67.35X_1 - 127.57X_2 + 61.78X_3 + 10.68X_1^2 + 134.35X_2^2 + 13.51X_3^2 + 79.81X_1X_2 - 4.11X_1X_3 - 68.72X_2X_3 
\]

(6)

\[
G'_0 = 5.52 - 0.33X_1 + 7.64X_2 - 0.58X_3 + 0.66X_1^2 - 0.47X_2^2 + 4.25X_3^2 - 0.68X_1X_2 - 0.84X_1X_3 + 1.90X_2X_3 
\]

(7)

\[
I-C = 65.76 + 3.80X_1 - 31.66X_2 + 24.20X_3 - 9.90X_1^2 - 12.75X_2^2 - 18.09X_3^2 - 24.50X_1X_2 - 13.50X_1X_3 - 8.50X_2X_3 
\]

(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.

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.

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).](image)

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.

**Funding**

This research received no external funding.
Acknowledgments

The authors declare no acknowledgments.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Vincken, J.P.; Heng, L.; De Groot, A.; Gruppen, A. Saponins, classification and occurrence in the plant kingdom. Phytochemistry 2007, 68, 275-297, https://doi.org/10.1016/j.phytochem.2006.10.008.

2. Guglu-Ustundag, Q.; Mazza, G. Saponins, properties, applications and processing. Critical Reviews in Food Science and Nutrition 2007, 47, 231-258, https://doi.org/10.1080/10408390600698197.

3. Reichert, C.L.; Salminen, H.; Weiss, J. Quillaja saponin characteristics and functional properties, Annual Review of Food Science and Technology 2019, 10, 43-73, https://doi.org/10.1146/annurev-food-032818-122010.

4. Shi, Z.Y.; Zeng, J.Z.; Wong, A.S.T. Chemical structures and pharmacological profiles of Ginseng saponins. Molecules 2019, 24, 443, https://doi.org/10.3390/molecules24132443

5. Cheok, C.Y.; Salman, H.A.K.; Sulaiman, R. Extraction and quantification of saponins: a review. Food Research International 2014, 59, 16-40, https://doi.org/10.1016/j.foodres.2014.01.057.

6. Ribeiro, B.D.; Barreto, D.W.; Coelho, M.A.Z. Use of micellar extraction and cloud point preconcentration for valorization of saponins from sisal (Agave sisalana) waste. Food and Bioprocess Technology 2015, 94, 601-609, https://doi.org/10.1016/j.fbp.2014.07.004.

7. Koczurkiewicz, P.; Klaś, K.; Grabowska, K.; Piska, K.; Rogowska, K.; Wójcik-Pszczoła, K.; Podolak, I.; Galanty, A.; Michalik, M.; Pękala, E. Saponins as chemosensitizing substances that improve effectiveness and selectivity of anticancer drug-Minireview of in vitro studies. Phytotherapy Research 2019, 33, 2141-2151, https://doi.org/10.1002/ptr.6371.

8. Abed El Aziz, M.M.; Said Ashour, A.; Gomha Melad, A.S. A review on saponins from medicinal plants: chemistry, isolation, and determination. Journal of Nanomedicine Research 2019, 8, 6-12.

9. Mohimipour, E.; Handali, S. Saponins: Properties, methods of evaluation and applications. Annual Review & Research in Biology 2015, 5, 207-220, https://doi.org/10.9734/ARRB/2015/11674.

10. Yassin, N.Z.; Melek, F.R.; Selim, M.A.; Kassem, A.A. Pharmacological activities of saponin containing fraction derived from GleditsiaacaspicaDesf. methanolic fruit extract. Der Pharm Lettre 2013, 5, 247-253.

11. Zhu, Z.; Wen, Y.; Yi, J.; Cao, Y.; Liu, F.; McClements, D.J. Comparison of natural and synthetic surfactants at forming and stabilizing nanoemulsions: Tea saponin, Quillaja saponin, and Tween 80. Journal of Colloid and Interface Science 2019, 536, 80-87, https://doi.org/10.1016/j.jcis.2018.10.024.

12. Mitra, S.; Dungan, S.R. Micellar properties of quillajasaponin. 1. Effects of temperature, salt, and pH on solution properties. Journal of Agricultural and Food Chemistry 1997, 45, 1587-1595, https://doi.org/10.1021/jf960349z.

13. de Faria, J.T.; de Oliveira, E.B.; Minim, V.P.R.; Minim, L.A. Performance of Quillajabarksaponin and β-lactoglobulin mixtures on emulsion formation and stability. International Journal of Food Properties 2017, 20, 1643-1654, https://doi.org/10.1080/10270274.2017.135837.

14. Salminen, H.; Bischoff, S.; Weiss, J. Impact of concentration ratio on the formation and stability of emulsions stabilized by Quillaja saponin-sodium caseinate mixture. Food Biophysics 2019, 14, 109-119, https://doi.org/10.1007/s11483-018-09563-x.

15. Reichert, C.L.; Salminen, H.; Bönisch, G.B.; Schäfer, C.; Weiss, J. Influence of concentration ratio on emulsifying properties of Quillaja saponin - protein or lecithin mixed systems. Colloids and Surfaces A: Physicochemical and Engineering Aspects 2019, 561, 267-274, https://doi.org/10.1016/j.colsurfa.2018.10.050.

