Article

Sugarcane light-colored lignin: A renewable resource for sustainable beauty

Inês F. Mota , Filipa Antunes , Joana F. Fangueiro , Carina A.E. Costa , Alírio E. Rodrigues , Manuela Pintado , Patrícia S. Costa *

Posted Date: 13 October 2023

doi: 10.20944/preprints202310.0867.v1

Keywords: Light-colored lignin; Cosmetics; Sugarcane bagasse

Article

Sugarcane Light-Colored Lignin: A Renewable Resource for Sustainable Beauty

Inês F. Mota [a], Filipa Antunes [a,b], Joana F. Fangueiro [a], Carina A.E. Costa [c,d], Alírio E. Rodrigues [c,d], Manuela E. Pintado [a] and Patrícia Santos Costa [a]*

[a] Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal;
[b] Amyris Bio Products Portugal Unipessoal Lda, Portugal
[c] LSRE-LCM - Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[d] ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
* Corresponding author email: pfcosta@ucp.pt.

Abstract: Lignin has emerged as a promising eco-friendly multifunctional ingredient for cosmetic applications, due to its ability to protect against ultraviolet radiation, antioxidant, and antimicrobial properties. However, its typical dark color and low water solubility limit its application in cosmetics. This study presents a simple process for obtaining light-colored lignin (LCLig) from alkaline black liquor of sugarcane bagasse (SCB), involving an oxidation treatment with hydrogen peroxide, followed by precipitation with sulfuric acid. The physico-chemical characterization, antioxidant and emulsifying potential, safety and stability in an oil-in-water emulsion were performed. A high-purity lignin (81.6%) with improved water solubility was obtained, as result of the balance between the total aromatic phenolic units and the carboxylic acids. In addition, the antioxidant and emulsifying capacities of the LCLig were demonstrated. The color reduction treatment did not compromise the safety of lignin for topical cosmetic applications. The emulsion was stable in terms of organoleptic properties (color, pH, and viscosity) and antioxidant activity, over 3 months at 4, 25 and 40°C.

Keywords: light-colored lignin; cosmetics; sugarcane bagasse

1. Introduction

The demand for natural and sustainable ingredients in personal care products is growing globally due to the increasing concerns about the safety and environmental impact of many synthetic ingredients used in the cosmetic industry. Additionally, evolving consumer behaviour and lifestyles are creating opportunities for natural and eco-friendly ingredients. In this context, lignin has gained special prominence as a bioactive, natural, renewable, biodegradable, and non-toxic cosmetic ingredient [1,2]. Research in this field has experienced significant growth in recent years, with numerous studies demonstrating the tremendous potential of lignin as a natural photo-protector, antioxidant, antimicrobial, and skin-whitening agent [2–5]. Robust scientific evidence supporting the safety of lignin has further fuelled the interest of both academic and industry researchers. Lignin has been shown to be non-irritating to the eyes and skin, as well as a non-mutagenic and non-genotoxic [2–4]. Despite the proven potential of lignin, its inherent dark brown color poses a challenge in color-dependent applications, thus hindering its valorisation [6]. Although lignin is nearly colorless in its natural state, the processing of biomass can generate various chromophores. The nature of these chromophores depends on factors such as the biomass source and extraction conditions. However, due to the structural complexity and variability of lignin, the exact mechanism of chromophores formation is not fully understood [7].

Efforts to remove or reduce chromophores from the lignin structure date back to the 1980's. Previous studies have explored methods such as chemical blocking of free phenolic hydroxyl groups to effectively reduce the color of lignin [8,9], and some have combined this with oxidation with

 doi:10.20944/preprints202310.0867.v1

chlorine dioxide, oxygen or hydrogen peroxide [10,11] or whitening agents (e.g. sodium borohydride) [12]. Alternatively, recent studies have focused on the removal or suppression of chromophoric structures through fractionation with solvents (e.g. methanol/water mixtures) or precipitation at different pH values [6,13–15], oxidation by UV radiation [16], biological color reduction by fungi [17], and modification of its chemical structure, morphology or size (e.g. acetylation, alkylation, ball milling, drying method) [6,18–21]. It has been suggested that mild delignification, lignin re-slurring in acidic water and ball milling can result in lighter lignins [6]. Zhang and co-workers have shown that methanol/water fractionation results in lighter lignins due to the removal of condensed and unsaturated structures [15]. Lignin oxidation with UV radiation was proposed by Wang and co-workers, where lignin was dissolved in tetrahydrofuran and irradiated with UV light for 200 h to protoxidize the phenoxyl groups into colorless aliphatic acid [16]. While substantial efforts have been invested, there remains a need to identify simpler, cost-effective, and environmentally friendly processes for reducing the color of lignin. Hydrogen peroxide, a well-known oxidant, is often used in lignin depolymerization experiments or studies involving pulp bleaching, either alone or in combination with other oxidants [22–24]. Hydrogen peroxide is a non-toxic chemical that readily decomposes into molecular oxygen and water. In an acidic medium, hydrogen peroxide acts as a strong oxidant. However, in an alkaline medium, it reacts mainly as a nucleophile with carbonyl and conjugated carbonyl structures (e.g. quinoid structures), effectively removing chromophore structures without degrading the lignin structural network [25]. Recent studies have achieved success in obtaining transparent wood through mild alkaline treatments combined with hydrogen peroxide concentrations up to 25 wt% [26–28]. Li and co-workers have demonstrated that over 80% of the bulk lignin can be preserved in transparent wood material obtained through a combined alkaline/hydrogen peroxide treatment of up to 3 wt% [29]. Building upon these findings, this study proposes, for the first time, the use of mild hydrogen peroxide treatment in alkaline liquor to reduce the color of lignin extracted from SCB. The resulting light-colored lignin (referred to as LCLig) was characterized in terms of composition, color, particle size, molecular weight, and functional groups. Its antioxidant and emulsifying properties were also investigated. Furthermore, the safety and stability of LCLig were evaluated in an oil-in-water (o/w) emulsion formulation.

2. Materials and methods

2.1. Materials

Hydrogen peroxide (H_2O_2, 35%) was purchased from Labchem. Sulfuric acid (H_2SO_4, 95-98%), acetone (>99.8%), dimethylformamide (DMF, 99.8%), anhydrous pyridine (99.8%), deuterated chloroform ($CDCl_3$, 99.8%), cholesterol, chromium (III) acetyl-acetonate, (phosphitylating reagent (2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane), dimethyl sulfoxide (DMSO, >99.9%), Folin & Ciocalteu's phenol reagent (2 M), 2,2'-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS, ≥98%), potassium persulfate (≥99.0%), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%), butylated hydroxytoluene (BHT, ≥99%), [2,2'-azobis(2-amidi-nopropane) dihydrochloride] (AAPH), acetonitrile (≥99.9%); L-lysine, cinnamic aldehyde, cysteine, trifluoroacetic acid and gallic acid, sodium carbonate were purchased from Sigma-Aldrich. Ethanol (>99.8%) was obtained from Honeywell. Methanol was purchased from VWR Chemicals (>99.8%). Dulbecco's Modified Eagle Medium (DMEM, GlutaMAX™), TrypLEX express enzyme, phosphate buffer, fetal bovine serum, penicillin-streptomycin (pen-strep) and dimethyl sulfoxide (DMSO) were obtained from Gibco. Human keratinocyte cell line (HaCAT) and PrestoBlue cell viability reagent Fluorescein [3',6'-dihydroxyspiro (isobenzofuran-1 [3H], 9' [9H] -xanten)-3-one] were obtained from Thermo Fisher Scientific. *Salmonella typhimurium* containing a deletion mutation for histidine (His), with the following mutations: the type frameshift (TA98), base-pair substitution (TA100) and reversion/transversion (TA102), 2-Aminoantracene, ampicillin were purchased from Moltox. Nutrient broth-2, and phosphate buffer were purchased from Oxoid. Liver homogenate, S9 (Aroclor 1254-induced Sprague Dawley rat liver) was obtained from Xenometrix. Glycerin, lanette

 doi:10.20944/preprints202310.0867.v1

and refined shea butter were supplied by Acofarma. Solagum AX was acquired from Seppic. Tegocare PBS 6MB was supplied from Evonik and squalene was from Amyris. Euxyl PE 9010 from LotionCrafter. Lanette® O (cetyl stearyl alcohol) from BASF.

