Quinoa starch particles as a new technology to stabilize and encapsulate lipophilic components using a Pickering route

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Abstract

Background: A Pickering emulsion is a system stabilized by particles, as opposed to a surfactant. Lipophilic modified quinoa starch is a novel technology with strong emulsifying and stabilizing properties owing to its specific particle size and wettability properties. These key features were studied in a cosmetic formulation environment through microstructure investigations of simple and complex emulsions.

Methods: Different batches of native and modified quinoa starch powders were characterized by HPLC, electronic microscopy, and DSC, and their performance was evaluated. The spatial arrangement of cosmetic emulsions stabilized with modified quinoa starch was investigated by confocal microscopy and rheological analysis with and without a gelling agent. More complex emulsion microstructures such multiple emulsions, were also explored.

Results: The OSA modification enhances the performance of the particle, until a substitution degree optimum point. The OSA modified grains maintain a microscopic aspect despite a shift in gelatinization temperature. OSA quinoa starch fully and homogenously covers the oil droplets and generates a network beneficial to emulsion stability, and compatible with other traditional ingredients. A water-in-oil-in-water emulsion was successfully generated, the observation of which showed the existence of inner droplets and highlighted the coverage of outer droplet by OSA quinoa starch.

Conclusion: OSA Quinoa starch fits perfectly with expectations from a cosmetic emulsifier, stabilizing emulsions over the long term. The mechanisms of stabilization involve strong interface anchoring and droplet network organization. The introduction of other ingredients helps optimize the emulsifier’s performance and enables the creation of challenging formulas like multiple emulsions.

Introduction.

In the cosmetic field, new pathways in formulation lead to breakthrough innovations in texture, tolerance, and sustainability. Traditionally, emulsions are stabilized by surfactants or polymers. The use of particles to stabilize emulsions, generating Pickering emulsions, has been extensively studied over the years but this approach is not yet widely implemented by cosmetic formulators for various reasons related to material or process features, or unwanted side effects.

Quinoa starch is a cutting-edge material acting as a new ingredient platform delivering several functionalities with a surfactants-free approach. Compared to most starches, it has a very small granule size of 1–2 µm, and through chemical or physical modification, this starch can be made more hydrophobic, increasing its affinity for the oil/water interface than its native counterpart. In general, the size of particles Pickering emulsions is in the sub-micron to micron range. However, micron-sized particles have additional benefit of creating systems of superior stability due to a higher energy of detachment. The emulsion droplet size is also directly related to the particle size, with droplet size increasing with increased particle size. However, there is an upper limit, and particles of several microns and above in size, like other botanical starches, will be less efficient as emulsion stabilizers. Interestingly, the stability of the oil droplets formed is extremely high even for large droplets (above 100 µm) [1-2]. In addition, thanks to the starch's gelatinization properties, particles swell and fuse at the interface to produce a barrier. Thereby, quinoa starch particles are not only suitable to form Pickering emulsions but also to create core-shell structures for micro-encapsulation purposes, while inducing a very soft and powdery skin feel.

Although academic papers have been published on quinoa starch stabilized emulsions, studying the influence of hydrophobic modification or particle concentration as a proof of concept [1-3], it appears essential to complete this research with data obtained in a real-life cosmetic formulation environment. Interactions and compatibilities with other ingredients can affect the mechanisms of stabilization and the properties of the Pickering agent in negative or positive ways; understanding the microstructure of modified quinoa starch emulsions is key to the ability to correctly formulate and process it, and therefore optimize its use.

The aim of this work was to study the Pickering emulsifier behavior of an OSA-modified quinoa starch, by investigating the relationship between physical-chemical features and performance in emulsions produced in realistic cosmetic formulation conditions. Different batches of native and modified quinoa starch powders were characterized by chemical analysis, electronic microscopy, and DSC. Then, cosmetic emulsions stabilized with modified quinoa starch were formulated with or without cosmetic traditional ingredients (e.g. xanthan gum), and the resulting microstructure were examined by confocal microscopy and rheological analysis. Finally, we investigated the unique microstructure of a double emulsion produced with this novel emulsifier.

