FROM MOLECULAR MODELLING TO RAMAN MICROSPECTROSCOPY: UNPRECEDENTED DEMONSTRATION OF THE HYGROSCOPIC PROPERTIES OF APIOGALACTURONANS

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INTRODUCTION

The issue of hydration is one of constant attention in cosmetics. Dehydrated skin is characterized by a lack of water, a state that can be induced by a variety of factors (environmental, emotional, medicinal, etc.). It is very frequent and can involve all skin types, whether dry, mixed or oily. Tightness, lack of suppleness, loss of volume, lines and wrinkles that appear are all features that characterize this state of skin.

To specifically meet the needs of dehydrated skin, the use of molecules with elevated hygroscopic potential is therefore necessary for effective, immediate and long-lasting hydration.

SILAB has extensive expertise in glycobiology and has been studying the natural properties of pectins for more than 25 years. Pectins are a family of macromolecules with highly varied compositions and structures. They are present in plant cell walls and in addition to their structural role, they can bind water, a capacity closely linked to the type of pectin [1, 2]. SILAB thus interested in pectins, and more precisely in apiogalacturonans (APG), a singular one that is currently only found in a few species of aquatic plants. As key structures in water regulation of aquatic plants, we hypothesized that it could have beneficial role for skin hydration. A special attention was paid to pectins found in the water lentil, *Spirodela polyrhiza*. Used for over 2,000 years in traditional Chinese medicine for its capacities to promote water metabolism, the cell wall of *Spirodela polyrhiza* contains high amount of APG [3, 4].

The aim of this study was to predict the hygroscopic potential of APG thanks to molecular modelling and evaluate their capacity to capture and to retain water into the skin for a high skin hydration.

MATERIAL & METHODS

in silico prediction of 3D structure and hygroscopic potential of APG

An APG polymer fragment was built based on structural information obtained from the literature [4, 5] (Figure 1). The α-D-galacturonic acid chain was constructed using the carbohydrate builder program glycam.org [6]. The α-D-apiose structure was obtained from an experimental X-ray crystallographic structure (PDB: 5IBQ). Since the parameters for the apiose residue are not characterized in any of the conventional force fields, the parameters were derived from *ab initio* calculations [7-11].

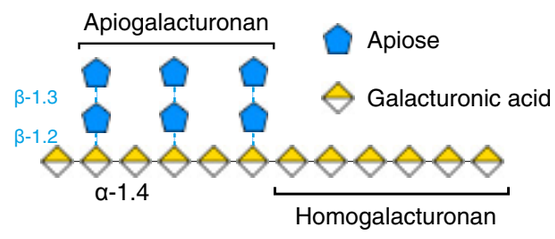

Figure 1. Schematic representation of the APG polymer fragment

The generated APG structures were solvated in a cubic box with edge distance of 8Å. Water molecules from TIP3P model were added with also 12 neutralizing Na+ counterions [12]. The solvated systems were energy-minimized, and heating equilibrated. A 50 ns simulation was performed. All the above-mentioned preparatory steps as well as simulations were performed using GROMACS 2020.4 software [13, 14]. Then, the hygroscopic potential was predicted *in silico* by analyzing the frequency of interaction of water molecules with each APG residue along the Molecular Dynamics (MD) trajectory.

Obtention of a natural active ingredient enriched in APG

Using mild acid hydrolysis coupled with optimized reaction temperature and pH, APG molecules of optimal size were obtained from *Spirodela polyrhiza*, while preserving the di-apiose ramifications. The resulting active ingredient is enriched to more than 50% in APG.

in vivo investigation of the APG hygroscopic potential

All the volunteers of this study were selected with a dehydrated skin (daily sensation of tightness and low levels of hydration (MoistureMeter D < 40 for Caucasian and Corneometer < 55 for Asian)).

The water capture in the skin was investigated directly *in vivo* by Raman microspectroscopy by using D_2O on the arm of 8 volunteers. The D_2O displays the same behavior than H_2O but with a characteristic Raman spectrum allowing it to be tracked specifically. An emulsion containing APG was applied at the surface of the skin for 3 hours and then, an occlusive patch containing D_2O was applied for 30 minutes. Acquisitions were conducted on different skin depths for 15 minutes.

in vivo demonstration of the moisturizing efficacy

in vivo measurements were conducted on Caucasian and Asian volunteers by using a Corneometer and a MoistureMeterD to investigate respectively the *Stratum corneum* (SC) and epidermis to upper dermis hydration levels.

