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Exploring the role of natural deep eutectic solvents in shaping cosmetic gels properties

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1. Introduction

The cosmetics industry is now well underway in its shift towards product eco-design, including sustainable formulas and processes. The preparation of bio-based ingredients must follow this movement towards sustainability. In complement to agrosolvents like bioethanol or dimethyl carbonate, Natural Deep Eutectic Solvents (NaDES), green solvents based on natural metabolites, are interesting candidates to replace conventional organic solvents in the extraction processes of biomasses.

Emerging in the 2010s, NaDES are composed of a hydrogen bond acceptor, usually choline chloride or betaine, and a hydrogen bonding donor, as sugars or polyols. Their association forms a network of hydrogen bonds resulting in the lowering of the melting point of the solids mixture [1-3], making the NaDES liquid at room temperature. NaDES are made of vegetal cellular constituent, which make them renewable and biodegradable [3]. Most used metabolites are choline derivatives, sugars, aminoacids, and organic acids. Moreover, their high dissolution power combined with their stabilizing ability make NaDES perfect media for sustainable valuation of vegetal biomasses [2]. Their composition makes also NaDES truly biocompatible allowing to avoid solvent removal step in extraction processes. NaDES were also described as skin penetration enhancers [4], which add to their attractiveness.

To extend the scope of these alternative solvents, a broader nomenclature has recently emerged: MINACs (Mixtures based on Natural Compounds) [5]. This category encompasses NaDES and Low Transition Temperature Mixtures (LTTMs), but also includes solvent systems that do not necessarily exhibit a deep depression of the melting or glass transition point. This flexibility allows for the inclusion of mixtures that may contain water or other additives, often required to facilitate solvent formation or to enhance the extraction efficiency of active compounds.

Nevertheless, when it comes to work with NaDES or MINAC, some questions can arise concerning their stability, their extraction capacities, their toxicity or their formulability. Through the example of developments in our labs based on a specific MINAC made of glycerol and glucose, named GG20 in the core of this paper, the critical point of the introduction of a MINAC in a cosmetic product will be highlighted to prepare future users of those new ingredients that will soon be widely present on the market.

MINAC made of glycerol and glucose at variable molar ratio is a well-known MINAC described in the literature as very promising to extract molecules of interest of biomasses [6-8]. Previously known as a NaDES, it has now been reclassified as a MINAC. Notably, MINAC including glucose and glycerol seem very interesting combination to extract antioxidant molecules from plants, like epigallocatechin-3-gallate from green Tea [6]. Studies performed inside our consortium demonstrated that GG20 MINAC was a solvent of choice to extract from microalgae Arthrospira platensis and Porphyridium cruetum and stabilize the family of molecule interesting for their cosmetic and colour properties: phycobiliproteins [7-8]. We also demonstrated its absence of toxicity on skin cells in culture [8]. To our knowledge, the compatibility GG20 with cosmetic products formulas like gel, creams, lotions etc was not published yet.

2. Materials and Methods

MINAC preparation: the MINAC used contains glucose as hydrogen bond acceptor and glycerol as a hydrogen bond donor (1:2 molar ratio) The eutectic point was reached by stirring and heating the mixture of raw materials at 80°C for 3 hours until a homogeneous colourless phase was formed. Water was added to reduce the viscosity of the system (20% wt/wt) and obtain a consistency compatible with extraction. This MINAC will be further called GG20.

Extraction of phycobiliproteins from microalgae (Arthrospira platensis and Porphyridium cruentum): For each microalgae strain, the biomass was simultaneously treated with a lipophilic NaDES to extract lipid contents and the hydrophilic MINAC GG20 to extract phycobiliproteins. In brief, for each microalga (A. platensis and P. cruentum), dried flakes were put in contact with the lipophilic NaDES and GG20 (1:1 w/w) using DAC mixing device (Speedmixer®, Hauschield, Germany) at a speed of 1500 rpm during 30 min. The resulting mixtures were centrifuged, and the hydrophilic phase containing the molecule of interest was kept. The GG20 extract was then filtered (2µm) to separate potential residual biomass. The samples were stored in an oven at 20°C protected from light.