Materials and Methods.

OSA modified quinoa starch production

Spray-dried native (unmodified) quinoa starch powders extracted from quinoa grains were obtained after elimination of fibers, lipids, and proteins.
To increase the quinoa starch's lipophilicity, octenyl succinic anhydride (OSA) was grafted to some hydroxyl groups of the starch backbone, yielding OSA-modified quinoa starch powders with different degrees of substitution (**Figure 1**).

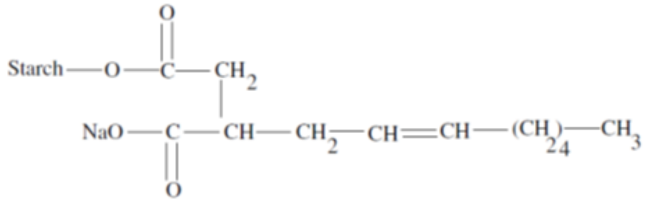

Figure 1: OSA esterification with starch backbone

OSA-modified quinoa starch characterization

Scanning electronic microcopy (SEM)

SEM was used to explore the aspect and the structure of quinoa starch powders and individual grains. SEM analysis was carried out using a field effect "gun" microscope (Zeiss SUPRA55-VP SEM). High-resolution observations were obtained using an Everhart–Thornley SE detector. The images were collected using a Secondary Electron Detector. Powders were directly attached to stud supports by means of a carbon tape and positioned directly under the electron beam.

Degree of Substitution (DS)

The DS is the average number of hydroxyl groups substituted with OSA, per glucose unit. This was evaluated by HPLC dosage:

$\% \, OSA_{grafted} = \% \, OSA_{total} - \% OSA_{free}$.

Gelatinization temperature by Differential Scanning Calorimetry (DSC)

When heated in water, starch granules become hydrated, swell, and are transformed into a paste. The granule structure collapses due to melting of crystallites, unwinding of double helices, and breaking of hydrogen bonds. These changes are collectively referred to as starch gelatinization and are accompanied by the loss of the characteristic birefringence of intact granules. The temperature of starch gelatinization is very characteristic of the state of the grain and its origin. Its endothermic transition point (T°C gelatinization) was determined by DSC measurement. Quinoa starch (3–5 mg, dry weight basis) was weighed into a high-pressure crucible and moistened with water (in amounts of about twice the starch mass). Thermal properties were analyzed in triplicate. The analysis temperature started at 25˚C for 5 min, rising up to 120˚C at a rate of 10˚C/min.

Emulsion preparation

- Simple Oil-in-Water (OW) emulsion

5% OSA quinoa starch were dispersed in purified water together with preservatives, using a rotor-stator device over 10 min at 2500 rpm for complete particle individualization. Then, 20% medium chain triglyceride oil (MCT) was introduced and emulsified using a rotor-stator device at 3000 rpm over 10 min. The entire process was performed at room temperature.

In some cases, 0.3% xanthan gum was added using a defloculator after OSA quinoa starch dispersion.

- Double Water-in-Oil-in-Water (WOW) Emulsion

Firstly, a primary Water-in-Oil (WO) emulsion was generated by emulsifying 40% water pre-structured with 0.1% xanthan gum into MCT oil supplemented with 3% polyglyceryl-3 polyricinoleate. Next, the secondary OW emulsion was generated using 40% the primary WO emulsion and 5% OSA quinoa starch, according to the same procedure used for the simple emulsion. The outer water phase contains 0.26% xanthan gum and 1% preservatives.

Emulsion characterization

Microscopic aspect and droplet size analysis

Emulsion droplet-size distributions were measured by static laser diffraction (Mastersizer 3000, Malvern Instruments).This was completed by optical microscopy (Axioscope 5, Zeiss) observation.

