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CHEMICAL SCIENCES

Nanoemulsion with wine lees: a green approach

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Abstract: Bioactive substances can be found in wine lees, a waste from the winemaking industry. This work developed two formulations, a nanoemulsion with coconut oil (NE-OC) and a nanoemulsion with coconut oil and 0.5% of wine lees extract (NE-OC-Ext), to investigate their effect on untreated, bleached, and bleached-colored hair. The oil-in-water (O/W) nanoemulsions were prepared with coconut oil, Tween™ 80, Span™ 80, Aristoflex™ AVC, Conserve NovaMit MF™, wine lees extract, and deionized water. The hydration measurements were carried out using a Corneometer® CM 825 with the capacitance method. Scanning electron microscopy (SEM) was used to characterize the effect of formulations on hair fibers. Differential Thermal Analysis (DTA) was to assess the thermal stability and compatibility of wine lees and coconut oil in formulations. Compared to NE-OC, NE-OC-Ext showed a greater hydration effect on bleached-colored hair. DTA showed that NE-OC-Ext presented a smaller number of exothermic degradation events than those of NE-OC, suggesting good interaction and compatibility of the wine lees extract in this formulation. This study highlights the value of wine lees, a residue from the winemaking process, and its possibility of use as raw material for the cosmetic hair industry since it shows a greater moisturizing potential in colored hair.

Key words: Hair, nanoemulsion, quercetin, wine.

INTRODUCTION

Vitis vinifera L. is an important crop and that is consumed the worldwide (Bustamante et al. 2008). In 2018, the global production of grapes was 77.8 million tons, of which 57% were wine grapes (OIV 2019). Globally, the grape processing industry produces many products and wastes, such as grape stalks, grape pomace, and wine lees. Therefore, pomace and wine lees represent a challenge for waste management (Nagai et al. 2019, Antonic et al. 2020).

In general, grape pomace and wine lees are sent to alcohol distilleries; however, this practice is not always followed by all wine producers, who generate even more residues and organic waste (Bustamante et al. 2008). The accumulation of these residues can be considered as pollutants with characteristics such as low pH, the presence of organic compounds, and resistance to biological degradation (Bustamante et al. 2008, Nagai et al. 2019, Antonic et al. 2020).

Wine lees are the residue formed at the bottom of the container which contains the wine, after its fermentation, during storage, or after authorized treatments in the wine making process, or as a residue obtained after filtering or centrifuging this product (Fia 2016). This residue is rich in bioactive phenolic compounds, and has great potential in the food, pharmaceutical, and cosmetic industries. However, wine lees are the least studied residue in the winemaking process (Romero-Díez et al. 2018).

Green chemistry techniques for the reuse of these residues are increasing, because they are low-cost materials that are rich in bioactive phytochemicals and have cosmetic potential (Ovcharova et al. 2016, Jara-Palacios et al. 2018). Moreover, consumer demand for natural and sustainable ingredients and products is leading to a new direction for the development of raw materials, product and waste management, improving the application of resources, especially those that can reduce the environmental impact (Yingngam et al. 2022).

Grape extracts have already been incorporated into cosmetic products such, as sunscreens, anti-aging products, skin depigmentation, oral care products, and skin penetration formulations (Hoss et al. 2021). They are an important source of bioactive compounds with antioxidant, anti-hyperpigmentation, and anti-aging properties, including phenolic compounds (Jara-Palacios et al. 2018, Hoss et al. 2021).

There is also a lack of research on combining natural products and nanotechnology for cosmetic purposes, for example in hair care formulations (Aziz et al. 2019). This is an important area to invest in, as the global hair care market size is expected to expand at a compound annual growth rate of 6.6% from 2021 to 2028 (Aziz et al. 2019).

Hair treatments are constantly performed by people to enhance their well-being, and these treatments can damage the integrity of cuticle, the outermost layer of the hair (Bloch et al. 2019). Therefore, hair health is important, and people are concerned about maintaining it. Cosmetic products need to have reparative properties to restore the hair fibers to their undamaged state (Marsh et al. 2015).

This aim of this work was to extract and characterize wine lees extract, which is a residue from the winemaking process, to develop and characterize a phytocosmetic formulation using nanotechnology, a nanoemulsion with wine lees extract, and to determine its effect on different hair fibers, such as untreated hair, bleached hair, and bleached-colored hair. This work is therefore part of the development of sustainable products, one of today's priorities.

The nanoemulsion was chosen as a nanosystem because it is an oil-in-water dispersion widely used in the cosmetic field because of its bioefficacy, biophysical, and sensorial benefits. Also, the nanodroplets can be diffused into hair fibers due to their small particle size enhancing cosmetic effects (Vijaya et al. 2016, Hu et al. 2012).

MATERIALS AND METHODS

Materials

Wine lees were provided by Adega Ana Vieira Pinto, located in Borba (Alentejo), Portugal, in January 2017. Grape varieties were Aragonez, Trincadeira, Alicante Bouschet, Touriga Nacional, Syrah, Carignan, and Cabernet. Coconut oil was supplied by Organic. Ammonium acryloyldimethyltaurate/VP copolymer (Aristoflex™ AVC) was purchased from Pharma Special (Brazil). Methylisothiazolinone/phenoxyethanol solution (Conserve NovaMit MF™) was purchased from Biovital (Brazil), and both sorbitan monooleate (Span™ 80) and polysorbate 80 (Tween™ 80) were purchased from Farmos (Brazil).