Topical gels preparation: Model cosmetic formulations based on COSMOS ingredients were prepared in triplicate as 150g batches with a bench mixer equipped with a dispersing turbine (Turbotest ®, VMI, France). Gels were composed of ultrapure water, antimicrobial agents (benzyl alcohol and dehydroacetic acid, 0.3% wt/wt in the final product) and a thickening agent. Two thickening agents derived from cellulose were tested: an ionic compound, sodium carboxymethylcellulose (CMC) at 3% wt/wt in the final product, and a non-ionic compound, hydroxyethylcellulose (HEC) at 5% wt/wt in the final product. The MINAC or MINAC extract were introduced at 1% or 10% wt/wt, concentrations relevant with their use as an active ingredient in topical formulations. A blank gel was prepared in which water replaces MINAC or MINAC extract was prepared for comparison.

Rheology study: A Kinexus pro+ rheometer (Netzsch, Germany) was used for the measurements. Analyses were performed at 25°C, with a gap of 0.1 mm and a textured plate-plate mobile of 4 cm of diameter. A study of the viscosity as function of the shear rate was performed between 0.01 and 1000 s-1. GG20 was analysed 24h after preparation only, while topical gels were observed 24h after preparation and after 28 days of storage in an oven at 20°C and in accelerated aging conditions (40°C, 75% humidity, dark) to evaluate the stability of the systems.

pH measurements: pH was measured with a Eutec pHmeter with an electrode specifically designed for viscous products, at 25°C.
Colorimetry: Color measurements were performed with a handheld chromameter C400 (Konika Minolta, Tokyo, Japan). All the measurements were performed at room temperature with the specific accessory provided to measure the color of transparent systems. Results are expressed in the CIELAB space. In this system, L represents the clarity of the color going from 0 for black to 100 for white, the a-axis is going from green to red for the negative to the positive value, the b axis is going from blue to yellow for the negative to the positive value. Dilutions were made with the extract including or not the preservatives like the gels replacing the amount of gelling agent by water. They were also analyzed to observe the impact of the different compounds of the gel on the MINAC based antioxidant extract.
Pigments determination: Titrations of phycocyanin, carotenoids and chlorophyll were carried on the samples after appropriate dilution by UV-vis spectroscopy using a microplate reader (Multiskan GO, Thermo Fisher Scientific, SAS) at 652, 620 and 562 nm corresponding respectively to allophycocyanin, phycocyanin and phycoerythrin.

3. Results&Discussion

Analysis of the MINAC-loaded gels

As a first step, 1 and 10% of MINAC were introduced in gels using the two different cellulosic gelling agents: sodium carboxymethylcellulose (CMC) 3% wt/wt and hydroxyethyl cellulose (HEC) 5% wt/wt. The gels were all transparent and the few bubbles that remain after the mixing disappear after one day of storage. The pH was around 6 for both blank gels and gels with MINAC, which is compatible with a skin application.

All gels show a shear-thinning behavior (Figure 1). For HEC, a slight increase of viscosity values, +17.2% (around 1 Pa. s) of the viscosity at low shear rates ($0.1s^{-1}$) at day 1 can be observed for the gel at 10% MINAC. Concerning gels prepared with CMC, all gels present a shear-thinning behavior, and the viscosity at day 1 is much higher, + 96.3% of viscosity at $0.01s^{-1}$ (more than 10 Pa.s), for the gel at 10% MINAC than the one at 1% or the blank gel. For both gelling agents, the higher viscosity of the gel at 10% compared to the 1% gel and the blank gel, combined with the shear thinning behavior are in agreement with the existence of a hydrogen bond network between the MINAC and the gelling agent, that breaks in the range of the high shearing rates. This phenomenon seems to be more pronounced with the ionic derivative.

