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Manuscript Draft

Manuscript Number: CARBPOL-D-17-01526R2

Title: Pickering emulsifiers based on hydrophobically modified small granular starches - Part I: Manufacturing and physico-chemical characterization

Article Type: Research Paper

Keywords: Rice; Quinoa; Amaranth; Starch granules; OSA; Pickering emulsions.

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Abstract: Small granular starches from rice, quinoa and amaranth were hydrophobized by esterification with octenyl succinic anhydride (OSA) in an aqueous alkaline slurry to obtain series of modified starches at defined intervals (i.e. 0.6, 1.2, 1.8, 2.4, 3.0%). The physical and the physico-chemical properties of the starch particles were characterized by proximate analysis including protein level, amylose level and dry matter. The shape and size of the starch granules were characterized by scanning electron microscopy and light scattering. The gelatinization properties were characterized by differential scanning calorimetry. The degree of modification was determined by titration with NaOH. With regard to the emulsion formulation and in order to assess the emulsifying capacity of the small granular starches, the effect of starch type, degree of modification and starch concentration on the resulting emulsion droplet size were evaluated by light scattering and optical microscopy. Emulsifying properties were found to depend on the degree of substitution, size of the granules and the starch to oil ratio of the formulation. Quinoa starch granules, in general, had the best emulsifying capacity followed by amaranth and rice. However, in higher starch concentrations (>400 mg/mL oil) and adequate levels of OSA (3.0%) amaranth performed best, having the smallest size of starches studied.

***Highlights (for review)**

- Small granular starches from rice, quinoa and amaranth were hydrophobized by OSA.
- Starch granules from small granule botanical sources have emulsification capacity.
- Quinoa and rice starch granules have emulsifying capacity in native and modified form.
- Emulsifying properties depend on modification level, size and concentration of starch.
- Quinoa starch granules had the best emulsifying capacity followed by amaranth and rice.

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We would like to thank the reviewers for their useful comments and suggestions on our manuscript titled: "Pickering emulsifiers based on hydrophobically modified small granular starches - Part I: Manufacturing and physico-chemical characterization". After careful revision and taking into consideration those comments, changes have been made in red in MS word following the editor guidelines and are properly discussed in the following paragraphs.

Reviewer No. 1

This manuscript deals with the manufacturing and physicochemical characterization of OSA modified starches with small granules. The range of techniques employed is appropriate. However, there are some questions that should be addressed in the list below.

1. In the recent years, there are many papers reported the preparation and physicochemical properties of OSA modified starches. And there are also many reports about the Pickering emulsions made from quinoa, rice and corn starch.

Answer:

Yes, we have clarified this in the manuscript as stated in lines 127-143.

The new text is: "Several reports have been published on emulsifying properties of different types of OSA modified starch granules (Timgren et al., 2013, Simsek et al., 2015, Yusoff and Murray, 2011). In addition, there has been a considerable amount of work on development, characterization and physical and physiological stability of emulsions stabilized by OSA modified quinoa, maize, tapioca, and rice starch granules (Timgren et al., 2011, Rayner et al., 2012a, Marku et al., 2012, Matos et al., 2013, Marefati et al., 2013, Marefati et al., 2017, Song et al., 2014, Yusoff and Murray, 2011, Simsek et al., 2015, Timgren et al., 2013).

Though, a comparison of small starch granules with varying OSA level in incremental steps has not been investigated. In addition, although Bhosale and Singhal (2006) have carried out some research on manufacturing and characterization of OSA modified amaranth where they investigated the emulsification capacity of those starches in molecular form, to the best of authors' knowledge, OSA modified amaranth starch granules have not previously been utilized to stabilize Pickering type emulsions. In addition, there are several studies on physicochemical characterization of rice starch, however, application of different conditions in them makes a direct comparison difficult. Therefore, we investigated the three different starches in the same conditions to be able to compare performance as Pickering emulsifiers as well as document the properties of the granules used."