Microstructure investigation with confocal microcopy

A Leica TCS SP8 confocal laser scanning microscope (CLSM) was operated in fluorescence mode. A 0.01% Nile Blue staining solution in water was used to stain the starch particles, and A 0.01% Nile Red staining solution in MCT oil was used to stain the oil phase. The oil and water phase of the emulsions were thoroughly mixed with 0.4% of Nile Red and/or Nile Blue solutions. The stained emulsions samples were scanned using 40_ (NA 1.25) oil immersion objective lens. Fluorescence from the sample was excited at 552 nm for the Nile red phase and 638 nm for the Nile blue one, with a respective detection range of 562 – 624 nm and 652 – 704 nm.

Emulsion network assessment by rheological measurement

Emulsion networks were assessed through an oscillatory test for viscoelasticity evaluation, carried out with a controlled shear deformation between 0.1% and 100% at a frequency of 1Hz. Elastic modulus G’, loss modulus G’’ and loss factor (tan δ) were measured at 25 ± 0.1˚C using a cone–plate geometry.

Results.

Native and OSA-modified quinoa starch powder characterization

SEM observation

Native and OSA-modified quinoa starch powders were examined under a scanning electron microscope (**Figure 2**).

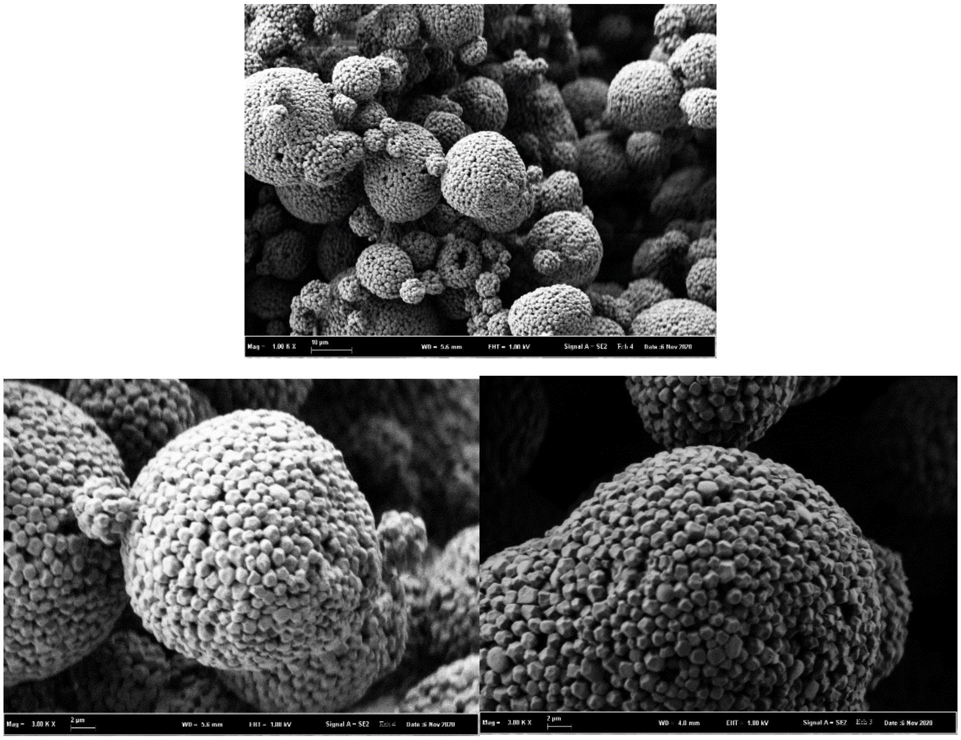

Figure 2: SEM pictures acquired at a magnification of x1000 and x3000. Top: global aspect of the powders at x1000. Bottom left: native quinoa starch powder; bottom right: OSA-modified quinoa starch powder.

SEM images showed spherical aggregates of quinoa starch grains resulting from the spray drying method used at the end of the production process. The median diameter of the spheres was about 30-40 µm, while individual quinoa grains exhibited a homogenous size profile, with diameters of 1-2 µm. The images highlighted that OSA modification did not affect the shape or size of the grains, maintaining their particle properties, crucial for Pickering emulsifying performance.

