ABSTRACT: The cosmetic industry provides a wide variety of shampoos to treat dandruff, containing insoluble ingredients such as Zn pyrithione. However, the solubility of this active ingredient is quite limited in both water and oil media; thus, antidandruff formulations must include a large amount of chemically synthesized stabilizing ingredients to avoid their precipitation. In this work, the stabilization of Zn pyrithione in O/W emulsions using a biosurfactant (BS) extract and Tween 80 is studied. The study includes an incomplete factorial design based on the tea tree oil/water ratio and both surfactant and biosurfactant concentrations. The formulations are characterized in terms of particle size, stability after 30 days, and solubility of Zn pyrithione. The formulation that provided the most favorable results contains Tween 80 (5%) and BS extract (2.5%), with an O/W ratio of 0.01. This provides the smallest particle size (40.5 μm), good stability after 30 days (91.0%), and the highest solubility of Zn pyrithione (59%). The results obtained enable the use of the combination of BS extract and Tween 80 as bio/surfactants of antidandruff shampoo formulations, along with another antiseptic agent such as tea tree oil. Furthermore, this is the first work where a biosurfactant is considered to be a stabilizing agent in antidandruff formulations.

INTRODUCTION

Dandruff is a generally known scalp disorder, also defined as the mildest version of seborrheic dermatitis. It is characterized by the formation of small flakes and sebum that occur in localized areas on the scalp, such as the forehead and the back of the ears beyond the hairline. Other symptoms derived from dandruff may be the feeling of a tight or dry scalp, producing irritation and itching. Therefore, it is stated as an abnormal condition that affects more than 50% of adults. What is more, its manifestation provokes a high socioeconomical impact, leading to the loss of self-esteem for the population that suffers from it.

Nowadays, there are a wide variety of marketed products to treat this problem. The most frequently used antidandruff treatment is the regular use of shampoos and conditioners that remove loosely attached flakes from the scalp. As a matter of fact, a successful antidandruff shampoo not only has to prove efficacy against dandruff removal to ensure patient compliance but also needs to offer excellent cosmetic and hair conditioning benefits, such as ease of combing, smoothness, softness, breakage reduction, and hair manageability. Recent developments in shampoo technology have already led to an increase in the efficacy of antidandruff agents, allowing shorter contact time and reducing irritation.

Those antidandruff shampoos contain agents that primarily exhibit an antimicrobial action against the growth of *Malassezia* species, the organisms responsible for dandruff. Among those agents, molecules such as azoles or hydroxypyridones and agents such as piroctone olamine, salicylic acid, selenium sulfide, ketoconazole, and zinc pyrithione can be found. Among these compounds, there is no big difference in efficacy. However, Zn pyrithione is preferred in terms of the overall performance, improving the appearance and feel of the hair. In this regard, Zn pyrithione has been established as an effective antiseborrheic agent. It is currently marketed in shampoos and hair dressings at concentration levels of about 0.5 and 2.0%, respectively. It has been successfully included in shampoos due to its strong antibacterial effect and ability to slow down the rapid turnover of epidermal cells, proving its efficacy in many studies and clinical trials. Moreover, this highly active compound has been listed in the Food and Drug Administration Final Monograph on Dandruff and Seborrheic...
Dermatitis as a Category I substance, meaning it is generally recognized as a safe and effective drug.\textsuperscript{12} However, this active ingredient is present in the form of dispersed fine solid particles since its solubility is quite limited in both water and oil media.\textsuperscript{13} Along this line, it has been stated that its solubility in water is about 6–20 ppm at pH 7 and increases to 35–50 ppm at pH 8.\textsuperscript{7,14}

On the other hand, there are other active agents available for use in antidandruff formulations such as tea tree oil. This oil extract has been demonstrated to be an excellent antiseptic, containing terpenes that penetrate the deeper layers of the scalp, developing more efficiently its disinfectant activity than other emollients.\textsuperscript{15}

Conversely, the stability of antidandruff formulations depends on the use of chemically synthesized surfactants. As a matter of fact, anionic surfactants have been used to solubilize Zn pyrithione formulations to stabilize these formulations. However, the presence of an anionic surfactant was proved to be insufficient to achieve dispersion stability.\textsuperscript{13} Moreover, it is noticeable that the surfactant concentrations used in these kinds of formulations are really high, around 30% in some cases,\textsuperscript{16,17} which can have harmful effects on the skin.\textsuperscript{18} Therefore, one possibility would be substituting these synthetic surfactants with their homologous biosurfactants, which present the same characteristics but better biocompatibility and lower toxicity than their chemical counterparts.\textsuperscript{19}