Wine lees extraction

Lyophilized wine lees (5 g) were extracted with 100 mL of MeOH: H_2O (1:1, v/v) by sonication for 1 hour. At the end of the process, the soluble part of the material was retained, while the insoluble part was subjected to a second extraction with 100 mL of acetone: H_2O (7: 3, v/v), by sonication for 1 h, in an attempt to extract molecules with

different polarities Afterward, the soluble part in the last solvent system was mixed with the previous soluble part, giving the wine lees extract (3 g).

Wine lees characterization

The wine lees extract was analyzed by mass spectrometry using an electrospray ionization source (ESI-MS) in negative mode. The spectrum was obtained using a Bruker spectrometer (model 9.4 T Solarix) coupled to a micrOTOF analyzer, which provides excellent mass resolution and mass accuracy. The mass range analyzed was 200-2000 m/z. The parameters used were a nebulizer gas pressure of 0.5-1.0 bar, capillary voltage of 3-3.5 kV, and a capillary transfer temperature of 523 K. The spectrum was processed using Bruker Compass DataAnalysis 4.2, and the double bond and ring equivalents of each molecule were determined from the Double Bond Equivalent (DBE) value.

The antioxidant activity of the wine lees extract was evaluated by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging method in 96-well plates with a capacity of 250 µL. A stock solution of wine lees extract was prepared at a concentration of 1 mg/ml in distilled water. Dilutions were made from this solution to obtain solutions at concentrations of 500, 250, 200, 100, 25 and 5 µg/mL. To each well, 175 µL of each wine lees extract solution, at different concentrations, and 50 µL of the DPPH solution, at a concentration of 0.3 Mm, in methanol were added. The negative control was a mixture of 125 µL of methanol and 50 µL of the DPPH solution. The analysis was performed in three triplicate (n= 9) (Brand-Williams et al. 1995).

The reactions took place at room temperature for 30 minutes and then absorbance readings were taken at 518 nm in a VersaMax™ Microplate Reader (ELISA). The antioxidant activity was defined according to Equation 1:

$$\% \text{ DPPH radical scavenging activity} = [(Ab - As) / Ab] \times 100 \quad (1)$$

where Ab is the absorbance of the control, and As is the absorbance of the sample.

The antioxidant activity can be expressed by the determination of EC_{50}, which is, the concentration of sample required to reduce the DPPH radical by 50% (Brand-Williams et al. 1995). The EC_{50} value of the wine lees extract was calculated by non-linear regression using the Graph Pad Prism® software.

In vitro cytotoxicity study of Wine lees

The cytotoxicity of the wine lees extract was evaluated in normal human keratinocytes (HaCaT cell line from the Rio de Janeiro Cell Bank – code 0341). Cells were grown at 1×10^4 cells/well in 96-well plates, in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, L-glutamine, and penicillin-streptomycin and incubated at 37°C, with 5% CO_2. The culture medium was changed every 3 days, and the cells were grown to confluence. Then, the cells were exposed to 200 µL of wine lees extract, and after 48 h of exposure, the MTT cell viability assay was performed (Mosmann 1983). MTT solution (200 µL - 5 mg/mL) was added to the plate and incubated for a further 3 h. Then, DMSO was added, and cell viability was measured at 570 nm using a microplate reader (TP-Reader, Thermoplate, Brazil) (Mosmann 1983).

Hydrophile-lipophile balance (HLB) study

A hydrophilic-lipophilic balance (HLB) study was performed to select the amount and type of surfactant for the formulations. In this study, 12 samples were developed, as shown in Table I. The emulsions were prepared by keeping the

Table I. Hydrophilic-lipophilic balance (HLB) Study.

Samples	% of Tween™ 80	% Span™ 80	% of Coconut oil	% of Water	HLB
1	0%	100%	7.5%	77.5%	4.3
2	10%	90%	7.5%	77.5%	5.4
3	15 %	85%	7.5%	77.5%	5.9
4	25 %	75%	7.5%	77.5%	7.0
5	35 %	65%	7.5%	77.5%	8.1
6	45%	55%	7.5%	77.5%	9.1
7	55 %	45 %	7.5%	77.5%	10.2
8	65 %	35 %	7.5%	77.5%	11.3
9	75%	25%	7.5%	77.5%	12.3
10	85%	15%	7.5%	77.5%	13.4
11	90%	10%	7.5%	77.5%	13.9
12	100%	0%	7.5%	77.5%	15

percentage of water (77.5%), surfactant blend (15%), and coconut oil (7.5%) constant. For the selected surfactants, Span ™ 80 (HLB 4.3) and Tween™ 80 (HLB 15.0), a range of HLB from 5 to 14 was calculated using Equation 2 (Streck et al. 2014).

$$HLB_T \times T\% + HLB_S \times S\% = HLB_{mix} \quad (2)$$

Where, the HLB_T, $HLB_{S,}$ and $HLB_{mix,}$ are the HLB values of Tween ™ 80, Span ™ 80, and the surfactant blend, respectively, T% is the mass percentage of Tween ™ 80, and S% is the mass percentage of Span ™ 80 in the blend of surfactants, respectively.