OSA grafting impact

Quinoa OSA starches exhibited significantly lower T°C gelatinization than their native counterparts (0 % grafted OSA), a feature emphasized as the DS increases (**Figure 3**). This is attributed to a disruption of the crystalline structure of amylopectin upon modification with OSA.

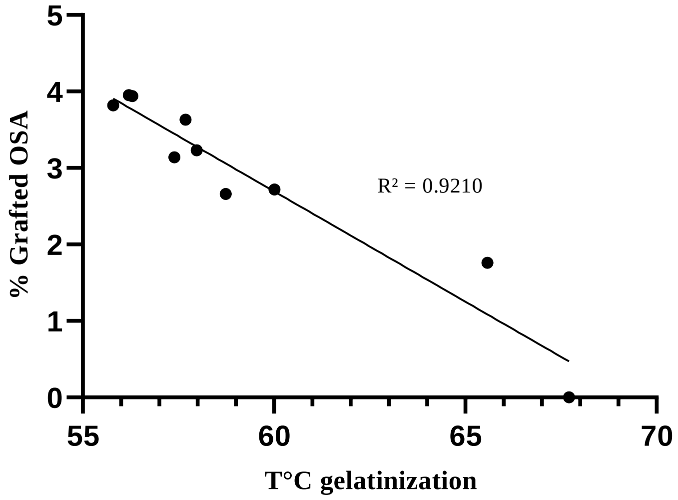

Figure 3: Influence of OSA grafting on gelatinization properties. Linear correlation between the % of OSA grafted and the T°C of gelatinization.

This shows that although the quinoa grains maintain their native shape and particle behavior (as shown in SEM pictures), the microstructure of the grains was in fact affected by OSA grafting.

Emulsion characterization

Influence of OSA modification in simple emulsions with xanthan gum

To produce effective stabilization of OW emulsions, it has been found that the surface character of hydrophilic quinoa starch particles needs to be made somewhat hydrophobic by suitable OSA modification. Under 3% of OSA grafted, emulsion droplets were coarse and non-cohesive, leading to instantaneous phase separation. Due to an inadequate wettability, some free quinoa starch grains were not anchored at the interface, and decanted to the bottom of the centrifugation flask. From 3% of OSA substitution, homogenous small droplets appeared, providing long-term stability to the emulsions. It is to be noted that further increasing the OSA modification seems not to provide any further benefit, as the droplet size reaches a plateau (**Figure 4**).

% OSA Grafted	Droplet size (Mode in µm)	Centrifugation (1600G during 20 min)	Optical microscopy (magnification x10)
0	No emulsion	-	-
1,.76	No emulsion	-	-
2.72	42.0	Free starch at the bottom / oily aspect on the top	
3.23	15.0	Homogeneous	
3.82	19.5	Homogeneous	

Figure 4: Impact of quinoa starch OSA modification in simple emulsions with gelling agent. The scale bar on optical microscopy images corresponds to 100 µm.

Emulsion microstructure investigation

The following results were obtained with a quinoa starch modified with at least 3% of OSA.

Basic features of simple emulsions stabilized with OSA quinoa starch

With higher optical microcopy magnification**,** micron-size OSA quinoa grains can be clearly observed at the oil droplet interface, showing good coverage. Additionally, the droplets can be seen to exhibit a flocculation state (**Figure 5**).