Conversely, an amphoteric biosurfactant (BS) extract has been obtained from corn stream residues. This BS extract is mainly composed of antioxidants, fatty acids, and lipopeptide biosurfactants,\textsuperscript{20,21} presenting an rHLB of 12 and good wetting activity in O/W formulations.\textsuperscript{22} It has been demonstrated to solubilize mica particles due to the adsorption onto the surface of the material, providing it with hydrophobicity, being able to form a stable emulsion.\textsuperscript{23} On the other hand, this BS extract has already been tested in damaged and undamaged hair, observing an improvement in the cuticle, with more homogeneous borders and thicker hair than untreated dyed hair.\textsuperscript{12,24}

The aim of the study is to obtain more natural and biocompatible formulations through the use of natural surfactants like that obtained from corn stream. It is expected that the biosurfactant under evaluation improves the stability and solubilization of Zn pyrithione in aqueous formulations in the presence of tea tree oil and with Tween 80 as a surfactant, which is widely used in shampoo formulations. For this purpose, a Box–Behnken factorial design was established with different formulations, using the concentration of Tween 80 and BS extract (%), together with the tea tree oil/water ratio, as independent variables of the study. Consequently, there were evaluated the particle size distribution of emulsions, the stability of formulations after 30 days of emulsion formation and the solubility of Zn pyrithione.

### EXPERIMENTAL SECTION

**Reagents.** Zn pyrithione powder was provided by Guinama S.L.U. (Lot 0081880, Spain), tea tree oil was provided by Gran Velada (Zaragoza, Spain), and Tween 80, synthesis grade, was provided by Scharlau S.L. (Spain).

**Biosurfactant Extract from Corn Stream. Extraction Procedure.** The biosurfactant (BS) extract was obtained from corn steep liquor (CSL) stream provided by Feed Stimulants (Netherlands). The CSL was diluted in water and extracted with an organic solvent using the protocol established by Vecino et al.\textsuperscript{25} The organic solvent was then subjected to vacuum evaporation and the oily biosurfactant extract was kept at −20 °C until its use.

**General Physicochemical Characterization of the BS Extract.** To corroborate that the biosurfactant extract obtained in this work possesses the same characteristics as those reported in previous works, this extract was subjected to several analyses, and the results were compared with some of the properties included in Table 1. Table 1 includes the properties of the biosurfactant extract under evaluation documented in previous works.

### Table 1. Physicochemical Properties of the Biosurfactant Extract from Corn Stream

| characteristic                     | physicochemical properties |
|------------------------------------|-----------------------------|
| elemental analysis                 | 74% C; 11.3% H; 1.5% N       |
| surface tension                    | 41 mN/m                      |
| CMC                                | 140 mg/L                     |
| pH                                 | 4.7                          |
| ionic charge                       | amphoteric                   |
| rHLB\textsuperscript{a,b}          | 12–13                       |
| wettability                        | good wetting agent in oil/water emulsions |
| foaming capacity                   | similar foaming capacity for other surfactants such as Span 20 |
| emulsifying activity               | poor emulsifier, but good cosurfactant activity in nanoemulsions prepared with Tween 80 |
| penetration into skin              | enhances the penetration of hesperetin in cream formulations and nanocrystal nanoemulsions |

\textsuperscript{a}rHLB, required HLB.

**Fourier Transform Infrared Spectroscopy (FTIR).** The biosurfactant under evaluation was characterized via identification of the functional groups and chemical bonds present in the biosurfactant extract. For this purpose, 1 mg of the BS extract was ground with 10 mg of potassium bromide and pressed (7500 kg for 30 s) to produce translucent pellets.

FTIR analyses were carried out on a Nicolet 6700 FTIR spectrometer (Thermo Scientific). The spectral measurements were made in absorbance mode in the range of 400–4000 cm\textsuperscript{-1}, with a resolution of 4 cm\textsuperscript{-1}, an average range of 32 data scanning, and a potassium bromide pellet for measuring background absorbance levels.