2.2. Lignin extraction and color reduction process

The SCB was air-dried overnight in a convection oven at 40°C. Then, the process of SCB delignification was performed with 2 wt% sodium hydroxide solution (Labchem) at 90°C for 0.5 h with a liquid-solid ratio of 15 using a high-pressure reactor (Parr instruments company, model 4551). After lignin extraction, the black liquor was separated from the solid and treated with hydrogen peroxide 35% prior to lignin precipitation. In a preliminary study, various loads of oxidant and sulfuric acid were tested (data not shown), and based on the results, 9% (v/v) of H_2O_2 and 2.5% (v/v) of H_2SO_4 were identified as the optimal process conditions. Therefore, the color reduction treatment was performed at 85°C (water bath, Julabo SW22) for 1.5 h and 5% $v_{H2O2}/v_{Black\ Liquor}$ oxidant load. Afterwards, lignin was precipitated with 2.5% (v/w) sulfuric acid, and thoroughly washed with tap water by means of vacuum filtration using a paper filter Whatman 113. The LCLig cake was spray dried (Büchi Mini Spray Dryer model B-290) using a water suspension at 3 wt% and the following operating conditions: 65% aspirator rate (equivalent to about 27.5 m^3/h), flow height 40-45 mm (equivalent to 667-831 L/h), pump speed 12% (equivalent to 4 mL/min) and inlet temperature 160°C. These operating conditions resulted in outlet air temperature approximately 85°C.

2.3. Moisture, ashes, total lignin, and carbohydrates content

The moisture and inorganic content of the lignin samples were determined gravimetrically at 105°C (Venti-Line Model, VWR) and 550°C (Nabertherm), respectively, until constant weight. The crucibles were previously calcined overnight at 550°C. Total carbohydrates, soluble and insoluble lignin were determined after acid hydrolysis as described by Ajao and co-workers [6] by high performance liquid chromatograph equipped with refractive index detector (HPLC-RI) and an Aminex HPX 87H column 300 × 7.8 mm (Bio-rad laboratories). The chromatograms were run in isocratic mode at 0.6 mL/min and 50°C. The mobile phase employed was 5 mM sulfuric acid solution and the injection volume was 10 µL. All samples were analyzed at least in duplicate.

2.4. Color

The color measurements were performed according to the *Commission Internationale de l'Eclairage* CIELAB color scale over a black background on a portable reflection spectrophotometer (CR-410 Chroma Meter, Konica Minolta) with a specular component excluded geometry. For blank calibration, the equipment was calibrated with a CR A44 Calibration Plate. The values of L* (lightness and darkness), a* (red and green) and b* (yellow and blue) were obtained.

2.5. Structural characterization

2.5.1. Attenuated Total Reflectance – Fourier-transform Infrared spectroscopy (ATR-FTIR)

The lignin samples were analyzed by the ATR-FTIR (Perkin Elmer Frontier) by direct transmittance in a single-reflection ATR System. The equipment was configured for 16 scans in a range of 4000-550 cm^{-1} and a resolution of 4 cm^{-1}. For all spectral manipulation, the Perkin Elmer FTIR Software (Perkin Elmer, USA) was used.

2.5.2. ^{31}P Nuclear Magnetic Ressonance (NMR)

The quantitative ^{31}P NMR analysis was performed as described by Costa and co-workers [30]. Briefly, approximately 40 mg of each lignin was weighted and dissolved in 0.4 mL of anhydrous pyridine and deuterated chloroform (1.6:1.0, v/v). The mixture was left at room temperature overnight with continuous stirring. Cholesterol (200 µL at 19 mg/mL, internal standard) and 50 µL of chromium (III) acetyl-acetonate (11.4 mg/mL, relaxation agent) were added and allowed to react for

 doi:10.20944/preprints202310.0867.v1

2 h. Phosphitylating reagent (2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, 100 µL) was added and the mixture was transferred to a 5-mm-OD NMR tube. The phosphitylated samples were analyzed by ^{31}P NMR spectroscopy using a Bruker AVANCE III 400 spectrometer operating at 400 MHz, at 298 K. The spectrum was acquired during 30 min with 10 s relaxation time, 45° pulse angle, and 4 s pulse delay.

2.6. Differential scanning calorimetry (DSC)

About 5 mg of sample was hermetically sealed in aluminum pans with pierced lids and analyzed under nitrogen atmosphere at a heating rate of 10°C/min from 30 to 210°C (Netzsch-Gerätebau GmbH DSC 204 F1 Phoenix®). Purging with nitrogen gas at a flow rate of 40 mL/min was performed to maintain an inert atmosphere.

2.7. Particle size

The size of lignin particles was measured in a MasterSizer Hydro 3000 equipped with a Hydro EV dispersion unit (Malvern Instruments) using an obscuration range of 5-10%. The sample was dispersed in water and ultrasonicated externally for 5 min. Prior to analysis, the sample was submitted to 1 min of ultrasounds and stirred at 2500 rpm. For the particle size distribution, it was considered spherical particles with a refractive index of 1.64 and absorption index of 0.01.

2.8. Gel Permeation Chromatography (GPC)

The number-average (Mn), weight-average (Mw) and polydispersity (PD) of the LCLlig was obtained as described by Mota and co-workers [31] with slight modifications. An Agilent 1260 Infinity II system equipped with UV detector and quaternary pump was used. Agilent gel Oligopore column 300 × 7.5 mm with a nominal particle size of 6 µm was sued in series with an Agilent gel Mesopore column 300 × 7.5 mm with nominal particle size of 3 µm, measuring molecular weights up to 4500 g/mol and 25000 g/mol, respectively. The columns were preceded by an Oligopore 50 × 7.5 mm guard column. The volume of injection was 20 µL and detection occurred at 268 nm. GPC analysis was performed in isocratic mode employing dimethylformamide, flow rate of 0.7 mL/min at 70°C. Sample was dissolved in the mobile phase solvent to obtain a concentration of 5 mg/mL, stirred until complete dissolution, and filtered through a 0.45 µm syringe filter before injection.

2.9. Solubility

The solubility of LCLig was determined in different solvents: deionized water, acetone, ethanol, methanol and DMSO. Sample (10 mg) was suspended in 1 mL of solvent, and the mixture was homogenized and ultrasonicated for 15 min. The soluble and insoluble fractions were separated by centrifugation at 15000 rpm (Megafuge 16R, Thermo Fisher Scientific); the insoluble fractions were oven dried overnight at 105°C (Venti-Line Model, VWR) and weighted for solubility determination. The experiments were carried out in triplicate.

2.10. Biological activity

2.10.1. Total phenolic content (TPC)

The TPC was assessed by the Folin-Ciocalteu method as described by Vilas-Boas and co-workers [32]. In brief, 20 µL of sample or gallic acid or solvent (Blank) mixed with 80 µL of Folin-Ciocalteu reagent (10 % v/v) and 100 µL of sodium carbonate (7.5 wt%) in a 96-well microplate. The reaction occurred for 1 h at room temperature. The absorbance was recorded at 750 nm using a microplate spectrophotometer (Epoch, Agilent). This assay was performed in triplicate for each sample and results are expressed as milligram gallic acid equivalents (GAE) per gram of sample.

2.10.2. Antioxidant activity

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 October 2023 doi:10.20944/preprints202310.0867.v1

The antioxidant activity was assessed by the oxygen radical absorbance capacity (ORAC) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation assays. The lignin samples were prepared as described by Antunes and co-workers [33] by dissolving 10 mg of each sample in 1 mL of methanol, sonicated for 15 min. The mixtures were centrifuged at 5000 rpm for 5 min. The lignin soluble fractions were quantified spectrophotometrically at 280 nm using a UV-visible spectrophotometer (UV-1900, Shimadzu).

The ORAC assay was performed as described in the literature with some modifications [34]. The reaction was carried out at 37°C in 200 mL of phosphate buffer (75 mM and pH 7.4) containing 20 µL of blank, sample or Trolox (1–8 µM, final concentration in the well) and 120 µL of fluorescein (final concentration in the well of 70 nM). The mixture was preincubated for 10 min before adding 2,2'-azobis(2-amidi-nopropane) dihydrochloride (AAPH, 60 µL; 12 mM) and fluorescence immediately record at intervals of 1 min during 140 min at an excitation wavelength of 485 nm with emission detected at 530 nm using a multidetector plate reader (Synergy H1, Biotek Instruments) controlled by the Gen5 Biotek software version 3.04. The microplate was automatically shaken before each reading. This assay was performed with a black polystyrene 96-well microplate (Nunc, Thermo Fisher Scientific). This assay was performed in triplicate for each sample and results are expressed as micromole Trolox equivalents (TE) per gram of sample.

The ABTS assay was performed as described in the literature with some modifications [35]. The ABTS radical concentration of was adjusted using methanol to an initial absorbance of 0.700 (± 0.020) at 734 nm. To 200 µL of this ABTS solution were added 15 µL of sample or butylated hydroxytoluene (BHT, reference antioxidant) or solvent (blank). The mixture was incubated for 5 min at 30°C, and the absorbance at 734 nm was measured in a microplate spectrophotometer). The radical stock solution was freshly prepared. The analyses were performed in triplicate. Results were expressed as half-maximal inhibitory concentration (IC50), which corresponds to the concentration necessary to inhibit 50% of ABTS radical scavenging.