2. Line 145-151: The authors isolated rice starch by NaOH-solution. "…the starch suspension was neutralized and spray dried using a spray dryer…at an inlet and outlet temperature of 180°C and 80°C, respectively." So the starch would be gelatinized during spray drying.

Answer:

During spray drying at 180/80°C no starch gelatinization can occur. We clarified this point as stated in lines 154-169.

The new text is: "At this point it should be noted that spray drying is widely used in the food industry as a gentle drying process which is even used for heat sensitive enzymes (You et al., 2017). In this work, despite the high inlet and outlet temperatures of the air in the spray dyer, moist droplets never reach the air temperature and experience a much lower temperature than the air during drying that is the wet bulb temperature due to evaporative cooling during the constant rate period, Singh and Heldman (2001) and thus gelatinization is avoided. Only at the end of the drying time (3 to 5 seconds) does the temperature begin to rise in the now almost dry particles. The temperature within the water droplets and thus the starch particles during the drying process is always below the outlet temperature of 80°C and after separation in the cyclone the temperature decreases rapidly to about 50°C (below the peak temperature of gelatinization measured in excess water). Furthermore the peak temperature of gelatinization depends on the mass fraction of water in relation to starch (BeMiller and Whistler, 2009). For example, the dried starch with a mass fraction of water of 0.12 gelatinizes at temperatures above 150°C. Since the mass fraction of water during spray drying is quickly reduced from 0.75 to 0.12 no gelatinization occurs. The absence of gelatinization was verified by the SEM photographs of the 3 starches and DSC thermographs described in section 2.4.2 and 2.4.3 below."

3. Line 156-157: "…the slurry went through enzymatic hydrolysis to assist protein separation …" What kind of enzyme did the authors used in the study?

Answer:

We clarified this as stated in line 176.

The text now reads: "Thereafter, for improvement of the protein separation from the starch suspension, the slurry went through enzymatic hydrolysis using a commercially available enzyme (Alcalase 2.4 L FG, Novozymes A/S, Bagsvaerd, Denmark) and then mixed with a screw loop mixer (type 50, DMT, Germany) and a high-pressure homogenizer (type 317HD4-3TBS, APV Gaulin, Germany)."

4. Line 161-162: "… the starch was dried using a spray dryer … at an input and output temperature of 180 °C to 80 °C respectively." The Peak temp of native quinoa and amaranth starches were only 69.8 and 70.3 °C, respectively. The starches would be gelatinized during spray drying.

Answer:

See answer to comment 2.

5. Line 175-176: "… then in a convection dryer at 30 °C for 4 h," What kind of convection dryer did the authors used? The temperature is only 30 °C, how to dry the samples?

Answer:

The type of the convention oven is stated in the text and we clarified the drying procedure in lines 195-199.

The text now reads:

"The third sediment was first dried at room temperature over the night and then in a laboratory convection dryer, WTB binder (Type MB6, Binder GmbH, Germany) at 30 °C for 4 h. At this conditions the acetone was quantitatively evaporated and the starch was dried below its equilibrium water content. Finally, in order for the starches to reach their equilibrium moisture content, samples conditioned at room temperature for 2 days".

6. Line 220: There are some writing errors, such as "(Phosphate buffer (95%, 0.5 mM, pH 7, 0.2 M NaCl)".

Answer:

We do apologize for the spelling error, we have carefully read and corrected the spelling.

7. Line 307-308: "The particle size distribution graph for the native rice granules showed a bimodal size distribution with small peak around 1 µm and a large peak around 7.6 µm. "Why did the rice granules show a bimodal size distribution? Did the authors compare this result with the other reports?

Answer:

We have no clarified this in the text as follows and the new text can be found in lines 331-336.

The new text is: "Comparing these results with previous results in the literature, unimodal and bimodal size distribution could be found (Wani et al., 2012, Zuo et al., 2009). The amount of the small granules could depend on botanical source, as well as the isolation process as in some industrial processes the fine granules are lost in the separation step. In addition, depending on the measuring technique, the small peak may not be resolved and instead a wider peak is seen."