**Surface Tension and Critical Micellar Concentration (CMC).** The surface tension and critical micelle concentration (CMC) were determined for the biosurfactant extract. The CMC is the minimum surfactant concentration needed to produce the maximum reduction in the surface tension of water. Above the CMC, the surface tension of a biosurfactant solution is constant and specific for each biosurfactant. Below the CMC, a linear relationship can be established between the surface tension and the concentration of a specific surfactant in an aqueous solution. Thus, for establishing this relationship, different solutions containing different concentrations of this biosurfactant were prepared and their surface tension was measured using a Kruss tensiometer (K20 EasyDyne), choosing the Wilhelmy plate method at room temperature (21 ± 1 °C). Measurements were made in triplicate to increase the accuracy of the results.

**Elemental Analysis.** The sample was decomposed by combustion and was then analyzed using chromatographic...
analysis with thermal conductivity detection. C, N, H, and S were determined using a Fisons Carlo Erba EA-1108 CHNS-O elemental analyzer. Hence, the amount of N can be correlated with the protein content by multiplying it by a factor of 6.25.30

**Preparation of Zn Pyrithione Emulsions.** Different formulations were prepared based on a Box–Behnken factorial design to study the solubility and stabilization of Zn pyrithione in aqueous solutions. The emulsions were composed of two different surfactants (Tween 80 and BS extract), Zn pyrithione as the active compound, tea tree essential oil as the oil phase, and demineralized water. For the Box–Behnken experimental design, three different independent variables were established: X₁ (Tween 80 concentration, %), X₂ (BS concentration, %), and X₃ (oil/water ratio). The amount of the different components was constrained as follows: Tween 80 concentration (0−5%, w/w), biosurfactant extract concentration (0−5%, w/w), and the oil/water ratio (0.01−0.1, w/w). The concentration of Zn pyrithione was kept at 0.5% (w/w) for all emulsions, a typical concentration in antidandruff shampoos.

During the experimental design, the values of the independent variables were coded, obtaining the standardized (coded) dimensionless independent variables used with limits of variation (−1, 1). This was applied so that the independent variables selected in the study were not influenced by their magnitudes; thus, they were normalized and a relationship between coded and uncoded variables was established using linear equations. Coded variables were then assigned values of −1, 0, and +1, corresponding to the lowest, central, and maximum limits of variation for each one.

Formulations were prepared by adding the different components in the stated concentrations up to 8 g at room temperature (22 ± 1 °C). Then, all components were mixed within 2 min with an Ultraturrax IKA T25 (Germany) at 20.000 rpm, to guarantee the complete formation of the emulsions.

**Emulsion Characterization. Size Measurement and Emulsion Stability.** The particle size distribution of the emulsions (mean diameter) was measured by light scattering using a LS 13 320 particle size analyzer (Beckman Coulter, Spain), which accounts from 40 nm up to 2000 µm. Individual formulations were prepared and evaluated at different times (at the first day, after 15 days, and after 30 days of experiment). A volume of 1.5 mL of each formulation was directly poured into the analyzer tubes. No dilutions were necessary.

Conversely, the emulsion stability of each formulation was measured taking into account the particle size distribution at the first day of production (size₁ day) and after 30 days of experiment (size₃₀ days), as follows

\[
\text{stability(%) = 100} - \left[ \frac{\Delta (\text{size}_{30 \text{ days}} - \text{size}_{1 \text{ day}})}{\text{size}_{1 \text{ day}}} \right] \times 100
\]

Zn Pyrithione Solubility. Zn pyrithione solubility was determined after 30 days by analyzing the Zn content of the sample by inductively coupled plasma optical emission spectroscopy (ICP-OES). An Optima 4300DV ICP-OES (PerkinElmer, Spain) equipped with a OneNeb nebulizer, baffled cyclonic spray chamber, and optics with an Echelle polychromator system was used. First, 1 mL of each formulation was calcined in an oven at 500 °C for 3 h. Afterward, the sample was mixed with a solution of nitric acid (2%), diluting the resultant products of the reaction in demineralized water up to 10 mL.