RESULTS & DISCUSSION

1. *in silico* prediction of 3D structure and hygroscopic potential of APG

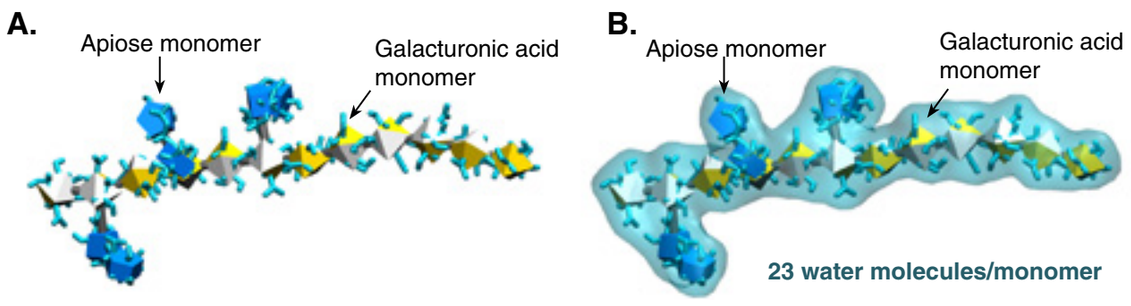

Figure 2. A. Tridimensional representation of an APG polymer fragment obtained by molecular modelling. B. Representation of the shell of water around the APG polymer fragment (derived from contacts during the MD simulation).

Molecular dynamic simulations revealed characteristic shell of water. There are 23 water molecules on average in contact with each residue of APG. From a theoretical point of view, this building fragment displays an interesting hygroscopic potential that seems twice higher than hyaluronic acid, a "gold standard" molecule for hydration [15].