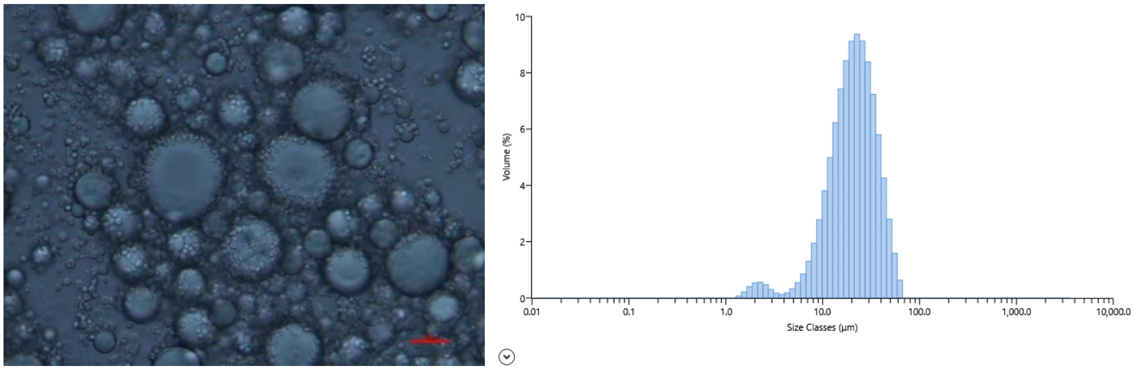

Figure 5: Droplet size and aspect of simple emulsions with xanthan gum Left: Aspect of a simple emulsion stabilized with OSA quinoa starch (optical microscopy at x100 magnification). Right: laser diffraction sizing analysis of the emulsion.

Typical droplet size distribution showed a main mode value of around 20 µm and a small peak representing a very small amount of residual free starch. As compared to a surfactant-based emulsion, the droplet size distributions produced using 5% of OSA quinoa starch for 20% of oil (approx. 238 mg/mL) were quite large (from approximately 3 to 60 µm in diameter). Consequently, these emulsions creamed readily if the formula did not contain a gelling agent, but otherwise were extremely stable to coalescence, with no significant change in droplet size distributions over several months. This can be explained by the high interface anchoring energy of micron-size Pickering particles, and probably also by the flocculation state of the emulsion.

Simple emulsion microstructures, with and without gelling agent

To further understand the emulsions' arrangement, confocal images were acquired on emulsions stabilized with 5% OSA quinoa starch, formulated with and without xanthan gum (**Figure 6**).

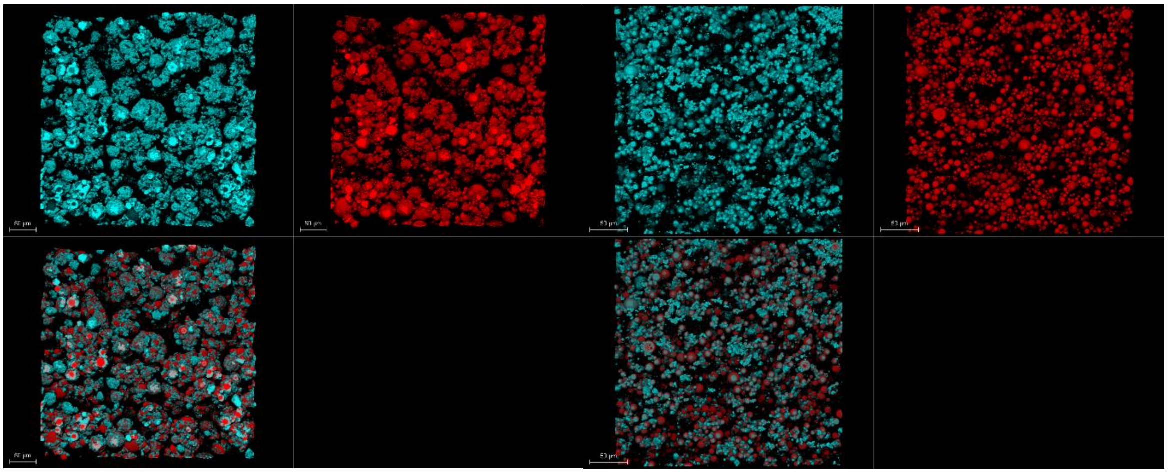

Figure 6: Two-color CLSM pictures of OSA quinoa starch-stabilized emulsions with (Right) and without (Left) xanthan gum, in volume style pictures. Top: the starch appears in blue and the oily phase in red; Bottom: combined image. The scale bar corresponds to 50 µm.