The conditions used for running the experiments were as follows: auxiliary gas flow (0.2 L/min), nebulizer gas flow (0.6 L/min), plasma gas flow (15 L/min), and radio frequency generator power (1300 W). The instrument was calibrated with a solution of nitric acid at 2%. Zn was detected at 206.2 nm. Once the Zn concentration (mg/L) contained in each sample is determined, the solubility of Zn pyrithione can be calculated, taking into account that Zn pyrithione presents 20.7% of elemental Zn. Therefore, the solubility (%) of each sample, has been calculated as follows

\[
\text{solubility (\%)} = \frac{\text{Zn}_{\text{soluble (mg/L)}} \times \text{volume (L)}}{\text{Zn}_{\text{total (mg)}}} \times 100
\]

Apart from this, the solubility improvement ratio was studied to compare the solubility obtained in these formulations.
(solubility) with the medium solubility of Zn pyrithione in water (20 mg/L), as follows:

\[
\text{solubility improvement ratio} = \frac{\text{solubility (mg/L)}}{20 \text{mg/L}}
\]  

Macroscopic and Microscopic Analysis. The macroscopic views of the emulsions were obtained with a Samsung dual pixel 12.0 MP (F/1.7) mobile camera using an optical zoom (4×).

For microscopic characterization of the different emulsion droplets, a Nikon Eclipse E800 optical microscope equipped with a Nikon camera was used. The emulsion was placed on the microscope stage and observed through a 4× objective lens. The ocular was changed until different magnifications were obtained for the samples, ranging from 4× till 20×. The photographs were taken using NIS Elements D2.30 SPI software (Nikon).

Statistical Analysis. The experimental data were analyzed by the response surface method with Statistica 7.0 software, by adjusting the obtained dependent experimental data to a quadratic function shown in eq 4:

\[
Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_1^2 X_1^2 + \beta_2^2 X_2^2 + \beta_3^2 X_3^2
\]

where \( Y \) is the dependent variable, \( \beta \) denotes the regression coefficients (calculated from experimental data by multiple regressions using the least-squares method), and \( X \) denotes the fixed independent variables, corresponding to \( X_1 \) (Tween 80 concentration, %), \( X_2 \) (BS concentration, %), and \( X_3 \) (oil/water ratio).

The particle size of the formulations \( (Y_1) \), the stability after 30 days of experiment \( (Y_2) \), and the solubility of Zn pyrithione \( (Y_3) \) were chosen as dependent variables. All theoretical values for these variables can be predicted by eq 4 in the range of the independent variables included in the study.

## RESULTS AND DISCUSSION

Biosurfactant Characterization and Properties. The BS extract under evaluation has been characterized using infrared radiation, to elucidate the molecular components of the biosurfactant extracted. Figure 1 shows the FTIR spectrum of the biosurfactant extract from corn stream under evaluation. The spectral analysis of the biosurfactant indicates the presence of protein-related weak bands: C=O bond at 1750–1650 cm\(^{-1}\) (amide bonds) and N–H bonds at 1587 cm\(^{-1}\) (amide and amine bonds). The absorbance band between 3010 and 2850 cm\(^{-1}\) and bands at around 1467–1365 cm\(^{-1}\) denote the presence of C–H stretching corresponding to the CH\(_2\) and CH\(_3\) groups of aliphatic chains. The bands at 1743–1708 cm\(^{-1}\) are stretching vibrations of C–O and C=O bonds in carboxyl esters.

Furthermore, the bands at 1164 and 1240 cm\(^{-1}\) indicate the presence of a fatty acid. This spectrum is similar to those obtained in previous studies, showing the reproducibility of the extraction process.

As a result, the FTIR spectrum of the biosurfactant confirmed that this extract contains lipids and peptides, corroborating the lipopeptide nature of the biosurfactant extract (see Figure 1). Moreover, the elemental analysis revealed that this biosurfactant extract was composed of 72%
Significant solutions. Moreover, the particle size and stability (after 30 days) were studied for all emulsions. The formulations were prepared according to the Box–Behnken experimental design (Table 2).

As mentioned above, the independent variables established in the assay were the Tween 80 concentration ($X_1$), the BS concentration ($X_2$), and the tea tree oil/water ratio ($X_3$). Conversely, the dependent variables studied were the particle size of each emulsion ($Y_1$), the stability of the emulsion after 30 days ($Y_2$), and the solubility of Zn pyrithione ($Y_3$). All experimental results obtained for these dependent variables are summarized in Table 2.