In the HLB study, 30 g of formulations were developed. The lipophilic phase, consisting of Span ™ 80, Tween ™ 80, and coconut oil, was slowly added to the hydrophilic phase, composed of water, under constant magnetic stirring, in a stirring plate (IKA, model C-MAG HS 7), for 15 min at room temperature (25 °C). They were then centrifuged (Daiki 80-2B) at 2,300 rpm for 10 min at 25 °C. After 24 h, the HLB value required to stabilize the systems was characterized macroscopically by visual inspection, where stability was verified by the presence of the creaming, coalescence, or phase separation (Coelho et al. 2018).

Development of nanoemulsions

The components used for the formulations are listed in Table II. The formulations developed were nanoemulsion with coconut oil (NE-OC) and nanoemulsion with coconut oil and wine lees extract (NE-OC-Ext). The oil-in-water (O/W) nanoemulsions were prepared with coconut oil (7.5%), Tween™ 80 (0.75%), Span™80 (14.25%), Aristoflex™ AVC (0.5%), Conserve NovaMit MF™ (0.1%), wine lees extract (0.1%) and deionized water (76.3%).

The oil phase consisted of coconut oil and the surfactants Tween™ 80 and Span™ 80, the concentration of which was determined in the HLB study. The percentages of the different components of the nanoemulsions were chosen according to Alves et al. (2020). The formulations were prepared by the fusion-emulsification method with high energy, using a mechanical stirrer (Fisatom - 718), where the oil phase was dispersed in the aqueous phase (Alves et al. 2020). The oil phase was composed of coconut oil heated at 30 °C, the nonionic surfactants

Table II. Formulations developed: nanoemulsion with coconut oil (NE-OC) and nanoemulsion with coconut oil and wine lees extract (NE-OC-Ext).

Components	NE-OC	NE-OC-Ext
Polysorbate 80 (Tween™ 80)	0.75 %	0.75 %
Sorbitamonoleate (Span™80)	14.25 %	14.25 %
Coconut oil	7.5 %	7.5 %
Ammonium acryloyldimethyltaurate/VP copolymer (Aristoflex™AVC)	0.5 %	0.5 %
methylisothiazolinone/phenoxyethanol solution (Conserve NovaMit MF™)	0.1 %	0.1 %
Wine lees extract	-	0.1 %
Deionized water	76.9 %	76.8 %

(Tween™ 80 and Span™ 80), and the preservative (Conserve NovaMit MF™). All were mixed and homogenized in a mechanical stirrer to form the oil phase. The aqueous phase consisted of Aristoflex™ AVC, water, and wine lees extract. The Aristoflex™ was added to the water phase and then slowly stirred on a mechanical stirrer at 25 °C. The wine lees extract was added to the water phase. The two phases, water and oil, were homogenized on a mechanical stirrer until a homogeneous mixture was obtained.

The mixture was processed in an ultrasonic processor (model UP 100 H, with 60% of power = 60 W, equipped with a 7-mm-diameter tip, Dr. Hielscher GmbH, Germany), and the input power level was 60% of the total input power. The processing time of the NE was 5 min, and the temperature was maintained at 5 °C by a cold bath (Coelho et al. 2018). The total amount obtained for each formulation was 10 g. Two phytocosmetic nanoemulsions were developed, NE-OC and NE-OC-Ext, with a concentration of 0.1 g of wine lees extract in 100 g of sample.

Characterization of nanoemulsions

Macroscopic analysis

Formulations were visually evaluated after processing (time 0) at 24 hours, 48 hours, 15 days, and 30 days to detect color, changes, instabilities, or homogeneity, such as creaming, coalescence, or phase separation.

Assessment of mean droplet size

The mean droplet size of the samples was characterized using laser diffraction with a Mastersizer (Malvern Instruments, model MAF5000). A quantity of the sample sufficient to obtain obscuration rates between 12 and 19% was introduced into the apparatus with distilled water. Measurements were performed in triplicate (Coelho et al. 2018).

Thermal Analysis

Thermal analysis of the nanoemulsions was performed by Coupled Thermogravimetry (TG-DTA) using a TG-DTA instrument (model STA 6000, Perkin Elmer). The aim of this analysis was to obtain information on the interactions between the components present in the formulations. The samples were heated at a rate of 10 °C.min^{-1} from 25 °C to 600 °C under a nitrogen atmosphere.

Evaluation of Cosmetic Hydration

The hair samples were donated by health female donor from a South America female.

The virgin and chemically treated hairs were first washed and defatted with a solution of sodium laureth sulphate 2% in water and then rinsed with distilled water before treatment with the formulations. This procedure is intended to

remove any adsorbed material, and thus avoid interferences in the test (Villa et al. 2013). Then, the hair samples were dried at 70 °C (Taiff Style 2000 W hairdryer), with 10 cm from the hair samples.