2.11. Emulsion stability index (ESI)

To evaluate the ESI, the volumetric method proposed by Choi and co-workers was adopted [36]. The freshly prepared emulsion was poured into a glass volumetric cylinder and allowed to stand at 25°C. This formulation was composed of 25 wt% cetyl stearyl alcohol (Lanette® O), 1-10 wt% of lignin and 65-75 wt%. of water. The emulsion stability was recorded visually by the formation of the clarified serum layer at the bottom of the emulsion after 24 h. Then, the ESI was calculated as follows (1):

$$ESI = \left(1 - \frac{Vw}{Ve}\right) \times 100 \quad (1)$$

where Ve is the volume of the o/w emulsion and Vw is the volume of the separated bottom layer after the desired storage period.

2.12. Safety assessment

2.12.1. Cytotoxicity

The cytotoxicity of LCLig was assessed in a human keratinocyte cell line - HaCAT - with PrestoBlue Fluorescence assay performed according to the manufacturer's protocol (Thermo Fisher Scientific 2010). Briefly, after reaching 80-90% confluence, HaCAT cells were detached with TrypLEX express enzyme (1x) (Gibco) and seeded to a final density of $1x10^5$ cells/mL in Dulbecco's Modified Eagle Medium (DMEM GlutaMAX™, Gibco), supplemented with 10% (v/v) fetal bovine serum (Gibco) and 1% of penicillin-streptomycin (Pen-Strep 100 IU/mL and 10 mg/mL, respectively) (Gibco) in 96 wells plates. After 24 h at 37°C, in a humidified atmosphere of 5% CO_2, cells were exposed to six serial dilutions of soluble light-colored lignin from 62.5 to 2000 µg/mL. LCLig stock solutions were prepared in DMEM prior to cells exposure. Positive control of cells death was DMSO (Gibco) 10%. After 24 h of exposure, the cells were incubated with PrestoBlue reagent (Thermo Fisher Scientific).

 doi:10.20944/preprints202310.0867.v1

Cell viability was evaluated by fluorescence spectroscopy after 2 h of incubation at 37°C, using a Synergy HT Multi-detection microplate reader operated by GEN5TM software, with excitation and emission wavelengths of 560 and 590 nm, respectively. Relative cell viability was expressed as percentage relative to the untreated control cells (1% DMSO), considering 100% viability in the solvent control.

2.12.2. Mutagenicity

Mutagenic potential was assessed by the Miniaturized Ames Test in a liquid 384-well microplate format (Moltox®). Three standard strains of *Salmonella typhimurium* containing a deletion mutation for histidine (His) were used, with the following mutations: the type of frameshift (TA98), base-pair substitution (TA100) and reversion/transversion (TA102). The strains were inoculated in nutrient broth-2 with 25 μg/mL of ampicillin. The incubation was performed for 10-12 h at 37°C in an incubator shaker (Innova 44, New Brunswick) at 120 rpm until a density of 1 – 2x10^9 bacteria/mL or approximately 1.0 - 1.4, OD 650 nm. The mutagenic potential of the samples was evaluated directly, and in the presence of metabolic activation with a liver homogenate (S9). For that, five LCLig concentrations were prepared in DMSO and added to the exposure medium (inoculum at 10% v/v). For the negative control DMSO was used, and for the positive controls without S9 activation, 2-nitrofluorene (100 μg/mL), 4-nitroquinoline-N-oxid and mitomycin-C (50 μg/mL each) in DMSO were used for the strains TA98, 100 and 102, respectively. In the presence of S9, the 2-aminoantracene (100 μg/mL) dissolved in DMSO was used for all strains. The reversion indicator media was added after 90 min of incubation, and 50 μL of the homogenous mixture was transferred to a 384-well plate, sealed into a plastic bag, and incubated at 37°C for approximately 24 h (TA102) and 48 h, which afterwards the growth of *Salmonella* his+ revertants was counted.

2.12.3. Skin sensitization

The skin sensitization potential was assessed through Direct Reactivity Peptide Assay (DPRA), following OECD Test guideline No. 442C (2020). The sample LCLig was dissolved in Acetonitrile:DMSO (2.3:0.7, v:v) in a concentration of 8 g/L and diluted up to 1.4, 0.70 and 0.35 g/L. The cysteine model peptide was prepared as 0.667 mM stock solution in 100 mM phosphate buffer (pH 7.5) and lysine model peptide was prepared as 100 mM stock solution in ammonium acetate buffer (pH 10.2). The diluted samples and controls (peptide) were incubated for 24 h, at room temperature, with cysteine or lysine peptides, at ratio of 1:10 or 1:50 (v/v), respectively. The cinnamic aldehyde 100 mM in acetonitrile was used as positive control. The analysis of the free peptides was performed using a high-performance liquid chromatography reverse-phase (HPLC, Agilent 1260 Infinity II, Agilent Technologies, USA) equipped with a diode array detector (Agilent 1260 DAD HS) and a Poroshell 120 EC C18 column (30 x 150 mm; 2.7 μm). The analysis was performed in gradient mode with mobile phase A (0.1% trifluoroacetic acid in water) and phase B (0.085% trifluoroacetic acid in acetonitrile) starting at time 0 with 10% B, going to 25% B in 15 min, 90% B in 3 min, 10% B in 1 min and kept 6 min at 10% B. Detection was carried out at 220 nm, with a flow rate of 0.35 mL/min, column temperature of 30°C and with sample injection volume of 5 μL. The peptides were quantified using calibration curves for cysteine and lysine in the range 0.033 to 0.528 mM. Peptide depletion was calculated for each concentration by comparison to the control sample. The overall peptide depletion was determined considering the average % of cysteine and lysine depletion.

2.13. Accelerated stability

The accelerated stability evaluation was performed according to the International Conference of Harmonization (ICH) guidelines ISO/TR 18811:2018, for 3 months at different temperature/related humidity: 4°C/60%; 25°C/60%; and 40°C/75% (Climacell Eco line). The organoleptic (appearance, color and odor) and physico-chemical (color, pH and viscosity) parameters, ABTS radical scavenging capacity, and microbial contamination (ISO 11930:2019 / ISO 17516) were monitored.

2.13.1. Oil-in-water (o/w) emulsion preparation

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 October 2023 doi:10.20944/preprints202310.0867.v1

The oil-in-water (o/w) emulsion composition is detailed in Table 1. It was prepared by incorporating the oil phase into the water phase under high shear mixing using an Ultra Turrax homogenizer (Ika® T25). In each phase all ingredients were heated up to 75°C and blended vigorously. Aqueous phase (Part A) and oily phase (Part B) were combined. After cooling down, Euxyl PE 9010 (Part C) was added to the emulsion. The pH was corrected to 4.5 - 5.5 with sodium hydroxide 50 wt% solution or lactic acid.

Table 1. Composition of light-colored lignin (LCLig) and blank emulsions.

Commercial name	INCI	Function	Blank (%)	LCLig (%)
Part A (aqueous phase)				
Deionized water	Aqua	Solvent	79.6	74.6
Glycerin	Glycerin	Humectant	5	5
Solagum™ AX	Acacia Senegal Gum; Xanthan Gum	Thickening and stabilizing agent	0.9	0.9
LCLig	-	Active ingredient	-	5
Part B (oily phase)				
Lanette	Cetyl stearyl alcohol	Emulsifier	2.5	2.5
Tego® Care PBS 6 MB	Polyglyceryl-6 Distearate; Polyglyceryl-6 Behenate	Emulsifier	4	4
Shea butter	Butyrospermum parkii butter	Emollient	2	2
Squalane	Squalane	Emollient	5	5
Part C				
Euxyl PE 9010	Phenoxyethanol and Ethylhexylglycerin	Preservative	1	1

2.13.2. Organoleptic evaluation

The appearance of the emulsions was monitored by visual evaluation of phenomena, such as phase separation without reversion, creaming or flocculation events. The color was evaluated according to the procedure mentioned above (section 2.4).

2.13.3. Physico-chemical evaluation

The pH was determined at 25°C using a pH meter (Mettler Toledo) immersing the glass electrode into the emulsions until stabilization. The viscosity was performed using a Viscometer B-One Plus (Lamy Rheology Instruments) with a spindle number R5 at 100 rpm during 60 sec. All the measurements were performed at 25°C with previous equipment calibration.

2.13.4. Antioxidant activity

The antioxidant activity was assessed following the abovementioned (section 2.10.2) methodology with different sample preparation. About 20 mg of o/w emulsion was dissolved in 1 mL of DMSO and centrifuged at 5000 rpm for 30 sec after ultrasonication for 15 min.

2.13.5. Microbial contamination

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 October 2023 doi:10.20944/preprints202310.0867.v1

The microbial contamination was performed according to the European Standard ISO 17516:2014 ("International Organization for Standardization (ISO) 17516:2014 — Microbiological Limits for Cosmetics" 2014) regarding total counts of yeast and mold, and total viable aerobic count (CFU/g).