The volume CLSM images obtained suggested that the droplets were covered at all depths by starch particles. The starch particles were also often clearly aggregated, but most of these aggregates were associated with droplet surfaces in some way. This is particularly the case in the emulsion without gelling agent, where this organization provided an expanded network of clusters. This result could be surprising but according to *Dickinson* [4], the idealized model involving the stabilization of a Pickering emulsion by a monolayer of uniform spherical particles is rarely realized in practice. Due to the tendency towards flocculation, three-dimensional networks of closely packed emulsion droplets seem to have formed after emulsification.

In the case of the emulsion formulated with xanthan gum, the aggregates still appeared but were smaller and much less connected, highlighting the impact of the addition of a thickener into the formulation. As the droplets also exhibited a smaller size, xanthan gum might encourage more efficient oil fragmentation, creating a higher surface area to be covered by the starch particles. Consequently, a larger quantity of starch was involved at the oil/water interface, and in a lesser extent into the clusters. This time, the network was supported both by xanthan gum's suspending properties and by the Pickering emulsion arrangement.

The network of the emulsion without gelling agent, observed in confocal microcopy above, was evaluated through measurement of viscoelasticity as a function of shear strain applied (**Figure 7**).

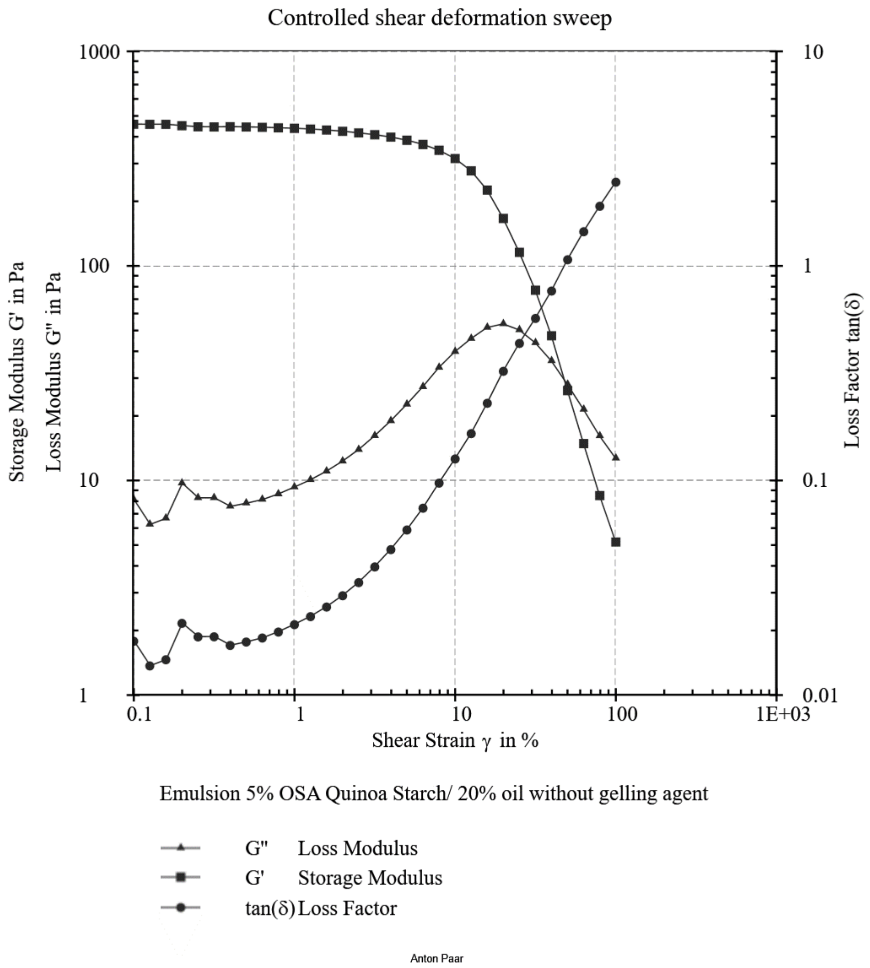

Figure 7: Shear strain amplitude sweep test of a simple emulsion without xanthan gum.