2. *in vivo* investigation of the hygroscopic potential

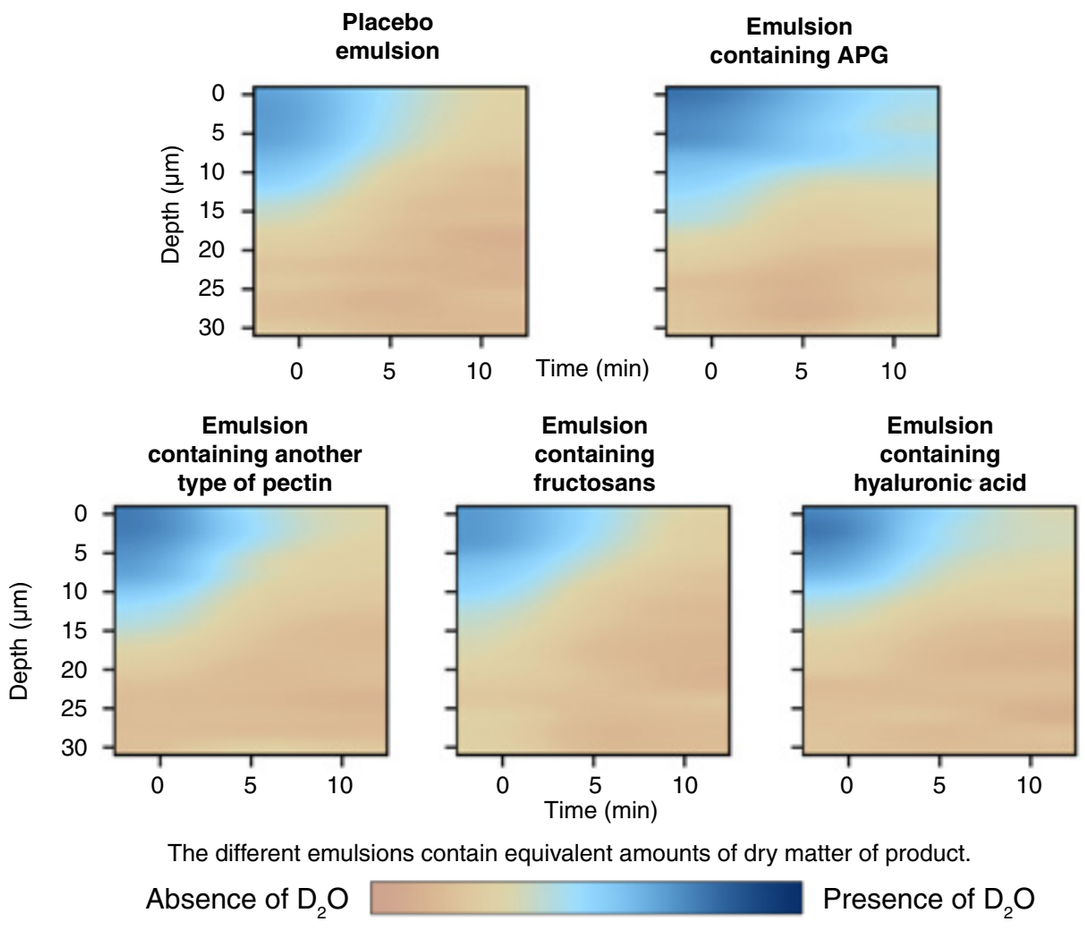

Figure 3. Effect of APG and other glycocompounds on the quantity and depth of D_2O taken up by the skin 3 hours after a single application.

APG take up and retain more water than a placebo, deeper and longer compared to other glycocompounds (homogalacturonans, fructosans and hyaluronic acid).

3. *in vivo* demonstration of the moisturizing efficacy

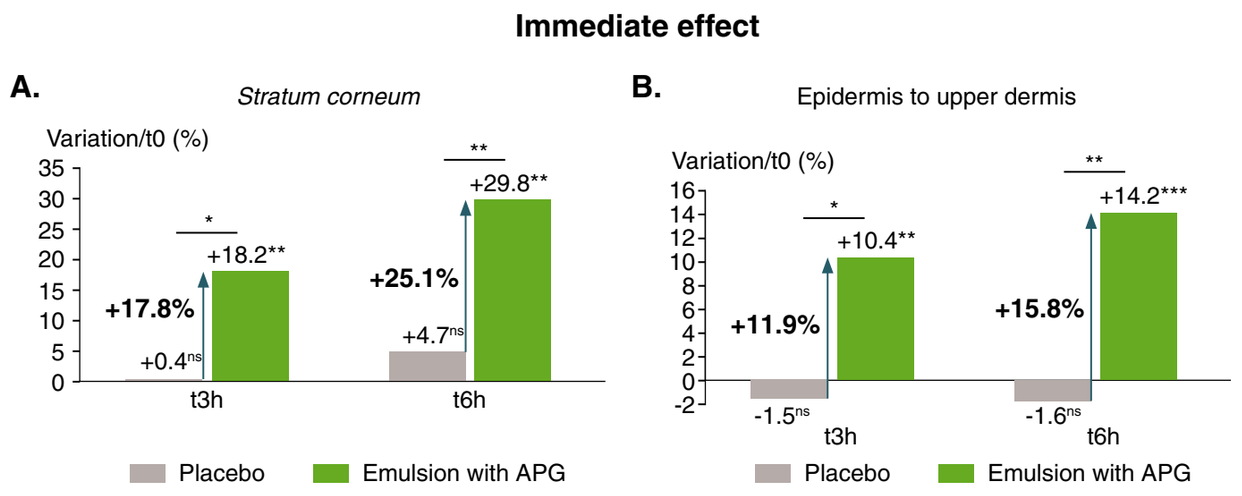

Figure 4. Effect of an emulsion containing APG on the hydration of Caucasian volunteers on the SC measured by Corneometer (A.) and from the epidermis to upper dermis by MoistureMeterD (B.).
****: P<0.001; **: P<0.01; *: P<0.05; ns: non-significant*