3. Results and discussion

3.1. Physico-chemical, structural, and thermal analyses

From Table 2, it can be concluded that the employed process conditions resulted in a substantial reduction in the color of LCLig. The purity, carbohydrates and ash contents of LCLig were 81.6 ± 3.6%, 3.50 ± 0.40% and 6.03 ± 0.01%, respectively (Table 2). The color of LCLig was evaluated quantitatively using the International Commission on Illumination CIE L*a*b* color space. For the LCLig the lightness value was 73, indicating a light color. The color coordinates a* and b* values were 2.5 and 24.5, respectively (Table 2). Comparing these results with the those reported by Antunes et al. (2023) [33] for a lignin obtained from sugarcane bagasse (L*60/a*6/b*19), it can be concluded that the proposed simple process successfully reduced the color of lignin extracted from SCB.

Table 2. Composition of light-colored (LCLig) lignin.

Sample	Total lignin (wt%)	Carbohydrates (wt%)	Ash (wt%)	Color CIELAB L*/ a* / b*	Appearance
LCLig	81.60 ± 3.60	3.50 ± 0.40	6.03 ± 0.01	73 / 2.5 / 24.5	

The ^{31}P NMR analysis allowed quantifying syringyl (S), guaiacyl (G) and *p*-hydroxyphenyl (H) free phenolic groups, as well as carboxylic, aliphatic, and condensed structures carrying out free phenolic groups [30] (Table 3). The content of total phenolic units of LCLig was 0.89 mmol/g_{lignin}. Free phenolic hydroxyl groups are one of the most important structural features regarding the reactivity and antioxidant properties of lignin [30,38]. Therefore, the content of phenolic hydroxyl groups, is usually associated with the dark brown color of lignin. So, reducing its color can cause a decrease of the antioxidant activity. The TPC variations might be attributed to condensation, re-polymerization, or char formation processes that may occur during the color reduction treatment [39]. The carboxylic acids content was 1.76 mmol/g_{lignin}, corroborating the FTIR analysis (Figure 1). According to the ATR-FTIR spectra (Figure 1), the lignin structure was not significantly affected by the removal of some chromophores compared to our previous results [33]. This result was expected since, in alkaline medium, hydroxide peroxide reacts with both aliphatic and aromatic structures of lignin, thus removing some chromophore structures without compromising the lignin structure network [25]. Nevertheless, the FTIR spectra revealed a pronounced band between 3650 and 3050 cm^{-1} attributed to hydroxyl groups in phenolic and aliphatic structures [40], probably due to the lignin depolymerization, thus triggering condensation reactions [22,41]. Some signatures were also observed in the carbonyl/carboxyl region, 1700-1680 cm^{-1}, corresponding to unconjugated C=O stretching (vibrations of unconjugated ketones, esters or carboxylic acids) [42]. The region between 3000 cm^{-1} and 2830 cm^{-1} corresponds to C-H stretch in methyl and methylene groups, and methoxyl groups, and the bands at 2918 cm^{-1} and 2853 cm^{-1} were detected [40]. Aromatic skeletal vibrations were assigned to bands 1593 cm^{-1} (characteristic of condensed G-units), 1509 cm^{-1}, 1459 cm^{-1} and 1421 cm^{-1} [40]. The band at 1593 cm^{-1} corresponded to the aromatic skeletal vibration characteristic of condensed guaiacyl (G) units, 1327 cm^{-1} related to aryl ring breathing with C-O stretch characteristic for syringyl (S) ring plus G ring condensed and 1228 cm^{-1} attributed to G units (condensed > etherified G units, not relevant band). The band at 830 cm^{-1} was assigned to C-H out of plane in positions 2, 5

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 October 2023 doi:10.20944/preprints202310.0867.v1

and 6 of S units and all positions of *p*-hydroxyphenyl (H) units [43,44]. The presence of *p*-coumaric esters (typical for GSH lignins) is shown in both samples at 1121 cm^{-1} and 1029 cm^{-1}, indicating the C-O stretching in C-O-C linkages between S and G units [43–45]. There was a remarkable band at 611 cm^{-1} (Figure 1) attributed to the C-H bending vibration of aromatic ring substituents, specifically, the C-H out-of-plane bending of meta-substituted aromatic rings in lignin [46].

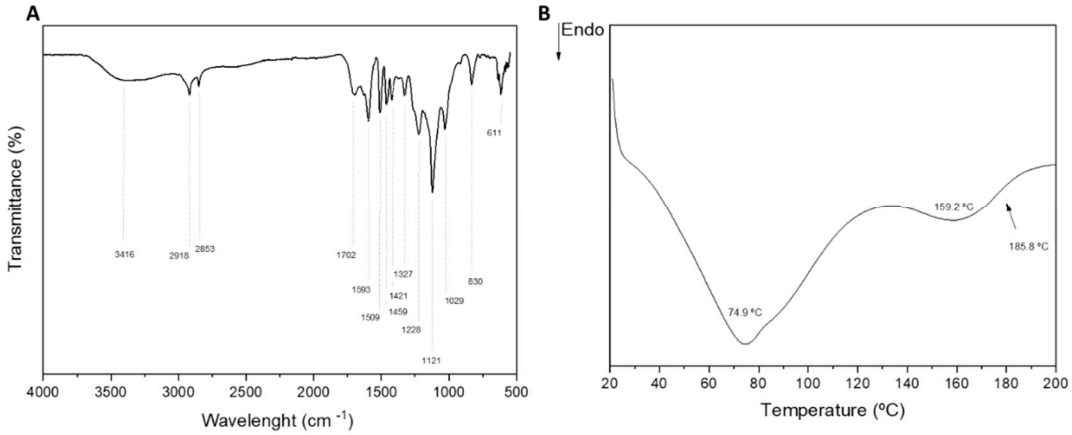

Figure 1. A) FTIR spectra and B) DSC thermograms of light-colored lignin (LCLig).

Table 3. Assignments and quantification of and aliphatic, carboxylic OH groups and phenolic OH groups identified by ^{31}P NMR in light-colored lignin (LCLig).

	Aliphatic OH (mmol/g_{lignin})	**Carboxylic acids (mmol/g_{lignin})**	**Phenolic units (mmol/g_{lignin})**				
			Condensed	**Non condensed**			**Total**
				S	**G**	**H**	
	δ 146.4 - 150.8 mg/L	δ 135.6 - 133.6 mg/L	δ 145.8-143.8 and 142.2-140.2 mg/L	δ 143.8 -142.2 mg/L	δ 140.2 -137.4 mg/L	δ 137.4 - 136.9 mg/L	
LCLig	3.73	1.76	0.13	0.10	0.63	0.04	0.89

From Figure 1, it can be observed that LCLig is stable up to 200°C, which is a relevant property for cosmetic and other industrial applications. Previous studies have already reported the thermostability of lignins up to temperatures of 200°C, with dehydration and decarboxylation processes being the most common events observed in this temperature range [47–49]. Nevertheless, the thermostability of lignin is affected by its degree of polymerization, degree of branching, and the presence of functional groups such as hydroxyl, methoxyl, and carboxyl groups [47]. According to the results (Figure 1 and Table 4), the profile of LCLig presented two main endothermic events. The first event, at 74.9°C, was caused by the water removal from the lignin (dehydration), as confirmed in the literature [47]. LCLig depicted a -65.76 J/g and a peak height of -0.6585 mW/mg (Table 4). The second event, at 159.2°C, described a different thermal profile from 140°C. These peaks are related to decarboxylation reactions involved in the removal of carboxyl groups from the lignin structure. Based on the NMR analysis (Table 3), LCLig is rich in carboxyl and aliphatic hydroxyl groups and, therefore, this is an expected peak (-8.367 mW/mg) (Table 4).

Table 4. DSC parameters analysed for light-colored lignin (LCLig).

Sample	**Peak max (°C)**	**Enthalpy (J/g)**	**Peak Height (mW/mg)**
LCLig	74.9 159.2	-65.76 -8.367	-0.6585 -8.367

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 October 2023 doi:10.20944/preprints202310.0867.v1

3.2. Particle size and molecular weight

The particle size distribution, weight-average (Mw) and number-average (Mn) molecular weights, and polydispersity index (Mw/Mn) are shown in Table 5. The particle size distribution was 9.54 ± 0.02 mm. This contrasts with some previous studies that reported a reduction in the molecular weight of lignin after bleaching due to its oxidative cleavage [22,50,51]. A possible explanation for these differences is that the mild conditions employed in the present study, such as low extraction temperatures and concentration of H_2O_2, may have prevented extensive fractionation or degradation of lignin, resulting in minimal changes in its molecular weight. This suggests that the use of mild treatment conditions can be beneficial in maintaining the structural integrity and properties of lignin, while still achieving the desired modifications [53].

Table 5. Particle size distribution, molecular-average (Mw) and number-average (Mn) weights and polydispersity index of the light-colored lignin (LCLig).

Sample	Particle size (mm)			Mw (g/mol)	Mn (g/mol)	PD
	Dv(10)	Dv(50)	Dv(90)			
LCLig	2.83 ± 0.01	8.07 ± 0.02	27.24 ± 0.32	14300 ± 2059	10598 ± 1577	1.35

Dv 50, Dv 10 and Dv 90 are standard percentile readings expressed in volume distribution where, Dv 50 is the size in microns at which 50% of the sample is smaller and 50% is larger. This value is also known as the Mass Median Diameter (MMD) or the median of the volume distribution. Dv 10 is the size of particle below which 10% of the sample lies and Dv 90 is the size of particle below which 90% of the sample lies.