The linear visco-elasticity region appeared quite short, due to the lack of stability of G'' within the low shear deformation domain, probably explained by its low value (<10 Pa). Indeed, before the yield point (G' and G" crossover), the storage modulus dominated and was dramatically higher than the loss modulus, as highlighted by the very low value of the loss factor. This confirmed that the emulsion microstructure formed a strong three-dimensional network thanks to the connection of starch particles and oil droplet in clusters, as visualized in optical and confocal microcopy. *Arditty et al* [5] explained that the elastic storage modulus G' was also influenced by the interfacial elasticity resulting from the strong adhesion between solid particles adsorbed at the oil–water interface compared to surfactant-based emulsions.

These results were consistent with previous reports [6], which had however been generated on high droplet volume fraction emulsions (>50%), which is known to strongly contribute to the storage modulus. This time, the results were generated in real-life cosmetic formulation conditions: standard oil volume fraction, standard emulsification tool, optimized starch/oil ratio.

Moreover, the rheological analysis had a pronounced G'' maximum during the yield point, which it would be interesting to understand. Following some rheological models, the rise of G" can be related to microfractures starting to develop in clusters. Deformation energy is lost because the freely movable bridge fragments around these fractures start to show internal viscous friction. When the strain amplitude continues to increase, a macrofracture of the global emulsion network runs through the entire sample, inducing the flow of the structure [7].

Multiple WOW emulsion microstructure

Thanks to the strong emulsifying and stabilization properties of OSA quinoa starch, a multiple WOW emulsion was successfully produced, and observed in confocal microscopy (**Figure 8**).

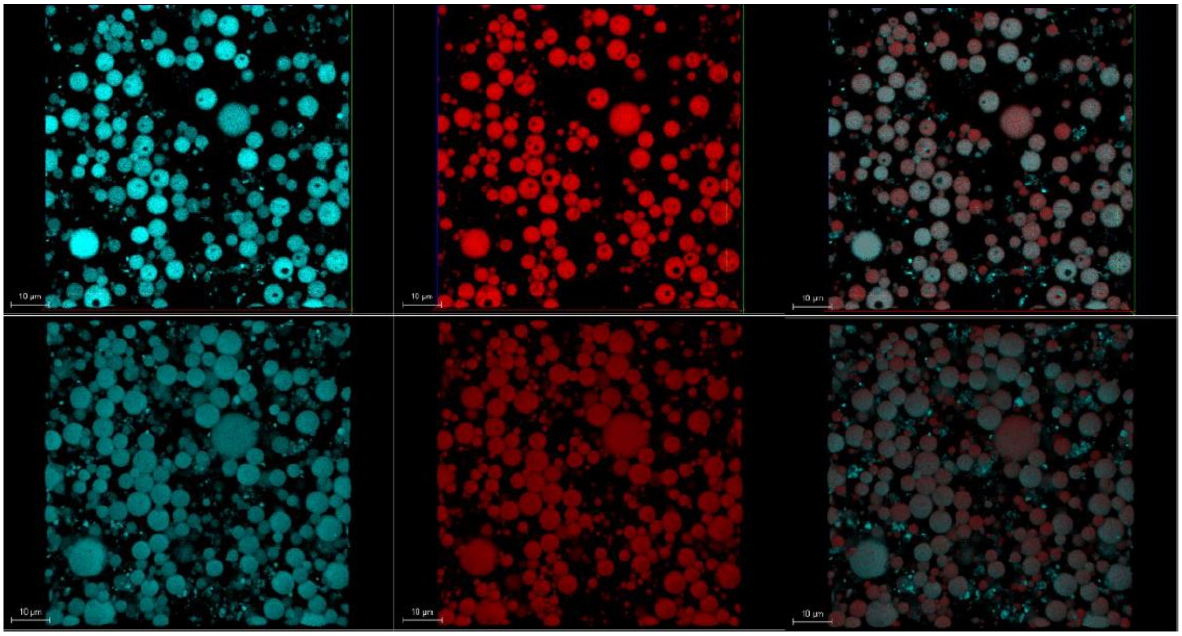

Figure 8: Two-color CLSM pictures of OSA quinoa starch-stabilized WOW emulsion Top: Vertical section; Bottom: volume representation. The starch appears in blue and the oily phase in red; the resulting coupled images are displayed on the right. The scale bar corresponds to 10 µm.