8. Line 323-324: "This can be due to higher protein levels in quinoa and rice which provides additional natural hydrophobic groups." The proteins have been removed in a low level as showed in Table 1. So I think this explanation is not right.

Answer:

We have provided more facts, calculations, evidence and reference to supporting literature to clarify this. The new text can be found in lines 365-382.

The new text reads:

"The amount of protein present as a minor component in starch granules is dependent on the botanical origin and purification method. According to Baldwin (2001), a substantial part of starch granule associated proteins are located at the surface of the starch granules. Due to the considerable surface area/g of the starches used in this study, which is 1.22, 1.98 and 2.86 m2/g for native rice, quinoa and amaranth respectively, the presence of these proteins may significantly influence the overall surface properties of starch granules. From the data presented in Table 1, the amount of protein was found to be higher in quinoa than rice (by a factor of 2) and amaranth (by a factor of approximately 6 and 17 for native and modified starches respectively). If we assume that all the proteins are at the surface of the starch granules, the amount of protein/unit area for native rice, quinoa and amaranth will be 2.7×10-3, 3.5×10-3, 0.4×10-3 g/m2 and for the modified rice, quinoa and amaranth we will have

2.2×10-3, 2.7×10-3, 0.1×10-3 g/m2. Considering the intermediate size of quinoa starch granules with more similarity in size and shape to amaranth, the better hydrophobicity and the higher emulsification capacity of quinoa starch could be explained by this protein level difference. This is further manifested in the case of the native starches where there was no chemical hydrophobization and the trace amount of protein present in amaranth did not create a stable emulsion. It is also due to this higher hydrophobicity that lower amounts of free (non-adsorbed) starches can be seen in particle size measurements for quinoa compared to other starches (see Fig. 4). "

9. Table 326-328: "This higher emulsifying capacity in native quinoa (as well as modified quinoa starches) could be attributed to higher protein level that may results higher hydrophobicity and better interfacial affinity which in turn can result in lower amount of free starch as well." This explanation is also not right.

Answer:

Please see the answer to comment 8 where we explain this better.

10. Line 344: Figure 4 is not clear.

Answer:

We have increased the resolution of the figure and used a different format to increase the resolution. Note figure 4 is now figure 3. We hope that the image quality is now sufficient.

Reviewer No. 2

In this paper, small granular starches from rice, quinoa and amaranth were characterized by proximate analysis, SEM, DSC and laser diffraction particle size analyzer. And next esterification with octenyl succinic anhydride (OSA) was carried out to increase the hydrophobic properties of these three starches. Finally, the effect of starch type, degree of modification and starch concentration on the resulting emulsion droplet size were evaluated by light scattering and optical microscopy. According to the experiments and results, the paper is not of sufficient novelty and quality to be published in Carbohydrate Polymers. And some suggestions were listed below:

Introduction

1. Line 102-129, it is not necessary to present the characterizations of these three different starches individually.

Answer:

Yes, is not absolutely necessary to present these as separates sections, and since many who are in the field of Pickering emulsions are not starch experts we have decided to include this in the introduction.

Materials & Methods

2. Line 2.6, the appearance of Pickering emulsions should be added in this paper.

Answer:

We have prepared a new set of samples and photographed them and put the photographs in Figure. 3, to avoid exceeding the total of 8 figure and tables.

We have provided some explanations for the observations in lines 342-382.