The hair samples were divided into 3 different groups: untreated hair, bleached hair, and bleached and colored hair. Each sample weighed approximately 1 g and measured 15 cm. Each group of hair was also divided into three parts. The first hair sample was washed with a 2% sodium laureth sulphate solution. The second and third parts of the hair samples were washed with 2% sodium laureth sulphate solution, followed by the application of 200 mg of NE-OC and 200 mg of NE-OC-Ext, respectively (Figure 1).

Measurement of hydration

Hydration measurements of strands of untreated hair, bleached hair, and bleached and colored hair were performed using the capacitance method, a Corneometer® CM 825 device (Courage and Khazaka, Germany), equipped with a Multi Probe AdapterW MPA 5 (Courage and Khazaka, Germany). Ten measurements were performed on each strand of hair with and without treatments, and the results are presented in "arbitrary units" (U.A.). Measurements were conducted at 25°C and 50% of relative humidity. This method uses the dielectric constant of water, which is relatively high (εr = 81C2. Nm^{-2}) compared to that of other substances in the skin (εr <7C2. Nm^{-2}). The capacitance value changes as function of the water content of the skin/hair and these differences can be measured and converted into a digital value proportional to the moisture content of the skin or hair. Statistical analysis was performed using ANOVA, Tukey's multiple comparison tests, to assess the final hydration of the hair strands (Villa et al. 2013).

Scanning electron microscopy (SEM)

To characterize the effect of the formulations in untreated, bleached, and bleached-colored hair, the samples were analyzed by scanning electron microscopy (SEM) (Hitachi TM 3030 Plus) at an accelerating voltage of 5 kV, and all samples were sputter-coated with gold prior SEM observation. Selected images at different magnifications were considered representative of the entire sample (Kaliyadan et al. 2016).

Statistical analysis

Experimental results are presented as mean ± standard deviation. Statistical analysis was performed using the one-way Analysis of Variance (ANOVA) with Instat3 software. $p > 0.05$ was considered statistically significant.

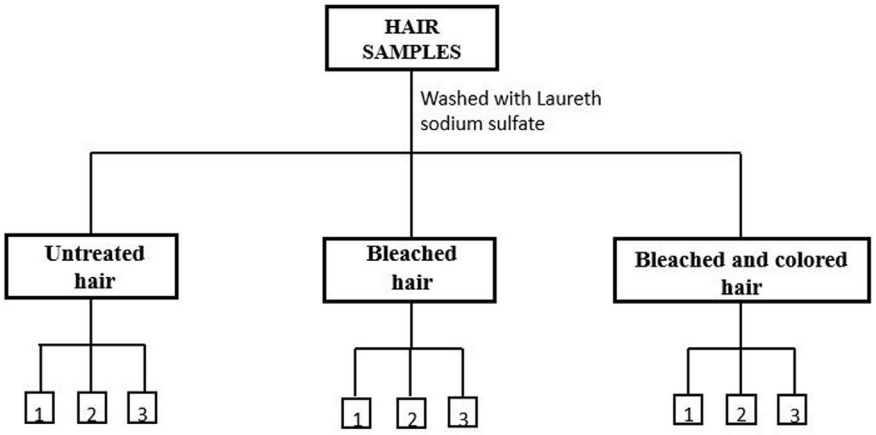

Figure 1. Flow chart of the treatment process (Sample 1: hair was washed with 2% laureth sodium sulphate solution; Sample 2: hair was washed with 2% laureth sodium sulphate solution and treated with 200 mg of NE-OC; Sample 3: hair was washed with 2% laureth sodium sulphate solution and treated with 200 mg of NE-OC-Ext).

RESULTS AND DISCUSSION

Wine lees extract characterization

The annotation process of ESI-MS profile (Figure 2) of wine lee extract was carried out and the major constituents were tentatively identified as a mixture of fatty acids, as palmitic acid ($C_{16}H_{32}O_2$, [M-H] 255.2327), hydroxypalmitic acid ($C_{16}H_{32}O_3$, [M-H] 271.2277), linoleic acid ($C_{18}H_{31}O_2$, [M-H] 279.2337), stearic acid ($C_{18}H_{36}O_2$, [M-H] 283.2646), linolenic acid ($C_{18}H_{29}O_2$, [M-H] 277.2178), oleic acid ($C_{18}H_{33}O_2$, [M-H] 281.2488), and the flavonol, quercetin ($C_{15}H_9O_7$, [M-H] 301.0351). Fatty acids are compounds commonly found in wine lees and are associated with yeast autolysis (Gómez et al. 2004, Pueyo et al. 2000). In addition to quercetin, phenolic acids, and flavonoids such as ellagic acid, *p*-coumaric acid, gallic acid, caffeic acid, chlorogenic acid, kaempferol, and anthocyanins have also been reported as constituents of wine lees extracts (Landeka et al. 2017, Barcia et al. 2014).