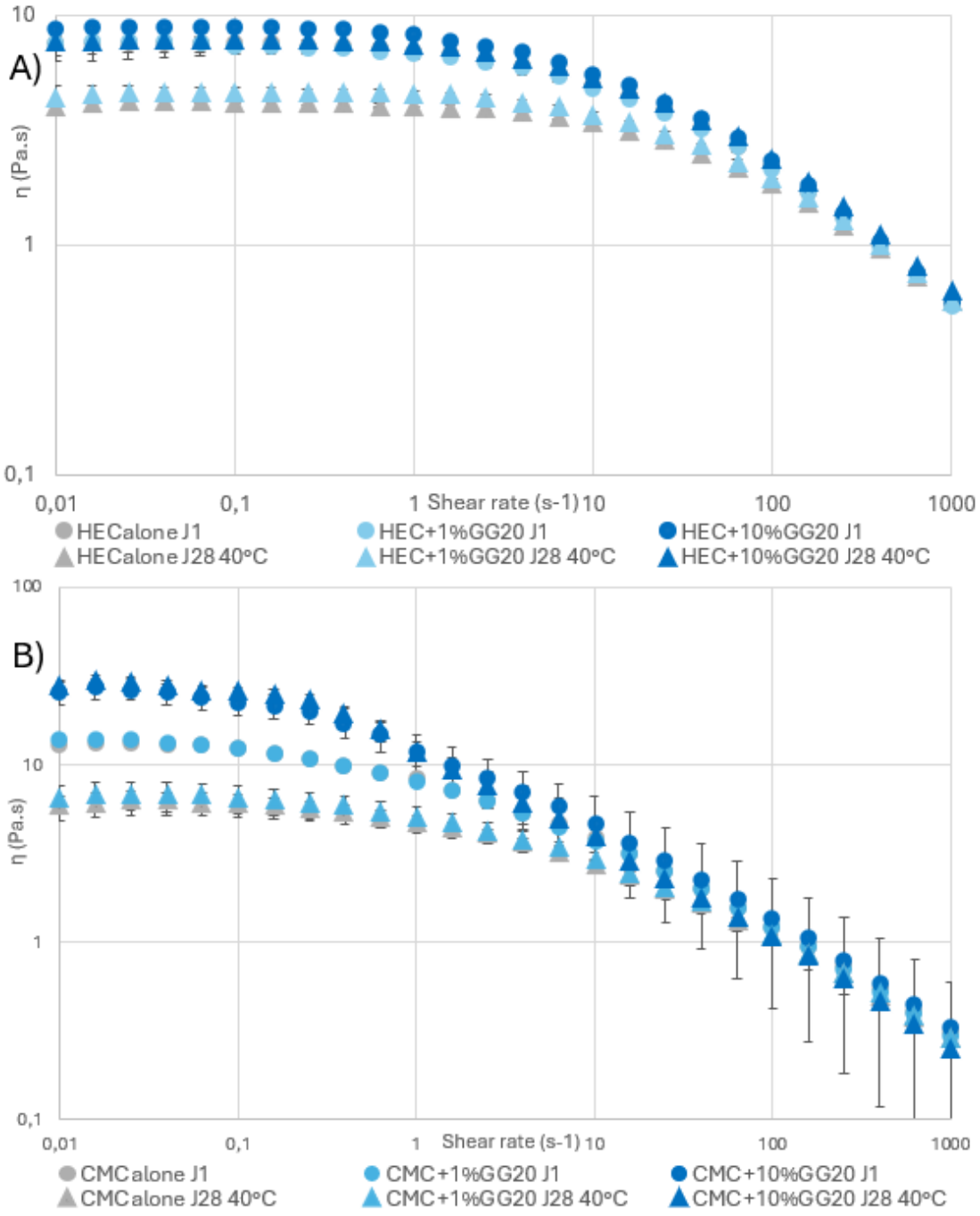

Figure 1:Impact of the addition of 1 and 10% of GG20 on the rheological behavior and stability of HEC gels (A) and CMC gels (B) (flow curves)

This implies that the MINAC interactions with the gelling agent may modify the behavior of the gel during the production and packaging steps. Interestingly, the difference in viscosity is not significant in the shear rates zone around 1000 s^{-1} describing the spreading of the product[8]. Therefore, the introduction of 10% of this MINAC will not have an impact on the spreading that can be detected by the consumer.

Rheological measurements one month after preparation allow us to evaluate the physical stability of the formulations. After 28 days at 40°C and 75% humidity, whatever the gelling agent, gels containing 10% of MINAC show a rheological behavior and viscosity values similar to the ones established at day 1. On the contrary, blank gels and gels containing only 1% wt/wt of MINAC show a decrease in their viscosity (-46.85% et -42.63% respectively of viscosity at 0.01 à1s^{-1}). These results indicates that the hydrogen bond network persists during the shelf-life of the 10% MINAC-gels and stabilizes the product.

This very interesting result shows that incorporating a high percentage of MINAC can increase the stability of gels.