The new text is as follows:

The appearance of the emulsions at all OSA modification levels and the morphology of the emulsions' droplets produced with 200 mg/mL oil of different starches at 3.0% is presented in Fig. 3. The starch particles can be seen on the surface of the emulsions' droplet which is the characteristic trait of Pickering emulsion. As can be seen in Fig. 3, these emulsions were not space filling and the droplets formed a sediment in the bottom of the test tubes due low oil fraction and high density of starch compared to the continuous phase respectively which agrees with the previous results for quinoa (Rayner et al., 2012b). The cumulative and volume frequency particle size distribution of emulsions produced from native and modified starches at all OSA modification levels (i.e. 0.6, 1.2, 1.8, 2.4, 3.0%) at the same oil/starch ratios (200 mg/mL oil) are presented in Fig. 4 and Table 2. There seems to be a negative correlation between the level of modification and droplet size and the amount of free starch at the same oil/starch ratios meaning that the greater the degree of OSA the smaller the resulting emulsions droplet were at the same starch to oil ratio and starch type as can be seen in the right column of Fig. 4. Moreover, the degree of modification appears to be more influential on emulsifying capacity of amaranth. In addition, the thickness of the emulsions layer increased as the modification level increased. According to (Schröder et al., 2017), inter-particle interactions that are key factor to control the stability of the Pickering emulsions results in formation of three-dimensional network of aggregated droplets in continuous phase as can be confirmed by the micrographs in Fig. 3. The emulsifying capacity was observed to be higher for quinoa and rice and lower for amaranth at lower OSA levels and this concentration of starch (200 mg/mL). In addition, among different native starches, native quinoa showed to have better emulsifying capacity (Fig. 4, Table 2). This higher emulsifying capacity in native

quinoa (as well as modified quinoa starches) could be attributed to higher protein level that may provide additional hydrophobic groups and results in higher hydrophobicity and better interfacial affinity which in turn can also result in lower amount of free starch as well. The amount of protein present as a minor component in starch granules is dependent on the botanical origin and purification method. According to Baldwin (2001), a substantial part of starch granule associated proteins are located at the surface of the starch granules. Due to the considerable surface area/g of the starches used in this study, which is 1.22, 1.98 and 2.86 m^2/g for native rice, quinoa and amaranth respectively, the presence of these proteins may significantly influence the overall surface properties of starch granules. From the data presented in Table 1, the amount of protein was found to be higher in quinoa than rice (by a factor of 2) and amaranth (by a factor of approximately 6 and 17 for native and modified starches respectively). If we assume that all the proteins are at the surface of the starch granules, the amount of protein/unit area for native rice, quinoa and amaranth will be 2.7×10^{-3}, 3.5×10^{-3}, 0.4×10^{-3} g/m^2 and for the modified rice, quinoa and amaranth we will have 2.2×10^{-3}, 2.7×10^{-3}, 0.1×10^{-3} g/m^2. Considering the intermediate size of quinoa starch granules with more similarity in size and shape to amaranth, the better hydrophobicity and the higher emulsification capacity of quinoa starch could be explained by this protein level difference. This is further manifested in the case of the native starches where there was no chemical hydrophobization and the trace amount of protein present in amaranth did not create a stable emulsion. It is also due to this higher hydrophobicity that lower amounts of free (non-adsorbed) starches can be seen in particle size measurements for quinoa compared to other starches (see Fig. 4)."

Results and discussion

3. 3.1.1, 3.1.2 since a lot of researches have already investigated the properties of rice starch, it is not necessary to do the researches again about the granules properties.

Answer:

We clarified why we have done in lines 139-143.

The new text is:

"In addition, there are several studies on physicochemical characterization of rice starch however, application of different conditions in them makes a direct comparison difficult. Therefore, we investigated the three different starches in the same conditions to be able to compare performance as Pickering emulsifiers as well as document the properties of the

granules used.”

4. Line 326 How to get the conclusion about the relationship between protein content and emulsifying capacity? Please add more experiments to support your idea.

Answer:

We presented some more supporting facts to back up this observation as you can see in answer to comment 8, reviewer 1.