However, the composition of wine lees varies according to the origin and the variety of the grapes, the stage of vinification, and the category of operation of the wine lees (Fia 2016). Therefore, it can be assumed that the low qualitative presence of phenolic compounds in the wine lees extract of the Grape varieties Aragonez, Trincadeira, Alicante Bouschet, Touriga Nacional, Syrah, Carignan, and Cabernet is due to these variations and to the extraction used.

Antioxidant activity of the wine lees extract

Table III shows the percentages of antioxidant activity of the wine lees extract using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging method. From the %AAO values, it was possible to obtain an EC_{50} of 119.7 µg/mL. The antioxidant activity of plant extracts is related to their chemical constituents. As already mentioned, among the secondary metabolites present in the wine lees extract, there is quercetin, a flavonoid widely known for its biological activities, including its antioxidant activity.

Quercetin can scavenge free radicals such as hydroxyl, peroxyl, and superoxide anions (Costa 2015). Three structural components contribute to the structure-activity relationship of quercetin: 1. The Presence of the catechol group in the B ring, which promotes the formation of more stable phenoxyl radicals after hydrogen radical donation; 2. The C2-C3 double bond in conjunction with the 4-carbonyl group allows the displacement of an electron from the phenoxyl radical in the B ring to the C rings; and 3. The presence of the 3-hydroxyl group in combination with the double bond between C2-C3 increases the stabilization by resonance of the displaced electrons on the molecule (Costa 2015).

Natural extracts rich in flavonoids, such as quercetin, have been used in the development of hair products. Hair may benefit from the

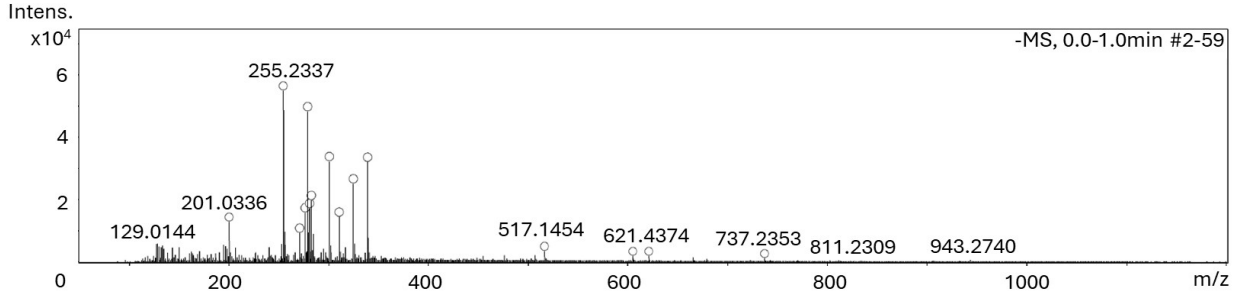

Figure 2. Electrospray ionization mass spectrometry (ESI-MS), spectrum of wine lees extract.

antioxidant activity of flavonoids, as oxidative stress has been associated with hair aging, including the deterioration of the hair fiber, hair loss and the appearance of gray hair. Therefore, the DPPH antioxidant activity test was carried out on the wine lees stratum, to confirm the presence of flavonoids with antioxidant activity, and suggest antioxidant benefits for hair cosmetics developed with this residue (Bassino et al. 2020).

In vitro cytotoxicity study of wine lees extract

The *in vitro* cytotoxicity test was performed to predict the safety of wine lees extract and to evaluate whether the human keratinocytes would be kept alive after treatment with a formulation containing the wine lees extract. Since the aim of this work was to develop a cosmetic formulation with wine lees extract for hair care treatment, the use of human epidermal keratinocytes (HaCat) was appropriate to evaluate the cytotoxicity (Fernandes et al. 2021). Keratinocytes are one of the cell types that is present in the hair cortex (Vellasco et al. 2009).

This test was performed with wine lees extract varying its concentration, ranging from 2 mg/mL to 0.125 mg/mL, to verify whether the cell viability depends on the extract concentration. The control was the cells not treated with wine lees extract, and the parameter of maximum cell viability was 100%. The treatment results of different wine concentrations of lees extract on HaCat cells are shown in Figure 3.

The viability of HaCat cell line was not affected after 48 h of exposure to wine lees extract samples at concentrations ranging from 1 mg/ml to 0.125 mg/mL. However, a statistically significant change ($p < 0.001$) was observed in the HaCat cell viability, where cell viability was reduced after exposure to wine lees extract at the concentration of 2 mg/ml.

Table III. DPPH radical scavenging activity of wine lees extract at different concentrations.

Concentration of wine lee extract µg/mL	% of radical scavenging activity
500	97.46 ± 2.78
250	88.52 ± 0.75
200	79.10 ± 0.53
100	49.50 ± 0.57
25	42.72 ± 0.77
5	13.89 ± 2.61

Values are means of three triplicate determinations (n = 9) ± standard error.

The results were considered satisfactory as over 50% of cell viability was found in the HaCaT line at all concentrations (Fernandes et al. 2021). It can be concluded that the application of wine lees extract on the human epidermal keratinocytes is safe at the concentrations studied, and it is possible to select an extract concentration for formulation.