Analysis of the Arthrospira platensis and Porphyridium cruentum MINAC extract

GG20 extracts obtained by solid/liquid/liquid extraction were stored for one month at 20 °C, and pigment content was analyzed to assess phycobiliprotein stability in MINAC. In both microalgae extracts, a decrease in the main phycobiliprotein was observed: 30% loss of phycocyanin for A. platensis and 17% loss of phycoerythrin for Porphyridium.

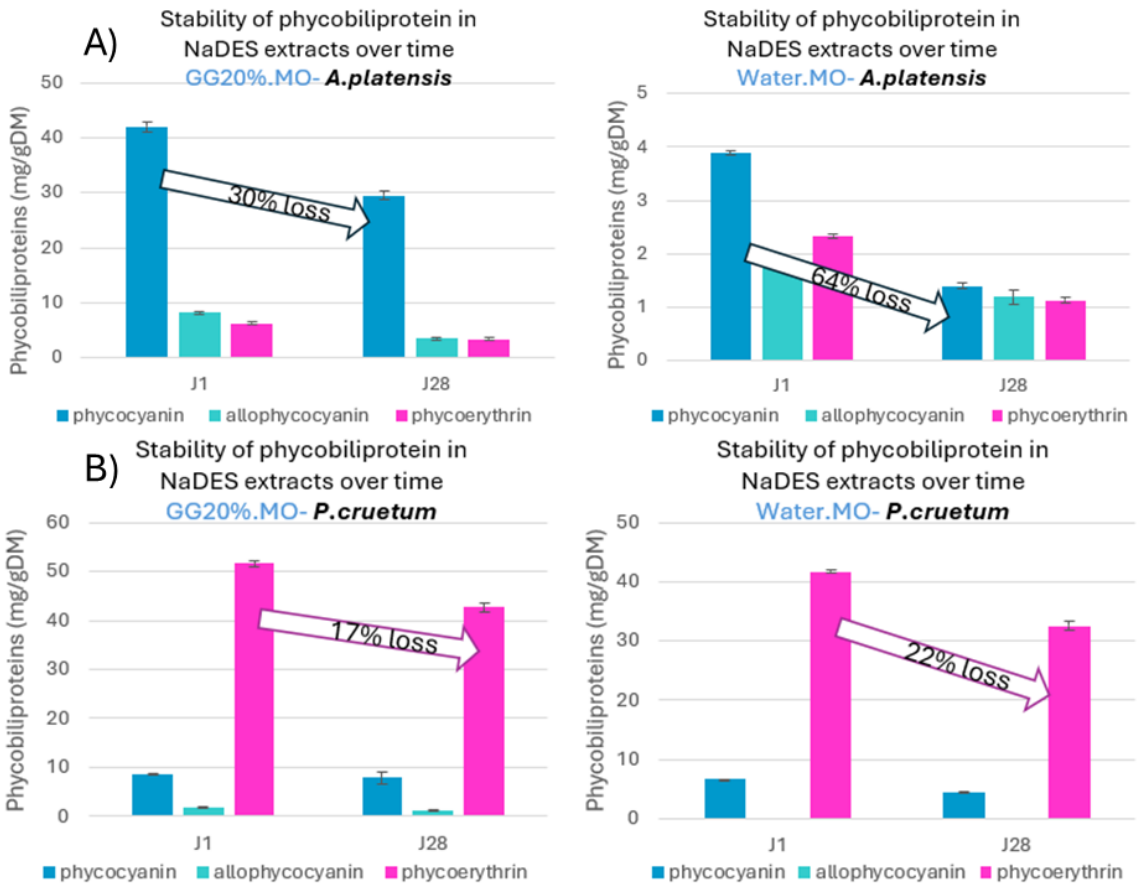

Figure 2: Stability of phycobiliproteins in GG20 and Water extract after one month in A) plantensis (Spirulina) and B) P. cruetum (Porphyridium)

For comparison, aqueous extracts were prepared using the same method. A clear difference appears in pigment extraction: GG20 extracts yielded over 900% more phycocyanin from A. platensis and over 20% more phycoerythrin from Porphyridium than water extracts. Stability of

the water extract compared to the MINAC extract was also significantly lower: phycocyanin loss reached 64% for A. platensis and phycoerythrin 22% for Porphyridium (Figure 2). Overall, MINAC notably improves phycobiliprotein stability compared to water. In particular, phycocyanin shows better preservation in MINAC, supporting further exploration of MINAC extracts in gel formulations.