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Pickering emulsifiers based on hydrophobically modified small granular starches – Part I: Manufacturing and physico-chemical characterization

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Abstract

Small granular starches from rice, quinoa and amaranth were hydrophobized by esterification with octenyl succinic anhydride (OSA) in an aqueous alkaline slurry to obtain series of modified starches at defined intervals (i.e. 0.6, 1.2, 1.8, 2.4, 3.0%). The physical and the physico-chemical properties of the starch particles were characterized by proximate analysis including protein level, amylose level and dry matter. The shape and size of the starch granules were characterized by scanning electron microscopy and light scattering. The gelatinization properties were characterized by differential scanning calorimetry. The degree of modification was determined by titration with NaOH. With regard to the emulsion formulation and in order to assess the emulsifying capacity of the small granular starches, the effect of starch type, degree of modification and starch concentration on the resulting emulsion droplet size were evaluated by light scattering and optical microscopy.

Emulsifying properties were found to depend on the degree of substitution, size of the granules and the starch to oil ratio of the formulation. Quinoa starch granules, in general, had the best emulsifying capacity followed by amaranth and rice. However, in higher starch concentrations (>400 mg/mL oil) and adequate levels of OSA (3.0%) amaranth performed best, having the smallest size of starches studied.

Key Words

Rice, Quinoa, Amaranth, Starch granules, OSA, Pickering emulsions

1. Introduction

Many food, pharmaceutical, and cosmetic products are formulations based on emulsions. Emulsions are mixtures of two immiscible phases where one phase is dispersed in the other in the form of small droplets. They can be water continuous in the case of oil-in-water emulsions (o/w) or oil continuous in water-in-oil emulsions (w/o). Due to the large interfacial area between the finely dispersed phase droplets and the continuous phase, emulsions are generally not thermodynamically stable, as there is a reduction in free energy if dispersed phase droplets coalesce, thereby minimizing the interfacial area. To prevent coalescence and stabilize the droplets, emulsifiers are used which act by decreasing the interfacial tension between the phases, increasing the steric hindrances and/or electrostatic repulsion between the droplets (Bergenstahl 2015). Typical examples include small molecular surfactants, proteins and hydrocolloids. In addition to low molecular mass and polymeric emulsifiers, particles can also be used to achieve droplet stabilization. Emulsions stabilized by particles are known as Pickering emulsions named after Pickering (1907). Pickering particles achieve droplet stabilization by dual wettability towards both phases. The adsorbed particles provide a steric barrier amongst the newly formed droplets which result in prevention of coalescence (Sjöö, Rayner et al. 2015). Compared to other stabilization mechanisms, Pickering emulsions are usually more stable against coalescence and Ostwald ripening (Aveyard, Binks et al. 2003, Yusoff and Murray 2011). This higher stability is due to higher energy of detachment of the particles thanks to the large particle sizes (>10 nm). Once these large particles are adsorbed at the oil-water interface, the energy needed to remove them is several thousand kT as long as the contact angle is not too close to 0º or 180º. As a result, presence of thick and irreversibly adsorbed barrier provides highly stable emulsions (Yusoff and Murray 2011, Rayner, Marku et al. 2014). The energy of detachment per particle can be calculated by the following equation:

$$\Delta G = r^2 \pi \gamma (1 - |\cos\theta|)^2 \qquad \text{Eq. 1.}$$

Where ΔG is the energy of detachment, r is the particle radius (m), γ is the interfacial tension between oil and water (N/m), and θ is the particle-oil-water contact angle measured through the water phase (Berton-Carabin and Schroën 2015).

In recent years there has been a push towards “green label” and “clean label” in many industries, especially in food, cosmetics and consumer products (Frost 2013). This trend has two main drivers, centering on the increase in consumer concerns for health and the environment. With respect to health, low molecular weight emulsifiers and surfactants have come under scrutiny in topical cream due to skin irritation (Wahlgren, Engblom et al. 2013), as well as in food where there is a proposed negative impact on gut health and inflammation (Chassaing, Koren et al. 2015). Furthermore, many

surfactants have an unknown fate in the aquatic environment. For these reasons, as well as an increasing interest in using ingredients that are biodegradable, based on renewable resources, and perceived as being natural and such, Pickering stabilizers based on biomass have been receiving increased interest. For recent and comprehensive reviews the interested reader is directed to Dickinson (2010), Rayner, Marku et al. (2014), Berton-Carabin and Schroën (2015), Rayner (2015). Examples of Pickering particles include clay, silica, alumina, titanium oxide, latex and starch (Binks and Lumsdon 1999, Ashby and Binks 2000, Binks and Lumsdon 2000, Binks and Lumsdon 2001, Stiller, Gers-Barlag et al. 2004, Chen, Vogel et al. 2011, Timgren, Rayner et al. 2011, Yusoff and Murray 2011).