Cefali et al. (2020) developed a formulation with a flavonoid-enriched extract obtained from grape that did not show cytotoxicity in human keratinocytes. According to the *in vitro* cytotoxicity test, the concentration of 1 mg/mL or 0.1% of wine lees extracts was chosen to be incorporated into the formulation.

Cosmetic products must undergo risk assessment procedures to ensure their safety. Several countries already use alternative methods - methods that replace, reduce, or refine the use of animals - to assess such products, one of which is the assessment of cytotoxic potential (Chiari et al 2012).

As mentioned, the cytotoxicity test with human epidermal keratinocytes (HaCat) was used to assess the safety of wine lees extract, which is an innovative raw material not yet used in cosmetic products and should be evaluated for its risk potential. As the hair consists of the outer layer of the cuticle, which is formed by dead cells, cytotoxicity tests for hair products

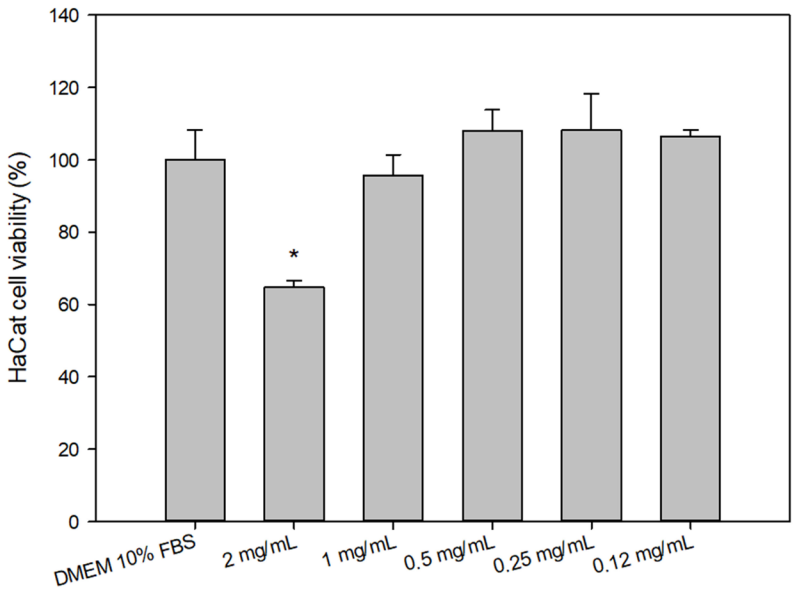

Figure 3. MTT HaCat cell viability studies after 48 h of exposure to wine lees extract, ranging from 0.125 mg/ml to 2 mg/ml. * Statistical difference.

should use keratinocytes, the cells present in the capillary cortex (Vellasco et al. 2009).

Hydrophile-lipophile balance (HLB) Study

The surfactant or mixture of surfactants is a critical step in achieving a homogenous system. The surfactant must have the same HLB value as the dispersed phase to stabilize it in the dispersed phase (Campos et al. 2012, Barradas et al. 2015, Coelho et al. 2018). Table IV shows the results obtained of the HLB study for emulsions. According to the results, it was observed that the HLB of coconut oil at 7.5% was 5.4, and the ratio of surfactants was 10 % of Tween ™ 80 and 90% of Span ™ 80. The appearance of sample 2 was homogeneous, confirming the absence of instability phenomenon, such as creaming, coalescence, and/or phases separation.

In fact, a mixture of surfactants, Span ™ 80 and Tween ™ 80, was required to stabilize the system, and achieve a suitable HLB value.

Table IV. Results of Hydrophile-lipophile balance (HLB) study.

Sample	HLB	Visual Aspect
1	4.3	Phase separation
2	5.4	Homogeneity
3	5.9	Instability phenomenon
4	7.0	Instability phenomenon
5	8.1	Instability phenomenon
6	9.1	Instability phenomenon
7	10.2	Phase separation
8	11.3	Phase separation
9	12.3	Phase separation
10	13.4	Phase separation
11	13.9	Phase separation
12	15	Phase separation

Nanoemulsion Development and characterization

Macroscopic analysis

NE-OC was semisolid, homogeneous, and white, while NE-OC-Ext was semisolid, homogeneous, and brown, the characteristic color of wine lees. Both systems maintained the same color and stability during the time analyzed, without any instability phenomenon (Figure 4).

Mean droplet size assessment

NE-OC presented a mean droplet size of 186.5 ± 12.4 nm and PDI of 0.27 ± 0.04, while NE-OC-Ext presented a mean droplet size of 185.7 ± 16.4

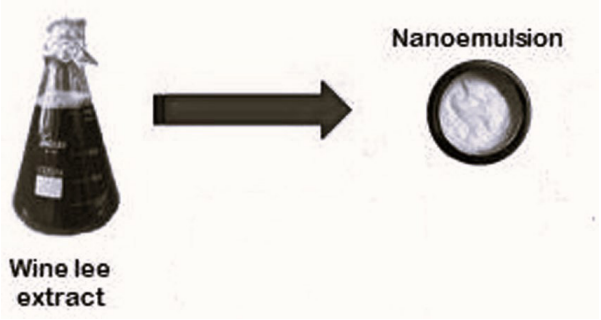

Figure 4. Wine lees extract and nanoemulsion.

nm and PDI of 0.29 ± 0.06 (Figure 5). After the incorporation of 0.1% of wine lees extract into the nanoemulsion, the droplet size remained constant compared to the white nanoemulsion, with no statistical difference ($p > 0.05$). This result showed that the droplet size remained below 200 nm, which is ideal for a cosmetic nanoemulsion, after the incorporation of the wine lees extract in formulations, and the droplets size stability was maintained (Marzuki et al. 2019). The PDI values also remained constant, around 0.2 with no statistical difference ($p > 0.05$), indicating a monodisperse distribution of nanoemulsion droplets (McClements 2012).