Analysis of the MINAC loaded gels

Following the gel observations with varying MINAC percentages, the goal was to evaluate the behavior and stability of antioxidant extracts in both CMC and HEC gels. The extract was initially incorporated at 1%, as previous tests showed no significant rheological differences between the blank gel and the one containing 1% MINAC.

Colorimetry measurements

Gels high viscosity combined to a low concentration in phycobiliprotein. It was then impossible to perform pigment determination with spectroscopic measurements. As the extract containing phycobiliproteins are blue and pink for respectively A. platensis and P. cruentum, the color variation of the samples in time was analyzed to highlight a possible degradation in time of our molecules.

First, the observation was done on the blank gel and the ones with 1% GG20. CMC gels are yellower after 28 days at 40°C and 75% humidity. This alteration due to the gel components must be taken into consideration in the analysis of the extract-loaded gels color after 28 days of shelf-life. For the HEC gels at day 1, the gel with 1% GG20 is yellower and greener than the blank gel but this difference narrows after 28 days at 40°C, 75% humidity. In this case, this color change must be taken into account for the analysis of the fresh gels.

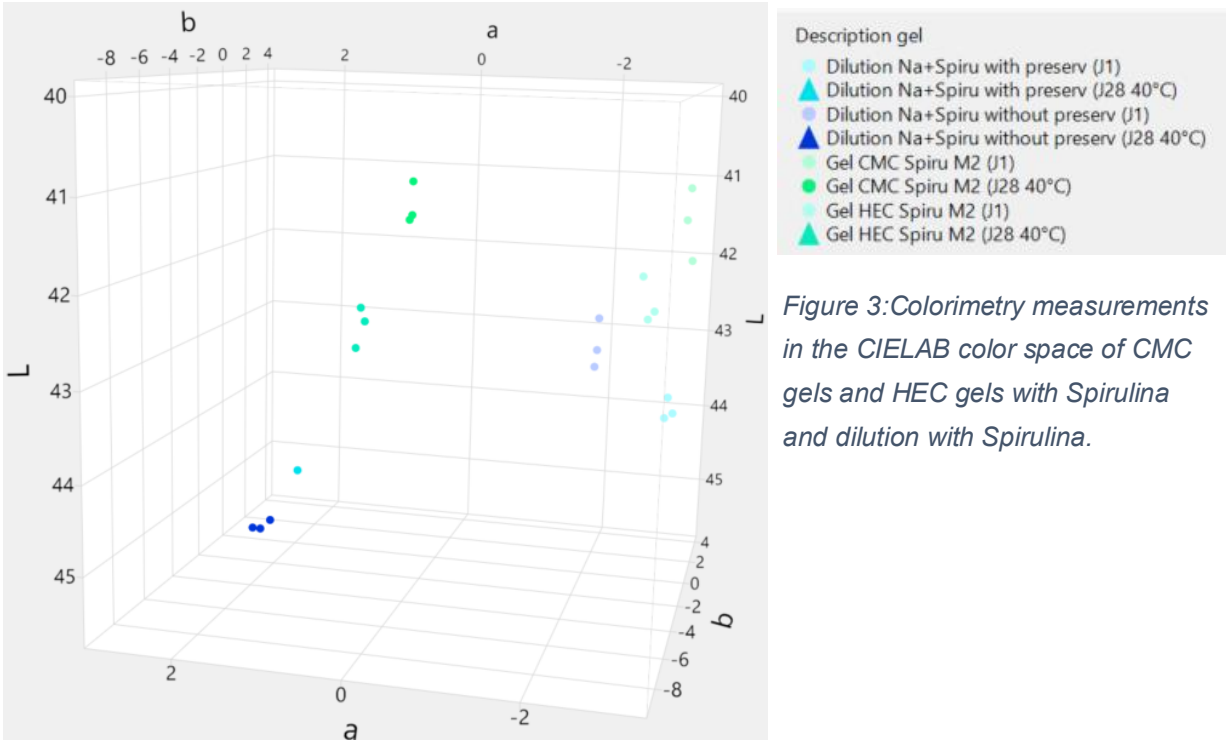

Figure 3:Colorimetry measurements in the CIELAB color space of CMC gels and HEC gels with Spirulina and dilution with Spirulina.