According to Table 2, the lowest values of the different variables will be coded as $-1$ (0% for Tween 80 concentration, 0% for BS concentration, and 0.01 for the oil/water ratio) the intermediate as 0 (2.5% for Tween 80 concentration, 2.5% for BS concentration, and 0.055 for the oil/water ratio) and the highest as 1 (5.0% for Tween 80 concentration, 5.0% for BS concentration, and 0.1 for oil/water ratio).

Regarding the particle size ($Y_1$), it is observed in Table 2 that the values ranged from 14.8 to 93.7 μm for the different formulations. In general, emulsions with a smaller particle size are preferred because they are usually more stable and penetrate better into the deepest layers of hair. However, in this case, the emulsion particle size is restricted by the particle size of Zn pyrithione powder, as it contains particles of 20 μm diameter. As a consequence, emulsion particle sizes are above this value. As a matter of fact, it is noted that the formulation with the lowest particle size (14.8 μm) corresponds to the control sample (formulation number 1) as it does not contain any bio/surfactant on the formulation and, consequently, almost no Zn pyrithione in suspension. The rest of the emulsion particle sizes are found to be in the range from 33.9 to 93.7 μm.

Another point to take into consideration is that the smallest sizes, in the presence of Zn pyrithione, correspond to the formulations containing the biosurfactant extract from corn stream, as can be observed in formulation numbers 6, 7, and 12, which possess a particle size of 40.5, 33.9, and 47.0 μm, respectively.

In regard to the stability of the emulsions ($Y_2$), the values shown in Table 2 were calculated according to eq 1, which takes into account the particle size distribution (mean diameter) of the emulsion at the first day of preparation and after 30 days. In this respect, if the mean diameter is constant through the experimental time, it means that the formulation remains stable, as particles do not agglomerate, keeping the colloidal system unchanged. In this sense, it can be observed that, except for formulation number 1, all stability values are above 50%, showing good stability of all emulsions. Moreover, it is necessary to remark that higher stability values were achieved for formulation numbers 3 (90.0%), 6 (91.0%), and 8 (93.2%). Once again, all of these formulations have in common the presence of the BS extract from corn stream, as can be observed in Table 2. These results are in concordance with a previous study, where the BS extract under evaluation was used to stabilize Pickering emulsions containing mica and vitamin E, observing a synergistic effect between mica and the BS extract, obtaining stability values around 80% after 30 days of emulsion formation.

Additionally, the solubility of Zn pyrithione in the different formulations ($Y_3$) is determined after analyzing the Zn content on each sample, following eq 2. The values are quite different for the formulations, with the percentage of solubility ranging

Table 4. Regression Coefficients and Their Statistical Significance for Variables $Y_1$, $Y_2$, and $Y_3$

| coefficient | $Y_1$ | $P_{<1}$ | $Y_2$ | $P_{<2}$ | $Y_3$ | $P_{<3}$ |
|-------------|-------|----------|-------|----------|-------|----------|
| $\beta_0$   | 73.5  | 0.0008$^a$ | 73.0  | 0.0009$^a$ | 6.1   | 0.0018$^a$ |
| $\beta_1$   | 5.1   | 0.0608   | 10.6  | 0.0172$^a$ | 4.2   | 0.0014$^a$ |
| $\beta_{11}$| -24.1 | 0.0063$^a$ | -3.3  | 0.2588   | 11.1  | 0.0004$^a$ |
| $\beta_2$   | 7.0   | 0.0330$^a$ | 15.8  | 0.0079$^a$ | 3.6   | 0.0020$^a$ |
| $\beta_{22}$| 2.6   | 0.3048   | -14.5 | 0.0199$^a$ | -11.1 | 0.0040$^a$ |
| $\beta_3$   | -5.7  | 0.0490$^a$ | -4.9  | 0.0748   | -1.8  | 0.0808$^a$ |
| $\beta_{33}$| -2.3  | 0.3507   | 11.3  | 0.0325$^a$ | 10.8  | 0.0004$^a$ |
| $\beta_{12}$| -9.5  | 0.0356$^a$ | -16.3 | 0.0148$^a$ | 0.9   | 0.0574   |
| $\beta_{13}$| 9.9   | 0.0332$^a$ | 0.0   | 1        | -18.4 | 0.0001$^a$ |
| $\beta_{23}$| -15.5 | 0.0139$^a$ | -1.8  | 0.4738   | -0.2  | 0.4686   |

$^a$Significant coefficients ($p < 0.05$).