Starch is an interesting material as it is one of our major food constituents, a carbohydrate produced by most green plants as an energy store consisting of a large number of glucose units joined by glycosidic bonds. Starch consists of two different polymers, amylose and amylopectin. Amylose is a mainly linear polymer consisting of units of α-D-glucopyranose with (1-4) glycosidic links and a few branches attached in (1-6) position. Amylopectin has a similar structure as amylose except that the polymer is more branched, and hence it is much larger. In native starches, amylose and amylopectin molecules, along with small amounts of water, are densely packed into partially crystalline, water-insoluble granules (Eliasson 2004).

Although the emulsifying capacities of native starch granules have been observed to be low (Li, Li et al. 2013, Timgren, Rayner et al. 2013), the hydrophobicity can be increased by chemical or physical modification. Starch can be chemically modified by treatment with different alkenyl succinyl anhydrides, for example 2-octen-l-yl succinic anhydride (OSA). The substitution with OSA can occur at the OH- group of carbon 2, 3 and 6 in the glucose molecule. The most widely described synthesis pathway is a reaction in aqueous medium under mild alkaline conditions with starch in its granular form (Trubiano 1986). In addition, modified starch produced through esterification is tasteless, colorless, odorless, inexpensive, non-allergic and approved food additive (E1450) and excipient with degree of modification lower than 3% based on the dry weight of starch, with no limit on application (Timgren, Rayner et al. 2011).

Generating Pickering particles based on starch can be achieved in 3 main ways. By dissolving and precipitation, size reduction of large granules by physical or chemical means, or isolating native starch granules from botanical sources which have small granules (Saari, Heravifar et al. 2016). Small particles (or granules) are of interest as the larger the particles, the larger the mass required to stabilize droplets of a given size. The sizes of starch granules are intrinsic to the botanical source they are isolated from. Starches have been classified in large granule (30-100 μm) including in

tubers such as potato and canna; medium granule (5-30 μm) including starches such as tapioca, barley, maize, sorghum, small granule (2-10 μm) including rice, oat, buckwheat, and extremely small starch granules (0.3-2 μm) such as quinoa, amaranth, cow cockle and pig weed. Some types of starch have bimodal sized starch granules including some species of rice, barley, sorghum and wheat (Hall and Sayre 1970, Hall and Sayre 1971, French 1973, Jane, Kasemsuwan et al. 1994, Lindeboom, Chang et al. 2004, Pérez and Bertoft 2010).

In this work 3 small granule starches of different botanical origin (i.e. rice, quinoa, amaranth) have been considered as potential candidates as Pickering emulsifiers.

Rice (*Oryza Sativa*) is a cereal grain which is the staple food for Asian countries (Singh, Okadome et al. 2000). Rice has small and polygonal granule between 3-9 μm (Juliano 1992, Wani, Singh et al. 2012). The total starch content of rice grain is 78-83% (Yadav, Sharma et al. 2010, Tran, Shelat et al. 2011, Ahmed, Tetlow et al. 2015). The reported amylose content ranges from 0.0-33.0% and the gelatinization temperature range (T_o-T_c) of 55-84.6 °C where temperature and T_o is the gelatinization onset temperature T_c is the gelatinization conclusion (Juliano 1992, Singh, Kaur et al. 2006).