3.3. Solubility

The data from Figure 2. indicate that the color reduction process induced a suitable water solubility in LCLig and comparable lower solubility in the remaining solvents tested. The treatment may break down certain chemical structures within the lignin that hinder its solubility in water. These variations are influenced by its ability to establish intermolecular interactions with aliphatic, carbonyl and hydroxyl groups present in the lignin structure [52]. Notably, the NMR analysis confirmed that LCLig has a relatively suitable concentration of phenolic hydroxyl groups that can contribute for this water solubility (Table 3) [53].

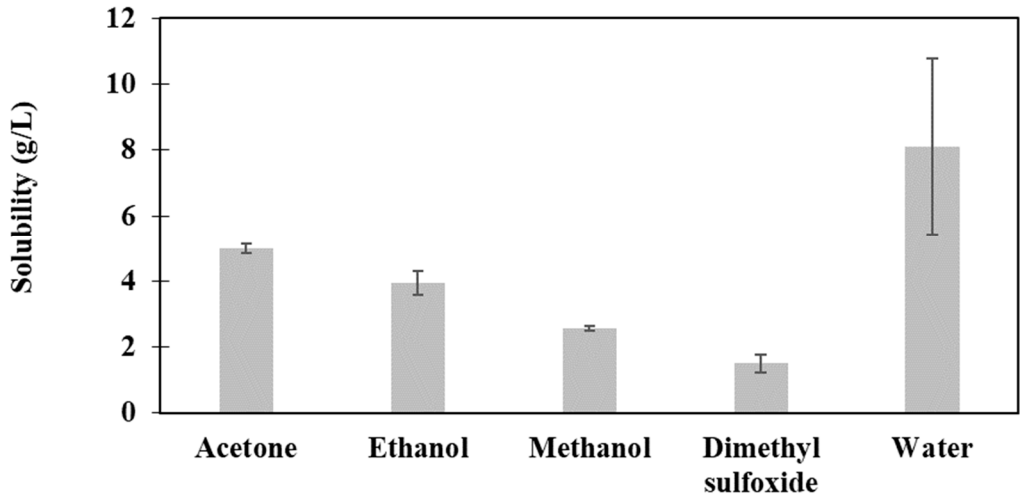

Figure 2. Solubility of the light-colored lignin (LCLig) in different solvents.

3.4. Total phenolic compounds (TPC) and antioxidant potential

 doi:10.20944/preprints202310.0867.v1

The TPC and antioxidant properties of LCLig are summarized in Table 6. The results revealed antioxidant activity as result of the TPC. When comparing these values to those reported in the literature for an alkaline lignin from sugarcane, a slight decrease in the TPC and antioxidant activity was observed [54]. This decrease appears to be related, at least in part, to the aliphatic hydroxyl groups as observed in the NMR results (section 3.1), which are known to have a negative contribution to the radical scavenging activity [4,55]. These findings are consistent with previous research on the modification of SCB lignins through oxidation, acetylation, or epoxidation [42,56].

Table 6. Total phenolic content (TPC), oxygen radical absorbance capacity (ORAC) and 2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS) of the light-colored lignin (LCLig).

Lignin	TPC (mg GAE/g)	ORAC (μmol TE/g)	ABTS * IC50 (mg/mL)
LCLig	169.3 ± 40.9	2571.5 ± 826	1.28 ± 0.13

GAE – Gallic acid equivalents; TE – Trolox equivalents; IC50 - half maximal inhibitory concentration. * IC50 for butylated hydroxytoluene (BHT) was 0.40 ± 0.02 mg/mL.

3.5. Emulsion stability index (ESI)

The emulsifying potential of lignin in an o/w emulsion was investigated, and the results are depicted in Table 7. According to the results (Table 7), a LCLig concentration-dependent effect was observed, with the ESI increasing from approximately 58 to 90% as the concentration of LCLig in the emulsion increased from 1.0 to 7.5 wt%. LCLig demonstrated effective stabilization of emulsions containing a mixture of fatty alcohols at a concentration of 2.5 g per g of oil phase. While the precise mechanism of lignin self-assembly and colloidal behavior in emulsions remains unclear [57], studies have suggested that lignin stabilizes emulsions through adsorption at the oil/water interface, preventing droplet coalescence by electrostatic and steric repulsion [58]. As per the findings of this study, a relatively high concentration of LCLig (ranging from 1 to 10 wt%) is required to effectively reduce the surface tension and solvate the oil droplets, as compared with typical emulsifiers. Specifically, for a mixture of fatty alcohols such as cetyl stearyl alcohol - commonly used in cosmetic products - a concentration of 10 wt% of LCLig was found to be effective in reducing the surface tension and stabilizing emulsions (Table 7). Indeed, the high ESI of LCLig even at lower concentrations (1.0%), suggests its potential as a co-emulsifier candidate in cosmetic formulations, potentially reducing the overall concentration of synthetic emulsifiers in cosmetic products.

Table 7. Emulsion stability index (ESI) for different amounts of light-colored lignin (LCLig).

LCLig (wt%)	ESI (%)
1.0	57.39 ± 1.33
2.5	65.49 ± 0.04
5.0	79.59 ± 2.56
7.5	85.52 ± 6.34
10.0	100.00 ± 0.00

3.6. Cytotoxicity, mutagenicity, and skin sensitization

The effects of LCLig on HaCAT cell viability were evaluated by PrestoBlue fluorescence assay with 6 different concentrations (61.5, 125, 250, 500, 1000 and 2000 μg/mL) for 24 h. As demonstrated in Figure 3, all the tested concentrations were statistically different from the control. The sample LCLig was not cytotoxic up to 250 μg/mL. The results revealed a dose-response effect, where higher concentrations of LCLig resulted in a lower cell viability. The maximum tested concentration of LCLig (2000 μg/mL) caused a significant decrease in cell viability to a value of 35.5% (p<0.0001).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 October 2023 doi:10.20944/preprints202310.0867.v1

Regarding the mutagenicity of the LCLig in *S. typhimurium* in the three tested strains with (S9+) and without metabolic activity, all the tested concentrations (0.4 to 250 µg/mL) revealed no significant reversion (p<0.05) (Figure 3). On the other hand, concerning the strain TA100 without metabolic activation, only the concentration of 250 µg/mL revealed a statistically significant increase in mutants relative to the control (p<0.05). In this respect, these results revealed that LCLig did not induce mutagenicity in all tested strains up to 250 µg/mL, being this maximum concentration more sensitive to base-pair substitution mutations.

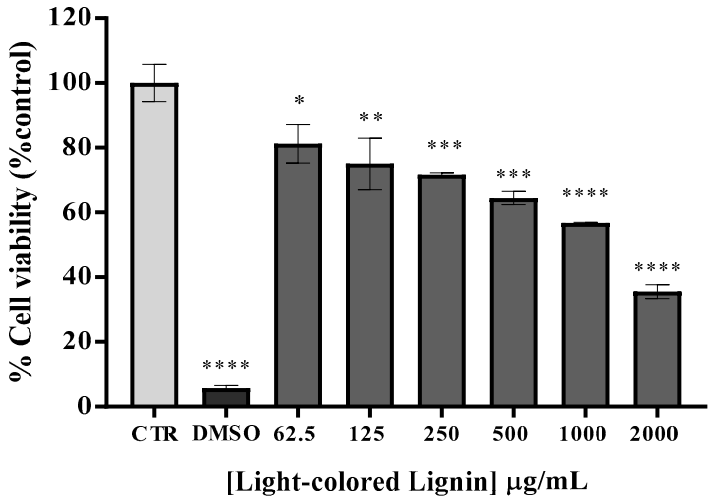

Figure 3. Safety assessment A) HaCAT line cell viability after 24 h of incubation with serial dilutions of light-colored lignin. Results are expressed as % of PrestoBlue reduction vs control (CTR, DMEM). DMSO (10%) represents the positive control. Results are expressed as the mean ± SD performed in triplicate. All the results are expressed as the mean ± SD of two independent assays, performed in quadruplicate. * p<0.05, ** p<0.01, *** p<0.001, **** p< 0.0001 (ANOVA, Tukey HSD); a* p<0.05 - different from non-treated cells (CTR).