Hu et al. (2012) developed silicone oil-in-water nanoemulsions with nonionic surfactants, with a droplet size of 300 nm, and they observed that the nanoemulsions improved the deposition of silicone oil on the hair surface compared to the conventional formulation. Since hair fibers are composed of dead cells, making self-repair impossible, pre- and post-treatment hair care formulations, such as nanoemulsions, help to reduce or prevent damage (Lohani et al. 2014). Nanoemulsions are used in many hair care products because they have many advantages, such as better penetration into hair follicles and hair spacing due to their small droplet size. For faster penetration into hair fibers, it is suggested that the oil nanodroplets should be 100 times smaller than the distance between hair scales (Lohani et al. 2014).

Thermal analysis

As shown in Supplementary Material - Figure S1, the TG curves indicate that the first stage of decomposition occurred between 80 °C and 100 °C with approximately 15% of mass loss for the NE-OC-Ext sample and 10% of mass loss for the NE-OC sample. For the NE-OC-Ext sample, the second event occurred between 180 °C and 400 °C with 60% of mass loss; and for the NE-OC sample, this second event occurred between 237 °C and 400 °C, with approximately 50% of mass loss. This degradation could be attributed to the decomposition of flavonoids and fatty acids (Ferreira et al. 2017). The last stage of decomposition (around 450 °C – 460 °C) results from the degradation of surfactants present in these formulations, with around 20% mass loss for the sample with wine lees (NE-OC-Ext) and 25% mass loss for the sample without wine lees (NE-OC) (Schmitt 2001). Finally, a lower residue was observed for the NE-OC-Ext (around 5%) compared to that of the NE-OC sample (around 15%), indicating a higher interaction of the wine lees extract with the surfactants and the synthetic polymer present.

Figure S2 shows the DTG curves which confirm the number of stages of decomposition mentioned in Figure S1. Figure S3 shows the DTA

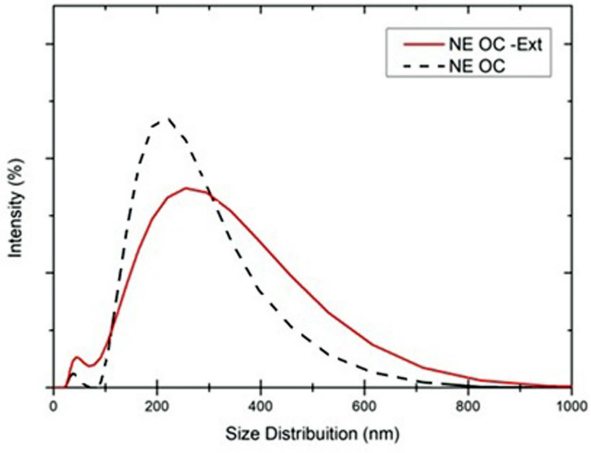

Figure 5. Size distribution of samples with and without wine lees (NE-OC-Ext) and (NE-OC), respectively.

curves, and for all the samples, they presented an endothermic event with a maximum of around 60 °C, which can be attributed to dehydration, while the other events were exothermic, indicating the degradation of components in the formulations. However, the NE-OC-Ext samples showed two exothermic events, while the NE-OC samples showed three exothermic events, conforming the interaction and compatibility with the wine lees extract in the formulation. Thus, thermal analysis can be useful for obtain information on the physicochemical and thermal behavior of the active substance with other compounds present in formulations and to evaluate their potential in product development (Mendonça et al. 2014, Almeida et al. 2014).

Evaluation of cosmetic hydration

Table V shows the hydration values of untreated hair, bleached hair, and bleached-colored hair with and without the application of NE-OC, and NE-OC-Ext. It was observed that there was a statistical difference between the hydration values of untreated hair without treatment, bleached hair untreated, bleached, and colored hair untreated, untreated hair with NE-OC, bleached hair with NE-OC, untreated hair with NE-OC-Ext, bleached hair with NE-OC-Ext, and bleached and colored hair with NE-OC ($p > 0.05$). There was also a statistical difference between the hydration values of untreated hair without treatment, bleached hair untreated, bleached, and colored hair untreated, untreated hair with NE-OC, bleached hair with NE-OC, and untreated hair with NE-OC-Ext ($p > 0.05$).

A statistical difference was observed between the hydration values of untreated hair without treatment, bleached hair untreated, bleached, and colored hair untreated, untreated hair with NE-OC, bleached hair with NE-OC, and bleached hair with NE-OC-Ext ($p > 0.05$). All samples showed a statistical difference in the hydration value compared to the sample bleached and colored hair with NE-OC-Ext ($p > 0.05$).