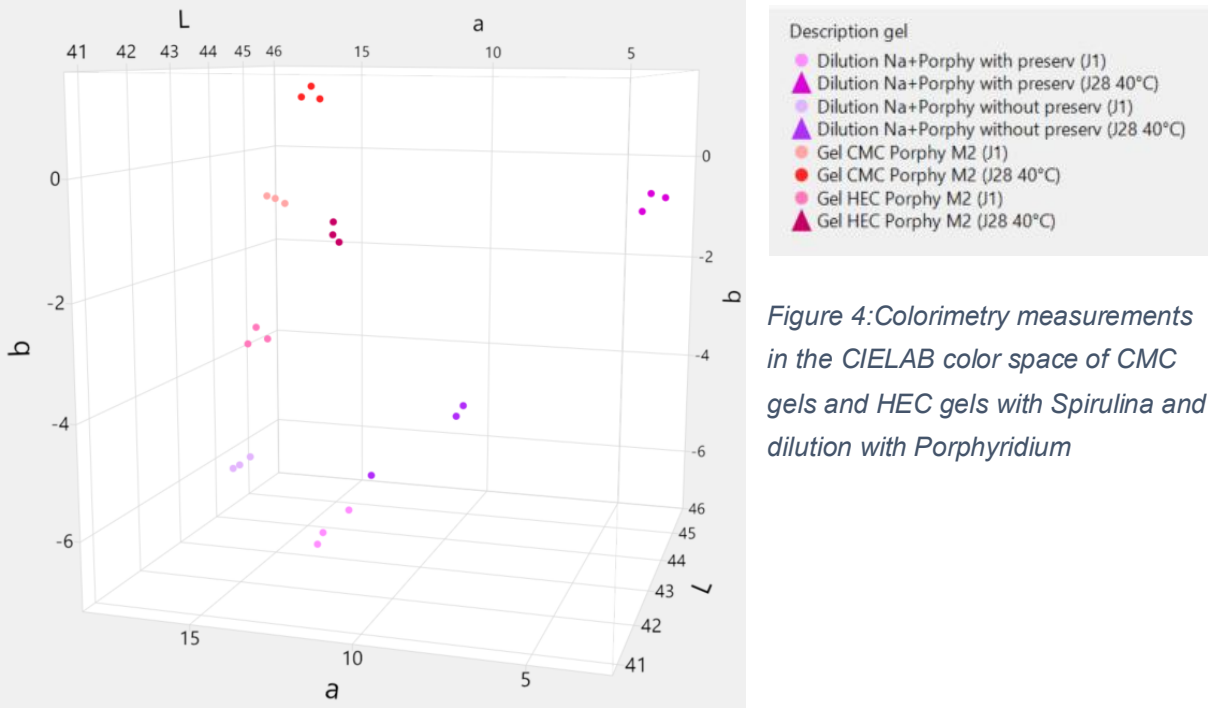

Figure 4:Colorimetry measurements in the CIELAB color space of CMC gels and HEC gels with Spirulina and dilution with Porphyridium

The preservatives also impact the colors of the gels, which can be seen through the difference of colors between the two types of dilution at day 1. With the A. platensis, the dilution with the preservatives is clearer, yellower and redder. With the Porphyridium, the dilution with the preservatives is darker and redder. However, the higher delta between J1 and J28 for the dilutions without this preservative, shows us the necessity to maintain it in the formula to help increase the stability of the antioxidant molecules. (Figure 3 and 4)

For both extract with the A. platensis and Porphyridium, at day 1, the CMC gels with the extract is yellower and slightly darker than the HEC gels with the extract. Between day 1 and day 28 at 40°C, a change in color is observed for both gels but it seems to have used the quite same vectors maintaining the slight difference observed at day 1. This translation is almost the same that the one observed in the dilution. However, the translation seems smaller in the gel which can implies that the gels allow to reduce the color destabilization during time As the MINAC-based extract is not stable on its own, the results observed with the colorimetry measure seems to show that the stability of the extract is improve when incorporated on gels.