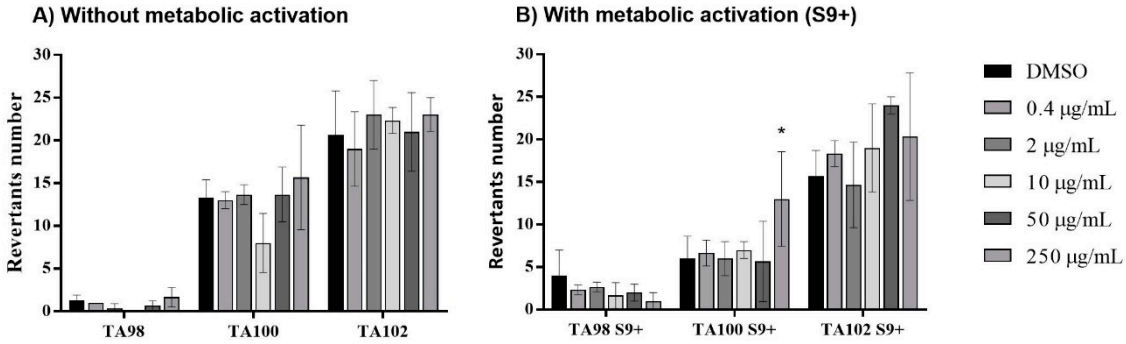

Figure 4. Number of revertant of Salmonella typhimurium individuals after exposure to different concentrations of the LCLig, A) without metabolic activation and B) with metabolic activation (S9+). Results are presented for the three mutant strains tested, TA98, 100 and 102. Results are expressed as the mean ± SD performed in triplicate. All the results are expressed as the mean ± SD of two independent assays, performed in quadruplicate. * p<0.05, (ANOVA, Tukey HSD) different from the control (DMSO). .

The skin sensitization potential was assessed through the Direct Peptide Reactivity Assay (DPRA), which quantifies the reactivity of chemicals towards synthetic peptides. The DPRA method contributes to the assessment of the skin sensitisation potential of chemicals and refers to the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 October 2023 doi:10.20944/preprints202310.0867.v1

molecular initiating event of the adverse outcome pathway, i.e. the covalent interaction with proteins [59]. Based on the results presented in Table 8, LCLig was not found to be a sensitizer up to the tested concentration 1.4 mg/mL.

Table 8. Cystein (Cys) and lysine (Lys) depletion (%), reactivity and prediction models for cinnamaldehyde (positive control) and sugarcane bagasse (SCB) light-colored lignin (LCLig).

Sample	Conc. (mg/mL)	Cys % depletion	Cys and lys % depletion	Reactivity (cys)	Reactivity class	DPRA Prediction
Cynamaldehyde (Positive control)	13.2	76 ± 3	66 ± 1	Moderate	High reactivity	Sensitizer
LCLig	1.40	6.0 ± 0.8	3.4 ± 0.4	Minimal	Minimal	No Sensitizer
	0.75	5.7 ± 0.6	2.8 ± 0.3			
	0.35	5.9 ± 0.4	2.8 ± 0.2			

Reactivity to Cys: Mean % Depletion ≤ 13.89% (No or minimal reactivity); Positive: 13.89% ≤ Mean % Depletion ≤ 23.09% (low reactivity); 23.09% ≤ Mean % Depletion ≤ 98.24% (moderate reactivity); Mean % Depletion ≥ 98.24 (high reactivity). Reactivity to Cys+Lys: 0% ≤ Mean % Depletion ≤ 6.38% (No or minimal reactivity); Positive: 6.38% ≤ Mean % Depletion ≤ 22.62% (low reactivity); 22.62% ≤ Mean % Depletion ≤ 42.47% (moderate reactivity); 42.47% ≤ Mean % Depletion ≤ 100% (high reactivity) A- Based on Cystein only prediction model; B- Based on mean of Cystein and Lysine prediction model. P- Positive; N – Negative.

3.7. Accelerated stability

The stability of an o/w emulsion containing 5 wt% LCLig was evaluated according to the ISO 22716:2007 guidelines for cosmetic industry. The initial color coordinates L*/a*/b* were 53/5.7/20.5. The stability tests were conducted on both the blank and 5 wt% LCLig o/w emulsion (Table 9). The control was set at 4°C (formulation which must remain unchanged during the whole life cycle of the product), and samples were maintained at 25°C (set as shelf sample) and 40°C (accelerated stability). The variation of several parameters is depicted in Table 9. Regarding the physical appearance of both emulsions (blank and 5 wt% LCLig o/w), a good physical stability was observed during the testing period for all temperatures (4, 25, and 40ºC). No phase separation, creaming, flocculation, or sedimentation was observed, indicating good physical stability of the emulsions. Both emulsions have maintained their homogeneity and smooth texture, as well good spreadability through the stability testing period. The color of the 5 wt% LCLig o/w emulsion was stable under all the tested conditions, as per the results presented in Table 9. The pH of the o/w emulsion containing 5 wt% LCLig revealed more consistent values compared with the blank o/w emulsion, indicating that LCLig was able to stabilize the pH of this emulsion. Maintaining an optimal pH range of 4.5-6.5 for cosmetic formulations is desirable, as it helps to protect the *stratum corneum* (outermost layer of the skin) and stabilizing the hydrolipidic film. Another notable finding was that the addition of LCLig did not affect the rheological properties of the final product, concretely, the viscosity of the formulation. The antioxidant activity of the 5 wt% LCLig o/w emulsion remained stable after a 3-month period, and both formulations showed no microbial contamination. This confirms the safety of the emulsion for topical application and good manufacturing practices.

Table 9. Stability results for blank and 5 wt% light-colored lignin (LCLig) o/w emulsions during 3 months at different conditions (4, 25, and 40°C).

Parameter	Blank o/w emulsion	5 wt% LCLig o/w emulsion

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 October 2023 doi:10.20944/preprints202310.0867.v1

	T (°C)	Initial	1 Month	2 Months	3 Months	Initial	1 Month	2 Months	3 Months
Physical appearance	4 25 40	Homogeneous and smooth consistency; good spreadability				Homogeneous and smooth consistency			
Color L* a* b*	4	90.42 -0.68 2.88	81.65 -0.84 3.32	82.92 -0.37 2.00	87.43 -0.68 2.46	55.1 7.4 25.7	53.00 5.70 20.46	52.54 5..38 23.29	53.13 7.19 23.48
	25		83.53 -0.96 4.05	84.99 -0.48 2.85	86.8 1-0.6 3.51		52.54 5.38 20.38	52.60 7.02 23.29	52.74 6.76 21.47
	40		81.87 -0.83 3.68	84.43 -0.53 2.89	88.07 -0.97 3.42		51.74 5.33 19.66	52.13 6.88 21.28	52.77 6.84 20.87
pH	4	5.33 ± 0.02	3.89 ± 0.02	4.13 ± 0.02	4.40 ± 0.02	5.4 ± 0.07	5.34 ± 0.07	5.26 ± 0.07	5.41 ± 0.07
	25		3.74 ± 0.02	3.87 ± 0.02	4.28 ± 0.02		5.31 ± 0.07	5.26 ± 0.07	5.33 ± 0.07
	40		3.73 ± 0.02	3.87 ± 0.02	4.59 ± 0.02		5.34 ± 0.07	5.27 ± 0.07	5.43 ± 0.07
Viscosity (mPa s)	4	1561 ± 8	1957 ± 93	1961 ± 83	1628 ± 54	1414 ± 17	1507 ±1	1870 ± 14	1491 ± 13
	25		1991 ± 95	1968 ± 43	1883 ± 06		1768 ±1	1730 ± 2	1815 ± 4
	40		1715 ± 127	1961 ± 83	1628 ± 54		1507 ± 1	1870 ± 14	1491 ± 13
	4	Not detected				0.64 ± 0.07	0.91 ± 0.03	0.91 ± 0.15	0.67 ± 0.03

 doi:10.20944/preprints202310.0867.v1

Antioxidant activity ABTS, IC50 (mg/mL)	25			0.89 ± 0.05	0.83 ± 0.05	0.73 ± 0.09		
	40			0.93 ± 0.04	0.55 ± 0.04	0.60 ± 0.03		
Total counts of yeast and mold (CFU/g)	4	Absent	<10					
	25							
	40							
Total viable aerobic count (CFU/g)	4	<10	<10					
	25							
	40							

4. Conclusion

In this study, a simple process was successfully applied to reduce the color of lignin extracted from SCB. A detailed characterization of the resulting LCLig was performed, showing 81.6% total lignin, 3.5% of carbohydrates and 3.6% of inorganics. Furthermore, LCLig had comparable molecular weight distributions with previous SCB derived lignin studies, indicating that the hydrogen peroxide treatment did not cause significant fractionation or degradation of the lignin structure. The analysis of LCLig structure revealed a comparable reduced total phenolic units and carboxylic acids, supporting its TPC and antioxidant activity. Furthermore, the color reduction treatment positively influenced the water solubility of LCLig. The sample LCLig was found to be safe for topical application based on the results of cytotoxicity, mutagenicity, and skin sensitization tests. In addition, it was shown to effectively stabilize o/w emulsions at a minimum concentration of 10 wt% or at 2.5 g per g of oil phase. Furthermore, its high ESI at lower concentrations (1.0%), suggests LCLig as a potential co-emulsifier, thus reducing the concentration of synthetic emulsifiers in cosmetic formulations. This is particularly relevant in the development of sustainable and eco-friendlier cosmetic formulations, as they rely less on synthetic emulsifiers, while maintaining good emulsification, stability, and performance. All these findings support the conclusion that LCLig is a promising candidate for cosmetic applications.