Figure 3. Microscopic images at 40X of the emulsions from (A) formulation number 6 (5% Tween 80, 2.5% BS, and 0.01 tea tree oil/water ratio) and (B) formulation number 8 (5% Tween 80, 2.5% BS, and 0.1 tea tree oil/water ratio).
from 1.2 to 59.0%. In fact, formulations with the worse solubility (1.2, 1.7, and 1.4%) have in common the absence of the BS extract, as can be observed in Table 2 (formulation numbers 1, 2, and 9, respectively). Therefore, it can be observed that the presence of Tween 80, even at the highest concentration (formulation number 2), gave a poor solubility of Zn pyrithione similar to the control (formulation number 1). Meanwhile, the capacity of the BS extract alone to solubilize Zn pyrithione is higher than that of Tween 80 alone, at the same O/W ratio (see formulation number 3).

Additionally, it is noted when formulation numbers 6 and 8 are compared that both possess the same ingredients but different O/W ratios, and the solubility of Zn pyrithione is improved (from 11.6 to 59.0) when higher O/W ratios are used.

Conversely, the best values (59.0 and 33.6%) were obtained for those formulations containing 2.5% of BS extract in their formulations (formulation numbers 6 and 7, respectively). It is interesting to remark that using the BS extract alone as the stabilizing agent and in the presence of the highest O/W ratio assayed (formulation number 7) the second highest exper-
imental value for the solubility of Zn pyrithione (33.6%) was achieved.

However, the synergistic effect of both surfactant and biosurfactant improves the solubility of Zn pyrithione, increasing the solubility in all cases, being this effect more noticeable at lower O/W ratios (see formulation numbers 5 and 6). The above-mentioned behavior confirms the capability of the BS extract under evaluation to stabilize emulsions containing minerals with poor solubility in water. Rincon-Fotán et al. observed that the BS extract under evaluation was able to solubilize an inert material (mica) by being adsorbed onto the surface of mica powder, keeping it in solution. If this behavior is applied to the current formulations, it can be speculated that the BS extract can be adsorbed onto the surface of Zn pyrithione powder acquiring hydrophobic properties, which indicates that it is stable at the oil–water interface, obtaining a better emulsion. Moreover, as the biosurfactant extract presents an amphoteric character, it can provide partial charges to this powder improving its stabilization. These charges would allow interactions with the dispersed medium of the emulsion, making Zn pyrithione soluble.

It is known that the maximum soluble concentration of Zn pyrithione in water is stated to be around 6–20 mg/L. In this respect, Table 3 shows the soluble concentration in mg/L obtained for each sample, which are in all cases above 20 mg/L, and the solubility improvement ratio, obtained from eq 3.

Therefore, in formulation number 6, a solubility of 2948 mg/L was achieved, improving the Zn pyrithione solubility 147 times. This emulsion is also characterized by higher stability (91.0%) and a smaller particle size (40.5 μm) in comparison with the rest of the formulations. Regarding its composition, it contains 7.5% of surfactant (5.0% of Tween 80 and 2.5% of BS extract) and a small content of oil (1%).

It must be mentioned that the studies related to the solubility of Zn pyrithione are quite scarce. In this way, different patents have been found in the literature, where Zn pyrithione is solubilized through the addition of different synthetic compounds. As an example, US Patent 3940482 conveys the possibility of solubilizing Zn pyrithione, thanks to the use of aliphatic polyamines or quaternary ammonium compounds, forming a complex with Zn pyrithione in water, obtaining values of 100 mg/L of soluble Zn pyrithione, which is well below the maximum value achieved in this assay (2948 mg/L). The problem that arises from this concept is that it is just stable at pH above 9.0, which can lead to a destabilization of Zn pyrithione (above pH 8.0). In this sense, another study (US3636213A) presents a method to solubilize heavy metal salts of 1-hydroxy-2-pyridineethanol by mixing the salts with an amine, and neutralizing the resultant alkaline solutions with mineral acids or simple carboxylic acids to form compositions of a pH acceptable for more general application to the skin and hair. The solubility of the zinc salt in all formulations having a pH of about 8 was proved to be around 200 mg/L. However, the most useful cosmetic and dermatologic compositions have a lower pH, as the pH of the scalp is stated to be 5.5, whereas for the hair shaft, it is about 3.7. Therefore, it is necessary to prepare shampoo formulations with a pH from 5 to 8. For instance, the use of suitable amines for solubilizing the metal pyrithiones, such as ethanolamine, diethanolamine, or diglycolamine (among others), has been proven.