Rheology

In terms of rheological behavior, all the gels have a shear-thinning behavior with a G" moduli higher than the G'moduli at day 1 meaning that these gels are considered viscoelastic liquid. For almost every gel the viscoelastic domain is large going from 0,01 to 100% deformation. The viscosity of the gels at day 1 is similar to the blank gel for each gelling agent. However, a lack of rheological stability is observed after 28 days at 40°C at 75% humidity, especially with the gel containing 1% MINAC extract with Porphyridium. A drop in viscosity leading to a Newtonian behavior of the gels with both gelling agents. A slight gap between the viscosity with the A. platensis extract and the control appears after 28 days but compared between the

drop of viscosity between day 1 and 28 of the control gels this appears to be unsignificant. (Figures 5 and 6)

Porphyridium extract seems to impact CMC and HEC gelling properties, as the previous results have shown that the incorporation of 1% of NaDES didn't impact the gelling properties. A first hypothesis is that Porphyridium extracts contains other types of sugars that stabilizes the phycobiliproteins, like sulfated polysaccharides, that can also be extracted with the NaDES. These sugars could destabilize the gelling agent. Further experiments need to be performed to validate this hypothesis.

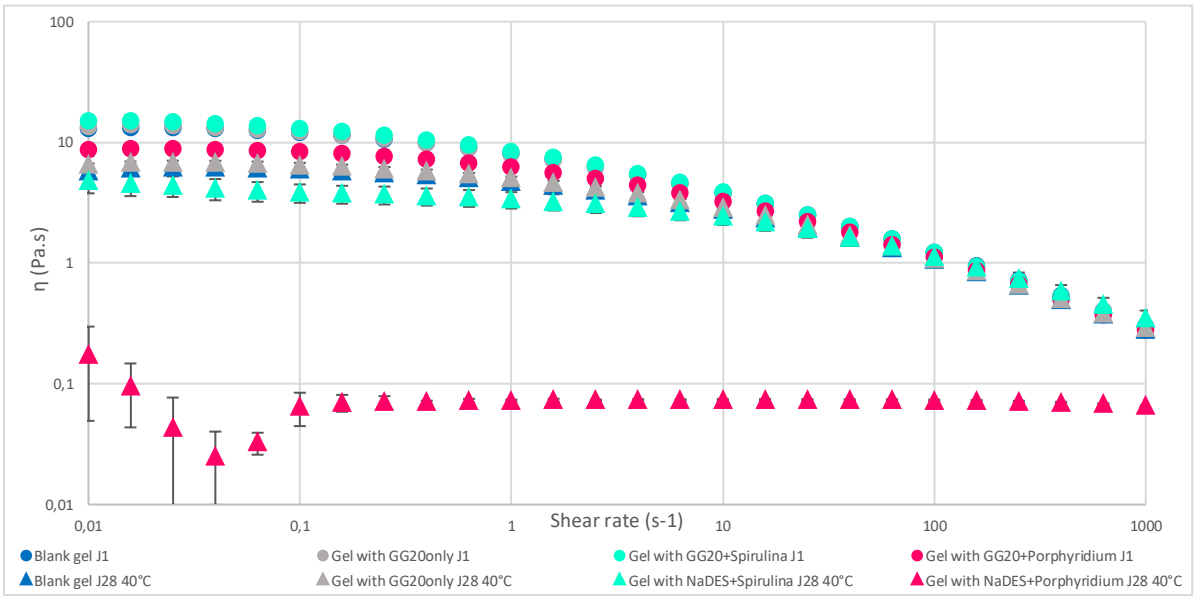

Figure 5: Flow curve of the gels with CMC (blank gel and gel with 1% GG20 with or without extract)