CRediT authorship contribution statement: Inês F. Mota: Conceptualization, Methodology, Investigation, Writing – review & editing. **Filipa Antunes:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Joana F. Fangueiro:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Carina A.E. Costa: Methodology,** Writing – review & editing**. Alírio E. Rodrigues:** Writing – review & editing. **Manuela Pintado:** Writing – review & editing, Supervision. **Patrícia Santos Costa:** Conceptualization, Writing – review & editing, Supervision.

Acknowledgments: This work was supported by Fundo Europeu de Desenvolvimento Regional (FEDER), through the Programa Operacional Competitividade e Internacionalização (POCI) under the project Alchemy:

Capturing High Value from Industrial Fermentation BioProducts (POCI-01-0247-FEDER-027578). We would also like to thank the scientific collaboration under the FCT project UID/Multi/50016/2019 and LA/P/0045/2020 (ALiCE), UIDB/50020/2020 and UIDP/50020/2020 (LSRE-LCM), funded by national funds through FCT/MCTES (PIDDAC).

References

1. G.J. Gil-Chávez, S.S.P. Padhi, C. V. Pereira, J.N. Guerreiro, A.A. Matias, I. Smirnova, Cytotoxicity and biological capacity of sulfur-free lignins obtained in novel biorefining process, Int J Biol Macromol. 136 (2019) 697–703. https://doi.org/10.1016/j.ijbiomac.2019.06.021.
2. P.M.A. Vinardell, M. Montserrat, Lignins and Their Derivatives with Beneficial Effects on Human Health, Int J Mol Sci. 18 (2017) 1219. https://doi.org/https://doi.org/10.3390/ijms18061219.
3. J.L. Espinoza-Acosta, P.I. Torres-Chávez, B. Ramírez-Wong, C.M. López-Saiz, B. Montaño-Leyva, Antioxidant, Antimicrobial, and Antimutagenic Properties of Technical Lignins and Their Applications, Bioresources. 11 (2016) 5452–5481. https://doi.org/10.15376/biores.11.2.
4. M.P. Vinardell, V. Ugartondo, M. Mitjans, Potential applications of antioxidant lignins from different sources, Ind Crops Prod. 27 (2008) 220–223. https://doi.org/10.1016/J.INDCROP.2007.07.011.
5. N. Ratanasumarn, P. Chitprasert, Cosmetic potential of lignin extracts from alkaline-treated sugarcane bagasse: Optimization of extraction conditions using response surface methodology, Int J Biol Macromol. 153 (2020) 138–145. https://doi.org/10.1016/J.IJBIOMAC.2020.02.328.
6. O. Ajao, J. Jeaidi, M. Benali, A. Restrepo, N. El Mehdi, Y. Boumghar, Quantification and variability analysis of lignin optical properties for colour-dependent industrial applications, Molecules. 23 (2018) 377. https://doi.org/10.3390/molecules23020377.
7. J. Zheng, L. Chen, X. Qiu, Y. Liu, Y. Qin, Structure investigation of light-colored lignin extracted by Lewis acid-based deep eutectic solvent from softwood, Bioresour Technol. 385 (2023) 129458. https://doi.org/https://doi.org/10.1016/j.biortech.2023.129458.
8. S. Falkehag, Modified lignin surfactants, US Patent US3763139A, 02 October 1973, 1973. https://patentimages.storage.googleapis.com/25/3b/5a/d99a7c889348dd/US3763139.pdf (accessed May 4, 2023).
9. S.I. Falkehag, H.H. Moorer, C.W. Bailey, Alkylene chlorohydrin,oxide or carbonate modified sulfonated lignins in a disperse or vat dye cake, US Patent 3672817A, 27 June 1972, 1972. https://patentimages.storage.googleapis.com/6d/48/24/516ce9e694a38f/US3672817.pdf (accessed May 4, 2023).
10. S.Y. Lin, Process for Reduction of Lignin Color, US Patent 4184845A, 22 January 1980, 1980. https://patentimages.storage.googleapis.com/f4/0a/fe/816bfc1b2194bb/US4184845.pdf (accessed May 4, 2023).
11. P. Dilling, P. Sarjeant, Reduction of lignin color, US Patent 4454066, 12 June 1984, 1972. https://patentimages.storage.googleapis.com/77/fd/89/204cb7b9726ae3/US4454066.pdf (accessed May 4, 2023).
12. H. Zhang, Y. Bai, W. Zhou, F. Chen, Color reduction of sulfonated eucalyptus kraft lignin, Int J Biol Macromol. 97 (2017) 201–208. https://doi.org/10.1016/j.ijbiomac.2017.01.031.
13. R. Li, D. Huang, S. Chen, L. Lei, Y. Chen, J. Tao, W. Zhou, G. Wang, Insight into the self-assembly process of bamboo lignin purified by solvent fractionation to form uniform nanospheres with excellent UV resistance, Colloids Surf A Physicochem Eng Asp. 642 (2022) 128652. https://doi.org/10.1016/j.colsurfa.2022.128652.
14. H. Zhang, S. Fu, Y. Chen, Basic understanding of the color distinction of lignin and the proper selection of lignin in color-depended utilizations, Int J Biol Macromol. 147 (2020) 607–615. https://doi.org/10.1016/J.IJBIOMAC.2020.01.105.
15. H. Zhang, Y. Bai, B. Yu, X. Liu, F. Chen, A practicable process for lignin color reduction: fractionation of lignin using methanol/water as a solvent, Green Chemistry. 19 (2017) 5152–5162. https://doi.org/10.1039/C7GC01974B.
16. J. Wang, Y. Deng, Y. Qian, X. Qiu, Y. Ren, D. Yang, Reduction of lignin color via one-step UV irradiation, Green Chemistry. 18 (2016) 695–699. https://doi.org/10.1039/c5gc02180d.
17. V. Saritha, Y.A. Maruthi, K. Mukkanti, Biological Decolourization of Higher Concentrations of Synthetic Lignin by Native Fungi, 1 (2010) 1–4.
18. B. Jiang, Y. Zhang, L. Gu, W. Wu, H. Zhao, Y. Jin, Structural elucidation and antioxidant activity of lignin isolated from rice straw and alkali-oxygen black liquor, Int J Biol Macromol. 116 (2018) 513–519. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2018.05.063.
19. H. Sadeghifar, Lignin as A Natural Sunscreen-An Overview, (2020) 20389–20391. https://doi.org/10.26717/BJSTR.2020.27.004430.