Other studies showed the possibility of producing Zn pyrithione in situ by the reaction of sodium pyrithione and zinc chloride, obtaining a maximum solubility of 1000 mg/L. Again, this assay has proven the possibility of solubilizing Zn pyrithione to a larger extent than in the mentioned studies, using more natural and biocompatible ingredients than synthetic amines or anionic surfactants.

Another example in which sodium pyrithione has been used shows the possibility of adding as well some organic amines to enhance solubility. Accordingly, the sodium salt of pyrithione is highly soluble in water and present fungicidal and bactericidal properties. However, it has been found to be toxic and unacceptable for use in cosmetics or dermatological compositions for topical application to the skin.

Contrarily, the different experiments performed in this study have shown promising results without the need to add organic compounds, to include Zn pyrithione in hair care formulations. All mentioned methods use different amines or acids to enhance the solubility of Zn pyrithione, resulting in a tough procedure where the different components must be mixed. However, using the BS extract under evaluation achieves solubility values of Zn pyrithione similar to or above those obtained in those studies, providing new formulations composed of greener compounds.

Emulsion Characterization. All formulations have been characterized by light microscopy and macroscopic analysis. Figure 2 shows the macroscopic images of the different formulations.

In this regard, it can be observed that the emulsions with an orange color contain the BS extract, as it is yellowish. It was observed that the emulsions just containing the BS extract, without Tween 80 (formulation numbers 3, 5, and 7) present less stable emulsions; however, emulsions containing Tween 80 and the BS extract as a cosurfactant showed good stability (formulation numbers 4, 6, 8, 12, 13, and 14), which is in agreement with the data reported in a previous work, where it was observed that the biosurfactant under evaluation improves the capacity to stabilize O/W emulsions when it acts as a cosurfactant. Moreover, the capacity of the BS extract to form stable nanoemulsions has been demonstrated.

Conversely, Figure 3A,B shows the microscopic analysis of some formulations with a favorable size and emulsion stability, as well as good solubility for Zn pyrithione (formulation numbers 6 and 8).

It is noticeable that the behavior observed in Figure 3A,B is quite similar, as both emulsions present small and quite homogeneous droplets, where the particles remain dispersed. However, it can be observed in Table 2 that the amount of Zn pyrithione solubilized was higher in formulation number 6 (59.0%), against formulation number 8 (11.6%). The main difference between them lies in the oil/water ratio, observing an improvement in Zn solubility when the oil content is lower in the formulation, which is not really appreciated in the emulsion microscopic images.

Statistical Analysis. The experimental results were subjected to a statistical analysis (Statistica 7.0 software) to obtain different theoretical equations that can predict the behavior of the dependent variables under study (Y1, Y2, and Y3). In this regard, Table 4 shows the different coefficients and their significance obtained for the statistical analysis of this Box–Behnken experimental design. Three different equations were obtained for each dependent variable, using the significant coefficients obtained in Table 4, as follows.
\[ Y_1 = 73.5 + 7.0X_2 - 5.7X_3 - 9.5X_2X_2 + 9.9X_3X_3 \]
\[ - 15.5X_2X_3 - 24.1X_1^2 \]  
\[ Y_2 = 73.0 + 10.6X_1 + 15.8X_2 - 16.3X_2X_2 - 14.5X_2^2 \]
\[ + 11.3X_3^2 \]  
\[ Y_3 = 6.1 + 4.2X_1 + 3.6X_2 - 1.8X_3 - 18.4X_2X_3 + 11.1X_2^2 \]
\[ - 11.1X_2^2 + 10.8X_3^2 \]

According to the \( p \)-values obtained, concentrations of Tween 80 \( (X_2) \) and the BS extract \( (X_3) \) were the most significant variables for particle size \( (Y_1) \) and stability of emulsions \( (Y_3) \), whereas the O/W ratio \( (X_1) \) was the least influential variable (Table 4). However, regarding the solubility of Zn pyrithione \( (Y_4) \), the O/W ratio \( (X_1) \) possesses a similar significance to Tween 80 \( (X_2) \) and the BS extract \( (X_3) \). This information can be corroborated in Figure S1 (see the Supporting Information), which shows the Pareto chart of effects for the different variables \( (Y_1, Y_2, Y_3, \text{and } Y_4) \).