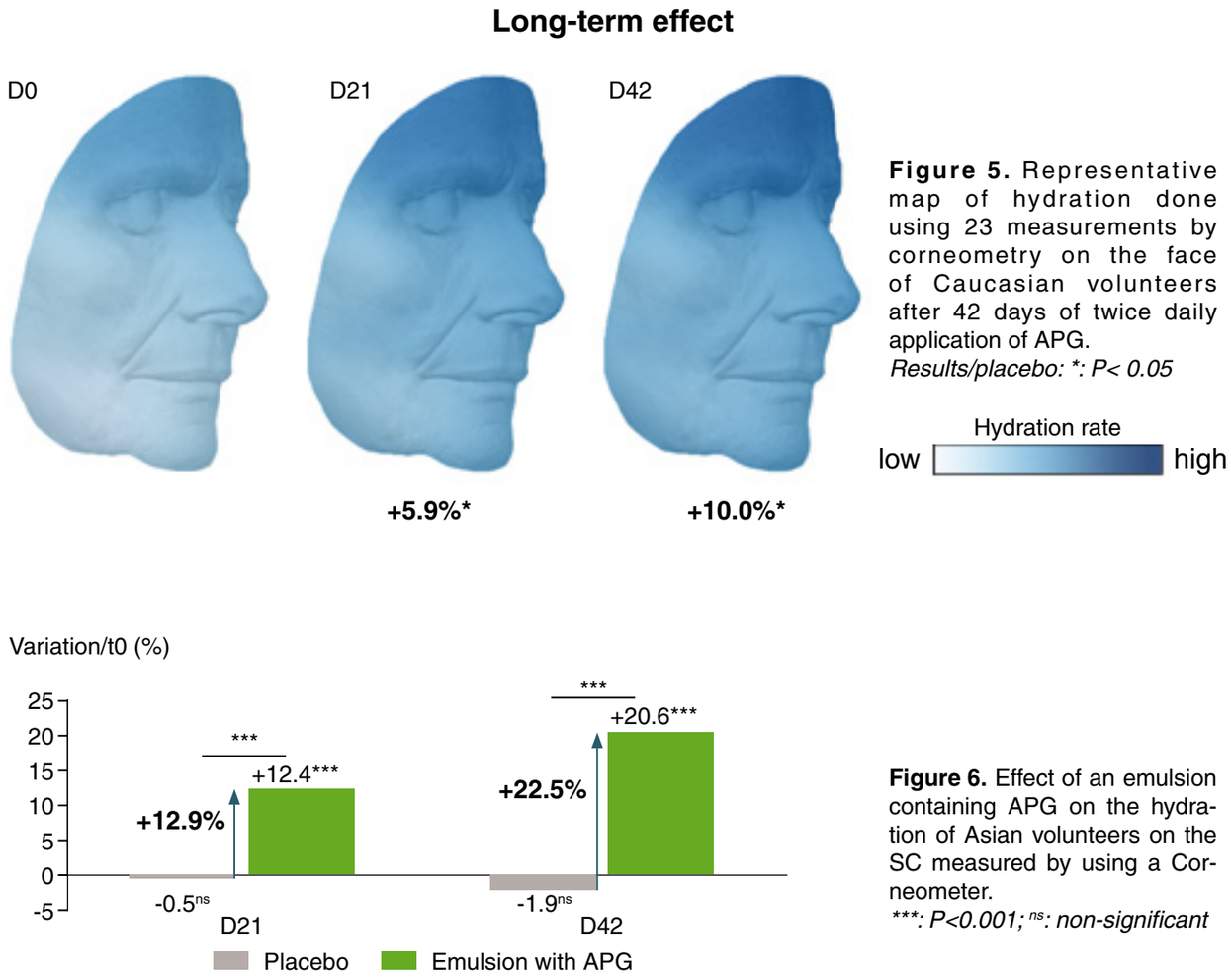

Figure 5. Representative map of hydration done using 23 measurements by corneometry on the face of Caucasian volunteers after 42 days of twice daily application of APG.
*Results/placebo: *: P< 0.05*

Figure 6. Effect of an emulsion containing APG on the hydration of Asian volunteers on the SC measured by using a Corneometer.
****: P<0.001; ns: non-significant*

An emulsion containing APG immediately improves hydration in Caucasian volunteers from the surface to the upper dermis. This effect continues after 21 and 42 days of twice daily application on Caucasian and Asian volunteers.

CONCLUSION

In an unprecedented manner, by combining the power of molecular modelling numerical tools and Raman microspectroscopy, SILAB has predicted and validated the hygroscopic potential of APG. The resulting natural active ingredient, enriched in APG, provides flash and long-lasting hydration, superficially and in depth. Complexion radiance is revived, lines smoothed, and facial volumes redefined.

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