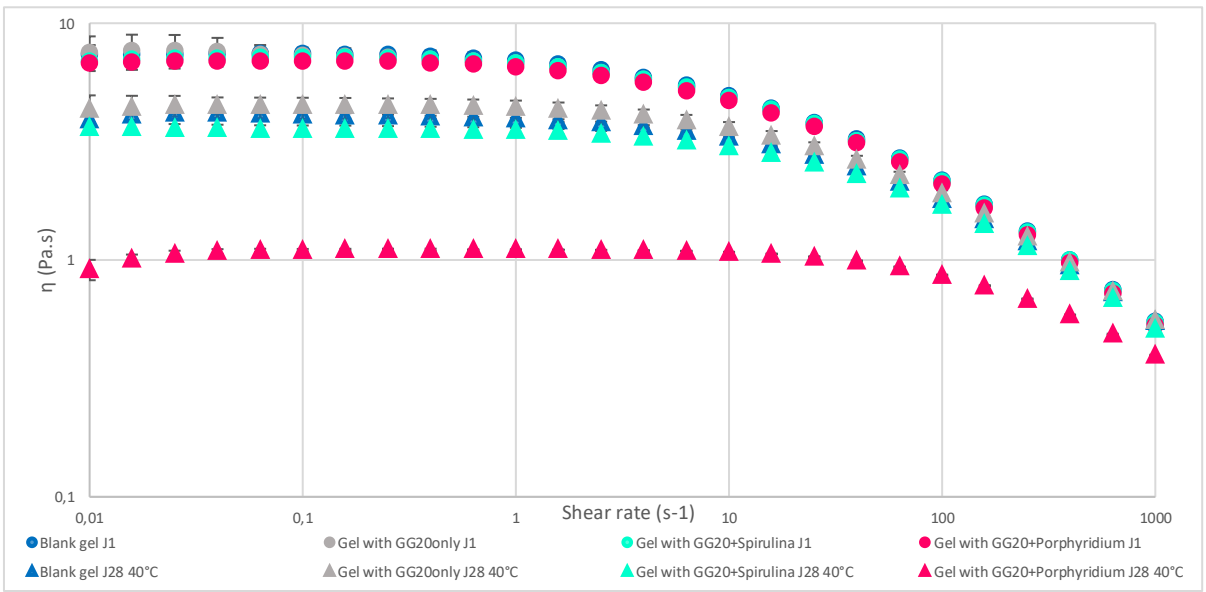

Figure 6: Flow curve of the gels with HEC (blank gel and gel with 1% GG20 with or without extract)

5. Conclusion

This study highlights the promising potential of MINAC-based extracts in cosmetic formulations. These mixtures offer improved preservation of active compounds compared to conventional water-based extracts. When used at low concentrations, MINAC extracts have minimal impact on formulation stability, which remains primarily dependent on the nature of the active molecules themselves. Incorporating the extracts directly into gels, rather than as diluted solutions, helps to better preserve the color of the final product, suggesting enhanced stability of the bioactive compounds. Moreover, using higher proportions of MINACs as partial water replacements can positively influence the product's texture, offering opportunities to develop firmer formulations while maintaining desirable sensorial properties.

6. References

1) Abbott, A.P., Boothby, D., Capper, G., Davies, D.L., Rasheed, R.K., (2004). Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. J. Am. Chem. Soc. 126, 9142–9147.
2) Fernández MLÁ, Boiteux J, Espino M, Gomez FJV, Silva MF (2018) Natural deep eutectic solvents-mediated extractions: The way forward for sustainable analytical developments Anal Chim Acta. 1038:1-10.
3) Liu Y, Friensen JB, McAlpine JB, Lankin DC, Chen SN, Pauli GF (2018) Natural deep eutectic solvents: properties, applications and perspectives, 81(3):679-690.
4) Fernández MLÁ, Boiteux J, Espino M, Gomez FJV, Silva MF (2018) Natural deep eutectic solvents-mediated extractions: The way forward for sustainable analytical developments Anal Chim Acta. 1038:1-10.
5) Bragagnolo F, Strieder M, Pizani R, Mesquita L, Gonzalez-Miquel M, Rostagno M (2024). Revisiting natural deep eutectic solvents (NADES) as extraction media and ready-to-use purposes. TrAC Trends in Analytical Chemistry. 117726.
6) Jeong K.M., Ko J, Zhao J., Jin Y., Yoo D.E., Young Han S., Lee J. (2017) Multi-functioning deep eutectic solvents as extraction and storage media for bioactive natural products that are readily applicable to cosmetic products, Journal of Cleaner Production, 151, 87-95,
7) Wils L, Leman-Loubière C, Bellin N, Clément-Larosière B, Pinault M, Chevalier S, Enguehard-Gueiffier C, Bodet C, Boudesocque-Delaye L. (2021) Natural deep eutectic solvent formulations for spirulina: preparation, intensification and skin impact. Algal Res 56: 102317.
8) Hilali S., Wils L., Chevalley A., Clément-Larosière B., Boudesocque-Delaye L (2022). Glycerol-based NaDES as green solvents for ultrasound-assisted extraction of phycocyanin from Arthrospira platensis-RSM optimization and ANN modeling. Biomass Conversion and Biorefinery, 12, 157-170.