The oil droplets of the secondary OW emulsion are fully and homogenously covered by OSA quinoa starch granules. The water droplets of the primary WO emulsion are clearly observed as black holes in the red-stained droplets of the secondary OW emulsion in vertical-section images. This multiple emulsion didn't exhibit flocculation behavior or any starch/droplets clusters. As above, this could be explained by the presence of xanthan gum in the outer water phase. Furthermore, a larger amount of OSA quinoa starch grains were involved at the interface, since the quantity of WO emulsion emulsified here oil 40% (vs. 20%).

Discussion.

In the first part of the study, we investigated the impact of OSA modifications quinoa starch grains. Our results indicated that OSA grafting did not affect the shape and aspect of quinoa starch grains, despite some disruption in their crystalline structure. Indeed, the starch’s degree of substitution measured by HPLC was strongly correlated to better emulsifying and stabilization performance, and easily recognized as a shift in gelatinization temperature. However, we also demonstrated that there is a limit in the DS impact on particle size, partially explained by the existence of an optimum in particle wettability.

Once formulated into a simple cosmetic emulsion with a gelling agent, the droplet size generated by emulsification with quinoa starch is close to optimum. In addition to the DS, all other parameters affecting the droplet size are optimized. Indeed, the presence of some residual free starch showed that there is no advantage in increasing the OSA quinoa starch content of the formula. Changing the process change was not considered, as this process accurately mimics the maximum mixing shear conditions available in a standard cosmetic context. Previous work indicated that 15-20 µm is a particle size usually obtained with a much higher starch/oil ratio [1][2][6][8]. Moreover, we have seen that the addition of gelling agent affected the arrangement of the emulsion by reducing droplet size, again optimizing the starch/oil ratio needed to generate the emulsion.

Looking deeper into the microstructure of OSA quinoa starch-stabilized emulsions, we highlighted the flocculation of the droplets through anchored and free starch particles, providing strong elastic properties to the emulsion. Fixing the droplets in place at the bulk scale, this network is responsible for the extreme stability of the creamed emulsions made without a gelling agent, and continues to play a stabilization role in more complex formulations. It also provides strong barrier properties against coalescence. The idealized model involving a monolayer of starch surrounding droplets is not always achieved; according to Tambe *et al.*, [9], aggregated particles adsorb more strongly at the interface, thus promoting stability. The colloidal particles would need to be in an incipient state of flocculation to provide emulsion stabilizing power.

Depending on the composition of the emulsion, this flocculated state is more or less accentuated and the addition of a gelling agent like xanthan gum reduces the bridging between the droplets while decreasing the size of the emulsion droplets.

The stability of the WOW emulsion over several months met cosmetic industry expectations, highlighting this OSA quinoa starch as a unique ingredient to easily produce this type of formulations. Widely known as difficult to stabilize with surfactants, the production of multiple emulsions in cosmetic area is unlocked here through the combination of amphiphilic molecules and particles, stabilizing respectively OW and WO emulsions through two different stabilization mechanisms.

Conclusion.

In addition to better understanding the key factors affecting the stability of Pickering emulsions, we showed that this OSA quinoa starch is a perfect fit for the expectations of the cosmetic industry. In contrast with other Pickering particles, a reasonable OSA quinoa starch content allows the emulsification and long-term stabilization of standard oil volumes. Easy to handle and adaptable to other cosmetics ingredients, OSA quinoa starch fully and homogenously covers the oil droplets and produces an emulsion network beneficial to emulsion stability. Additionally, this technology shows the ability to create a sealed barrier which will allow the encapsulation of oily components, opening a wide range of possibilities for additional applications.

Conflict of Interest Statement. NONE.

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