Furthermore, the statistical analysis provides information of the correlation established between the experimental and theoretical values, which are really well adjusted, with a correlation value \( (R^2) \) of 0.81 for \( Y_1 \), 0.77 for \( Y_2 \), and 0.94 for \( Y_3 \) (see Figure S2 in the Supporting Information).

On the other hand, Figure 4 shows different graphics obtained using the oil/water ratio \( (X_1) \) as a fixed variable at its minimum (0.01) and maximum (0.1) values. Figure 4A,B represents the variation of the particle size at low and high oil/water ratios, respectively. In this regard, it can be observed that at lower O/W ratios (Figure 4A) the presence of the BS extract \( (X_3) \) increases the particle size of emulsions, whereas at higher O/W ratios (Figure 4B), the concentration of Tween 80 \( (X_2) \) is the variable that increases the particle size of emulsions to a large extent. Therefore, at lower O/W ratios (Figure 4A), higher concentrations of Tween 80 and lower to intermediate concentrations of the BS extract can be used to obtain a smaller particle size, whereas at higher O/W ratios (Figure 4B), the BS extract can be added in higher concentrations than Tween 80.

Regarding the stability after 30 days, Figure 4C,D presents its variation as a function of BS \( (X_3) \) and Tween 80 \( (X_2) \) concentration at lower and higher O/W ratios, respectively. In general, it can be observed that the increase in Tween 80 and BS concentrations better stabilizes the emulsions in all cases, regardless of the O/W ratio.

Finally, 3D graphics were obtained for the solubility of Zn pyrithione (Figure 4E,F). Figure 4E shows the variation of the solubility at low O/W ratios, observing values of maximum solubility of Zn pyrithione at high concentrations of Tween 80, in the presence of intermediate to high concentrations of the BS extract. Nonetheless, in the case of emulsions containing higher O/W ratios (Figure 4F), best results were obtained in presence of the BS extract but with a reduced concentration of Tween 80. All of these results corroborate the significant effect of the O/W ratio on the Zn pyrithione solubility.

**SUMMARY AND CONCLUSIONS**

In conclusion, a BS extract from corn stream was demonstrated to produce formulations with small particle size, higher stability, and, overall, good solubility of Zn pyrithione. In fact, the BS extract was able to enhance the solubility of Zn pyrithione in aqueous formulations, up to 147 times in comparison with its solubility in water, with the optimum formulation of Tween 80 (5%) and BS extract (2.5%) and an O/W ratio of 0.01.

Moreover, some of the different oil-in-water formulations of the study have been proven to be quite stable even after 30 days, up to values of 93.2%.

Conversely, this work supposes an advance in terms of biocompatibility, as the current ingredients used in the industry for stabilizing Zn pyrithione are from synthetic origin.

In fact, the biosurfactant under evaluation allowed achieving solubility values of Zn pyrithione similar to or above the ones obtained in those studies carried out with persistent organic surfactants. Thus, the inclusion of the biosurfactant extract and Tween 80 in antidandruff shampoo formulations, along with another antiseptic agent such as tea tree oil, could be presented as an interesting option.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03679.

Pareto chart of standardized effects (Figure S1) and the correlation between observed and predicted values (Figure S2), for the three dependent variables (particle size, stability after 30 days, and solubility of Zn pyrithione) (PDF)

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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This study was supported by the Spanish Ministry of Economy and Competitiveness (MINECO) under the project CTM2015-68904 (FEDER funds). X.V. is also grateful for her Juan de la Cierva contract (IJC-2016-27445). L.R.-L.
acknowledges the Spanish Ministry of Education, Culture and Sport for her pre-doctoral fellowship (FPUI15/00205), and M.R.-F. also acknowledges the University of Vigo for her pre-doctoral fellowship.

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