16. Benahmed-Djilali, A.; Chemoul, T.; Kal, S.; Nabiev, M.; Besombes, C. Propriétés d’une pommade antibactérienne formulée à base de saponines extraites des feuilles de noyer. Phytothérapie 2017, 1-9, https://doi.org/10.1007/s10298-017-1145-9.

17. Chung, C.; Sher, A.; Rousset, P.; Decker, E.A.; McClements, J. Formulation of food emulsions using natural emulsifiers: utilization of quillajasaponin and soy lecithin to fabricate liquid coffee whiteners. Journal of Food Engineering 2017, 209, 1-11, https://doi.org/10.1016/j.jfoodeng.2017.04.011.

18. Kaur, K.; Kumar, R.; Mehta, S.K. Formulation of saponin stabilized nanoemulsion by ultrasonic method and its role to protect the degradation of quercitin from UV light. Ultrasrconics Sonochemistry 2016, 31, 29-38, https://doi.org/10.1016/j.ultraschon.2015.11.017.

19. Doost, A.S.; Camp, J.V.; Dewettinck, K.; Van der Meeren, P. Production of thymol nanoemulsions stabilized using Quillaja Saponin as a biosurfactant: Antioxidant activity enhancement. Food Chemistry 2019, 293, 134-143, https://doi.org/10.1016/j.foodchem.2019.04.090.
20. Cibulski, S.P.; Mourglia-Ettlin, G.; Teixeira, T.F.; Quirici, L.; Roehe, P.M.; Ferreira, F.; Silveira, F. Novel ISCOMs from Quillajabrasiliensis saponins induce mucosal and systemic antibody production, T-cell responses and improved antigen uptake. Vaccine 2016, 34, 1162-1171, https://doi.org/10.1016/j.vaccine.2016.01.029.
21. Yang, C.; Wu, H.; Wang, J. Formulation and evaluation of controlled-release of steroidal saponins-loaded collagen microspheres, Journal Materials  Technology Advanced Performance Materials 2019, 34, 534-539, https://doi.org/10.1080/10667857.2019.1591727.
22. Krstonosic, V.; Dokic, L.; Nikolic, I.; Milanovic, M. Influence of xanthan gum on oil-in-water emulsion characteristics stabilized by OSA starch. Food Hydrocolloids 2015, 45, 9-17, https://doi.org/10.1016/j.foodhyd.2014.10.024.
23. Hamiouda, S.; Yahoum, M.M.; Lefnaoui, S.; Hadjsadok, A.; Moulai-Mostefa, N. New alkylated xanthan gum as amphiphilic derivatives: Synthesis, physicochemical and rheological studies. Journal of Molecular Structure 2020, 1207, https://doi.org/10.1016/j.molstruc.2020.127768.
24. Burgos-Díaz, C.; Pons, R.; Espuny, M.J.; Aranda, F.J.; Teruel, J.A.; Manresa, A.; Ortiz, A.; Marqués, A.M. Isolation and partial characterization of a biosurfactant mixture produced by Sphingobacterium sp. isolated from soil. Journal of Colloid and Interface Science 2011, 361, 195-204, https://doi.org/10.1016/j.jcis.2011.05.054.
25. Rao, M.A. Rheology of fluid and semisolid foods: principles and applications. Boston (MA): Springer; 2007; https://doi.org/10.1007/978-0-387-70930-7.
26. Mironenko, N.V.; Brezhneva, T.A.; Poyarkova, T.N.; Selemenev, V.F. Determination of some surface-active characteristics of solutions of triterpene Saponin derivatives of oleanolic acid. Pharmaceutical Chemistry Journal 2010, 44, 157-160, https://doi.org/10.1007/s11094-010-0421-x.
27. Stanimirova, R.; Marinova, K.; Tcholakova, S.; Denkov, N.D.; Stoyanov, S.; Pelan, E. Surface rheology of saponin adsorption layers. Langmuir 2011, 27, 12486-12498, https://doi.org/10.1021/la202860u.
28. Holmoberg, K.; Jonsson, B.; Kronberg, B.; Lindman, B. Surfactants and polymers in aqueous solution. 2nd edition, New York: John Wiley & Sons; 2003.
29. Wojciechowski, K. Surface activity of saponin from Quillaja bark at the air/water and oil/water interfaces. Colloids and Surfaces B: Biointerfaces 2013, 108, 95-102, https://doi.org/10.1016/j.colsurfb.2013.02.008.
30. Ren, Y.; Ellisa, P.R.I.; Ross-Murphy, S.B.; Wang, Q.; Wood, P.J. Dilute and semi-dilute solution properties of an exopolysaccharide from Echerichia coli strain S61. Carbohydrate Polymers 2002, 52, 189-195, https://doi.org/10.1016/S0144-8617(02)00289-8.
31. Rodd, A.B.; Dunstan, D.E.; Boger, D.V. Characterisation of xanthan gum solutions using dynamic light scattering and rheology. Polymer 2000, 42, 159-174, https://doi.org/10.1016/S0144-8617(99)00156-3.
32. Chung, C.; Sher, A.; Rousset, P.; McClements, D.J. Impact of oil droplet concentration on the optical, rheological, and stability characteristics of O/W emulsions stabilized with plant-based surfactant: Potential application as non-dairy creamers, Food Research International 2018, 105, 913-919, https://doi.org/10.1016/j.foodres.2017.12.019.
33. Sun, C.; Gunasekaran, S.; Richards, M.P. Effects of xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions. Food Hydrocolloids 2007, 21, 555-564, https://doi.org/10.1016/j.foodhyd.2006.06.003.