20. M.H. Tran, D.-P. Phan, E.Y. Lee, Review on lignin modifications toward natural UV protection ingredient for lignin-based sunscreens, Green Chemistry. 23 (2021) 4633–4646. https://doi.org/10.1039/D1GC01139A.
21. H. Zhang, X. Liu, S. Fu, Y. Chen, High-value utilization of kraft lignin: Color reduction and evaluation as sunscreen ingredient, Int J Biol Macromol. 133 (2019) 86–92. https://doi.org/10.1016/J.IJBIOMAC.2019.04.092.
22. Z. Ahmad, W.W. Al Dajani, M. Paleologou, C. Xu, Sustainable process for the depolymerization/oxidation of softwood and hardwood kraft lignins using hydrogen peroxide under ambient conditions, Molecules. 25 (2020) 1–19. https://doi.org/10.3390/molecules25102329.
23. S. Tripathi, N. Sharma, I. Alam, N.K. Bhardwaj, Effectiveness of different green chemistry approaches during mixed hardwood bamboo pulp bleaching and their impact on environment, International Journal of Environmental Science and Technology. 16 (2019) 4327–4338. https://doi.org/10.1007/s13762-018-1887-4.
24. K. Dölle, A. Honig, Laboratory Bleaching System for Oxygen and Ozone Bleaching, Asian Journal of Chemical Sciences. 4 (2018) 1–12. https://doi.org/10.9734/ajocs/2018/40620.
25. J.F. Kadla, H. Chang, The Reactions of Peroxides with Lignin and Lignin Model Compounds, in: Oxidative Delignification Chemistry, American Chemical Society, 2001: pp. 108–129. https://doi.org/doi:10.1021/bk-2001-0785.ch006.
26. Y. Li, Q. Fu, R. Rojas, M. Yan, M. Lawoko, L. Berglund, Lignin-Retaining Transparent Wood, ChemSusChem. 10 (2017) 3445–3451. https://doi.org/10.1002/cssc.201701089.
27. P. Bisht, K.K. Pandey, H.C. Barshilia, Photostable transparent wood composite functionalized with an UV-absorber, Polym Degrad Stab. 189 (2021) 109600. https://doi.org/10.1016/j.polymdegradstab.2021.109600.
28. I. Wachter, S. Tomas, P. Rantuch, J. Martinka, A. Pastierova, Effect of UV Radiation on Optical Properties and Hardness of Transparent Wood, Polymers (Basel). 13 (2021) 2067. https://doi.org/10.3390/polym13132067.
29. Y. Li, Z. Cai, M. Liao, J. Long, W. Zhao, Y. Chen, X. Li, Catalytic depolymerization of organosolv sugarcane bagasse lignin in cooperative ionic liquid pairs, Catal Today. 298 (2017) 168–174. https://doi.org/https://doi.org/10.1016/j.cattod.2017.04.059.
30. C.A.E. Costa, P.C.R. Pinto, A.E. Rodrigues, Evaluation of chemical processing impact on E. globulus wood lignin and comparison with bark lignin, Ind Crops Prod. 61 (2014) 479–491. https://doi.org/https://doi.org/10.1016/j.indcrop.2014.07.045.
31. I.F. Mota, P.R. Pinto, A.M. Ribeiro, J.M. Loureiro, A.E. Rodrigues, Downstream processing of an oxidized industrial kraft liquor by membrane fractionation for vanillin and syringaldehyde recovery, Sep Purif Technol. 197 (2018). https://doi.org/https://doi.org/10.1016/j.seppur.2018.01.001.
32. A.A. Vilas-Boas, D.A. Campos, C. Nunes, S. Ribeiro, J. Nunes, A. Oliveira, M. Pintado, Polyphenol extraction by different techniques for valorisation of non-compliant portuguese sweet cherries towards a novel antioxidant extract, Sustainability (Switzerland). 12 (2020). https://doi.org/10.3390/su12145556.
33. F. Antunes, I.F. Mota, J.F. Fangueiro, G. Lopes, M. Pintado, P.S. Costa, From sugarcane to skin: Lignin as a multifunctional ingredient for cosmetic application, Int J Biol Macromol. 234 (2023) 123592. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2023.123592.
34. M. del M. Contreras, B. Hernández-Ledesma, L. Amigo, P.J. Martín-Álvarez, I. Recio, Production of antioxidant hydrolyzates from a whey protein concentrate with thermolysin: Optimization by response surface methodology, Lwt. 44 (2011) 9–15. https://doi.org/10.1016/j.lwt.2010.06.017.
35. B. Goncalves, V. Falco, J. Moutinho-Pereira, E. Bacelar, F. Peixoto, C. Correia, Effects of elevated CO2 on grapevine (Vitis vinifera L.): volatile composition, phenolic content, and in vitro antioxidant activity of red wine., J Agric Food Chem. 57 (2009) 265–273. https://doi.org/10.1021/jf8020199.
36. S.J. Choi, J.W. Won, K.M. Park, P.-S. Chang, A New Method for Determining the Emulsion Stability Index by Backscattering Light Detection, J Food Process Eng. 37 (2014) 229–236. https://doi.org/https://doi.org/10.1111/jfpe.12078.
37. International Organization for Standardization (ISO) 17516:2014 — Microbiological limits for cosmetics, 2014.
38. X. Lu, X. Gu, Y. Shi, A review on lignin antioxidants: Their sources, isolations, antioxidant activities and various applications, Int J Biol Macromol. 210 (2022) 716–741. https://doi.org/10.1016/J.IJBIOMAC.2022.04.228.
39. E. Cequier, Extraction and characterization of lignin from olive pomace : a comparison study among ionic liquid , sulfuric acid , and alkaline treatments, (2019) 241–252.
40. C. Heitner, D. Dimmel, J. Schmidt, Lignin and Lignans:Advances in Chemistry, 1st ed., CRC Press, 2010. https://doi.org/10.1201/EBK1574444865.
41. L. An, C. Si, G. Wang, W. Sui, Z. Tao, Enhancing the solubility and antioxidant activity of high-molecular-weight lignin by moderate depolymerization via in situ ethanol/acid catalysis, Ind Crops Prod. 128 (2019) 177–185. https://doi.org/https://doi.org/10.1016/j.indcrop.2018.11.009.
42. R. Kaur, S.K. Uppal, Structural characterization and antioxidant activity of lignin from sugarcane bagasse, Colloid Polym Sci. 293 (2015) 2585–2592. https://doi.org/10.1007/s00396-015-3653-1.

43. X.-F. Sun, H. Wang, G. Zhang, P. Fowler, M. Rajaratnam, Extraction and characterization of lignins from maize stem and sugarcane bagasse, J Appl Polym Sci. 120 (2011) 3587–3595. https://doi.org/10.1002/app.33551.
44. L. Oliveira, D. Evtuguin, N. Cordeiro, A.J.D. Silvestre, Structural characterization of stalk lignin from banana plant, Ind Crops Prod. 29 (2009) 86–95. https://doi.org/https://doi.org/10.1016/j.indcrop.2008.04.012.
45. S.Y. Lin, C.W. Dence, eds., Methods in Lignin Chemistry, Springer Berlin Heidelberg, Berlin, Heidelberg, 1992. https://doi.org/10.1007/978-3-642-74065-7.
46. H. Yang, Z. Dong, B. Liu, Y. Chen, M. Gong, S. Li, H. Chen, A new insight of lignin pyrolysis mechanism based on functional group evolutions of solid char, Fuel. 288 (2021) 119719. https://doi.org/https://doi.org/10.1016/j.fuel.2020.119719.
47. Mihai Brebu, C. Vasile, Thermal Degradation of Lignin – A Review, Cellulose Chem. Technol. 44 (2010) 353–363.
48. J.C. Domínguez, M. Oliet, M. V Alonso, M.A. Gilarranz, F. Rodríguez, Thermal stability and pyrolysis kinetics of organosolv lignins obtained from Eucalyptus globulus, Ind Crops Prod. 27 (2008) 150–156. https://doi.org/https://doi.org/10.1016/j.indcrop.2007.07.006.
49. N. Ramezani, M. Sain, Thermal and Physiochemical Characterization of Lignin Extracted from Wheat Straw by Organosolv Process, J Polym Environ. 26 (2018) 3109–3116. https://doi.org/10.1007/s10924-018-1199-2.
50. W.G. Glasser, About Making Lignin Great Again—Some Lessons From the Past, Front Chem. 7 (2019) 1–17. https://doi.org/10.3389/fchem.2019.00565.
51. C. Cheng, J. Wang, D. Shen, J. Xue, S. Guan, S. Gu, K. Luo, Catalytic Oxidation of Lignin in Solvent Systems for Production of Renewable Chemicals: A Review, Polymers (Basel). 9 (2017) 240. https://doi.org/10.3390/polym9060240.
52. V. Ponnuchamy, O. Gordobil, R.H. Diaz, A. Sandak, J. Sandak, Fractionation of lignin using organic solvents: A combined experimental and theoretical study, Int J Biol Macromol. 168 (2021) 792–805. https://doi.org/10.1016/j.ijbiomac.2020.11.139.
53. E.I. Evstigneyev, S.M. Shevchenko, Structure, chemical reactivity and solubility of lignin: a fresh look, Wood Sci Technol. 53 (2019) 7–47. https://doi.org/10.1007/s00226-018-1059-1.
54. F. Antunes, I.F. Mota, J.F. Fangueiro, G. Lopes, M. Pintado, P.S. Costa, From sugarcane to skin: Lignin as a multifunctional ingredient for cosmetic application, Int J Biol Macromol. 234 (2023) 123592. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2023.123592.
55. L. Cesari, F. Mutelet, L. Canabady-Rochelle, Antioxidant properties of phenolic surrogates of lignin depolymerisation, Ind Crops Prod. 129 (2019) 480–487. https://doi.org/https://doi.org/10.1016/j.indcrop.2018.12.010.
56. R. Kaur, S.K. Uppal, P. Sharma, Antioxidant and Antibacterial Activities of Sugarcane Bagasse Lignin and Chemically Modified Lignins, Sugar Tech. 19 (2017) 675–680. https://doi.org/10.1007/s12355-017-0513-y.
57. L.B. Brenelli, L.R.B. Mariutti, R. Villares Portugal, M.A. de Farias, N. Bragagnolo, A.Z. Mercadante, T.T. Franco, S.C. Rabelo, F.M. Squina, Modified lignin from sugarcane bagasse as an emulsifier in oil-in-water nanoemulsions, Ind Crops Prod. 167 (2021) 113532. https://doi.org/10.1016/J.INDCROP.2021.113532.
58. L. Bai, L.G. Greca, W. Xiang, J. Lehtonen, S. Huan, R.W.N. Nugroho, B.L. Tardy, O.J. Rojas, Adsorption and Assembly of Cellulosic and Lignin Colloids at Oil/Water Interfaces, Langmuir. 35 (2019) 571–588. https://doi.org/10.1021/acs.langmuir.8b01288.
59. R. Kreiling, H. Gehrke, T.H. Broschard, B. Dreeßen, D. Eigler, D. Hart, V. Höpflinger, M. Kleber, J. Kupny, Q. Li, P. Ungeheuer, U.G. Sauer, In chemico, in vitro and in vivo comparison of the skin sensitizing potential of eight unsaturated and one saturated lipid compounds, Regulatory Toxicology and Pharmacology. 90 (2017) 262–276. https://doi.org/https://doi.org/10.1016/j.yrtph.2017.09.023.