NE-OC-Ext showed a hydration effect on bleached-colored hair, probably because the bleaching agent penetrates the cuticle, removing all pigment and promoting increased damage to the hair fiber. After this process, the hair loses capillary mass or becomes very fragile and dry (Jeong et al. 2010). Therefore, NE-OC-Ext could promote a higher hydration effect, confirming its conditioning activity, especially on bleached-colored hair.

Vegetable oils have been used in hair cosmetics because they have a lubrication effect on the hair fiber and reduce abrasive damage. In this context, the use of coconut oil in hair products is a good alternative: it can prevent damage to the cuticle cells as the lauric acid chains, one of its constituents, can penetrate

Table V. Hydration effect of nanoemulsion with coconut oil and wine lee extract in the untreated hair, bleached hair and bleached and colored hair. AU - Arbitrary units.

Hair Types	Types of Treatment		
	A	B	C
Untreated Hair	7.3 ± 0.5 AU	7.2 ± 0.58 AU	7.6 ± 0.58 AU
Bleached Hair	7.3 ± 1.2 AU	7.3 ± 0.58 AU	7.7 ± 0.57 AU
Bleached and colored Hair	7.2 ± 0.58 AU	8.3 ± 1.2 AU	9.6 ± 0.57 AU

Startup hair (washed with sodium laureth sulfate) and dried.
Hair with nanoemulsion with coconut oil.
Hair with nanoemulsion with coconut oil and wine lee extract.

the hair fiber, reducing the effect of swelling of the cuticle by water and the fatigue imposed on the capillary fibers (Fregonesi et al. 2009).

Vegetable oils containing of fatty acids have been reported to increase hair gloss and a reduce split ends in bleached hair treated, which it is attributed to the diffusion of these oils into the hair fiber (Fregonesi et al. 2009). The fatty acid components of wine lees extract, and coconut oil may be responsible for the hydrating effect on bleached-colored hair. Furthermore, the hydration values for untreated hair and bleached hair followed the values found by Villa et al. (2013), who observed an increase in hydration for all hair treated with enzymatic hydrolysates.

Scanning electron microscopy (SEM)

The SEM analysis allows the assessment of gradual changes in the hair surface structure. The hair surface is responsible for the diffusion of compounds, such as cosmetic products deposited on its surface, which can promote changes in the cuticle area (Monteiro et al. 2003).

Figure S4 shows the SEM analysis of untreated hair (Figures S4a, b), untreated hair treated with NE-OC (Figures S4c, d), and untreated hair treated with NE-OC-Ext (Figures S4e, f). In general, untreated hair or virgin hair has a sealed cuticles along with its surface (Figures S4a, b). After treatment with NE-OC and NE-OC-Ext, there were no changes in the hair surface.

Figure S5 shows SEM analysis of bleached hair (Figures S5a, b), bleached hair treated with NE-OC (Figures S5c, d), and bleached hair treated with NE-OC-Ext (Figures S5e, f).

Bleaching caused slight cracks and breaks as shown by the red arrows on the hair surface (Figures S5a-f), confirming that the bleaching treatment promoted damage in the hair fiber, but without exposing the cortex. Micrographs S5c-f show the deposition of the nanoemulsion in the cuticle junction. This process was more pronounced in the hair samples treated with the NE-OC-Ext formulation.

Figure S6 shows the SEM analysis of bleached-colored hair (Figures S6a, b), bleached-colored hair treated with NE-OC (Figures S6c, d), and bleached-colored hair treated with NE-OC-Ext (Figures S6e, f). In bleached-colored hair, the cuticle scales are poorly defined due to the damage caused by the oxidative treatment, which results in protein loss from the hair. Some breaks, holes, and complete disappearance of the cortex can be observed in hair fibers (Figures S6a, b). In the micrographs S6c-f, deposits of nanoemulsion can again be seen in at the junction of cuticle. This process was more pronounced in the hair samples treated with the NE-OC-Ext formulation, which increased hair hydration.

Thus, bleaching and bleaching-coloration caused damage to the outermost layer of the hair, resulting in a more fragile hair fiber and less protection of the cortex against damage (Bloch et al. 2019, Kaliyadan et al. 2016). NE-OC-Ext adhered more to the bleached and bleached-colored hair fibers, suggesting that the wine lees extract favors the deposition of the formulation and increases the hydration value in the bleached-colored hair fibers.

CONCLUSIONS

In this work, it was possible to develop a nanocosmetic formulation containing wine lees extract with moisturizing potential in colored hair and, according to the thermal analysis, there was compatibility between the wine lees and the formulation. Wine lees, a residue from the winemaking process, are rich in bioactive compounds, such as fatty acids and quercetin, and can be used as a raw material for the

cosmetics industry. In addition, reusing waste can help protect the environment. In this sense, the present work describes, for the first time, the incorporation of wine lees in a nanoemulsion for hair cosmetic treatment.

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SUPPLEMENTARY MATERIAL

Figures S1-S6.

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