Quinoa (*Chenopodium quinoa Willd*) is a native pseudocereal of Andes in South America which has been cultivated for 3000-4000 years and constituted an important component in the diet of the Incan civilization (Lindeboom, Chang et al. 2005, Li, Wang et al. 2016). Recently quinoa has attracted interest due to its unique characteristics including: high nutritional value due to the quality of protein and fatty acids and its ability to grow under extreme conditions such as salinity, acidity, drought, flooding and frost (Gonzalez, Roldan et al. 1989, Przybylski, Chauhan et al. 1994, Li, Wang et al. 2016). Starch is a major component of quinoa seed which comprises approximately 55-60% of the dry matter (Mundigler 1998, Lindeboom, Chang et al. 2005). The starch is present in the form of small polygonal granules in diameter 0.6-3 μm with mean diameter of 1.5 μm (Atwell, Patrick et al. 1983, Lorenz 1990, Tang, Watanabe et al. 2002, Lindeboom, Chang et al. 2005). The amylose content of quinoa is reported to vary between 3.5-27% (Inouchi, Nishi et al. 1999, Qian and Kuhn 1999, Tang, Watanabe et al. 2002, Lindeboom, Chang et al. 2005) and the gelatinization temperature ranges from 50-74.9 °C (Atwell, Patrick et al. 1983, Qian and Kuhn 1999, Li, Wang et al. 2016).

Amaranth (A*maranthus*) is another ancient pseudocereal domesticated in South America (Mundigler 1998) which currently constituents a large part of diet in Asia and Africa in addition to South America (Qian and Kuhn 1999). The total starch content has been reported to be 67.2% (Mundigler 1998). The starch has small polygonal granules with a mean diameter around 0.8-1.3 μm among different amaranth

cultivars (Bhosale and Singhal 2006, Kong, Bao et al. 2009). The amylose content of amaranth has reported to be in the range of 0-28% (Inouchi, Nishi et al. 1999, Qian and Kuhn 1999, Kong, Bao et al. 2009) and the gelatinization temperature ranges from 63.4-86.9 °C (Inouchi, Nishi et al. 1999, Qian and Kuhn 1999, Kong, Bao et al. 2009).

Several reports have been published on emulsifying properties of different types of OSA modified starch granules (Yusoff and Murray 2011, Timgren, Rayner et al. 2013, Simsek, Ovando-Martinez et al. 2015). In addition, there has been a considerable amount of work on development, characterization and physical and physiological stability of emulsions stabilized by OSA modified quinoa, maize, tapioca, and rice starch granules (Timgren, Rayner et al. 2011, Yusoff and Murray 2011, Marku, Wahlgren et al. 2012, Rayner, Sjöö et al. 2012, Marefati, Rayner et al. 2013, Matos, Timgren et al. 2013, Timgren, Rayner et al. 2013, Song, Pei et al. 2014, Simsek, Ovando-Martinez et al. 2015, Marefati, Bertrand et al. 2017). Though, a comparison of small starch granules with varying OSA level in incremental steps has not been investigated. In addition, although Bhosale and Singhal (2006) have carried out some research on manufacturing and characterization of OSA modified amaranth where they investigated the emulsification capacity of those starches in molecular form, to the best of authors' knowledge, OSA modified amaranth starch granules have not previously been utilized to stabilize Pickering type emulsions. In addition, there are several studies on physicochemical characterization of rice starch, however, application of different conditions in them makes a direct comparison difficult. Therefore, we investigated the three different starches in the same conditions to be able to compare performance as Pickering emulsifiers as well as document the properties of the granules used.

2. Materials & Methods

2.1 Isolation of starch granules

2.1.1 Rice

Rice starch was isolated in a semitechnical scale. 8 kg of rice were steeped in 16 kg of a 0.4% NaOH-solution for 16 h at 4 °C to soften the endosperm and enhance protein solubilization. Then the supernatant was separated, 30 kg of fresh water added and the rice wet milled with a colloid-mill (150 µm). Afterwards, the protein and fiber were separated from the starch by repeated centrifugation (decanter) and wet-sieving (vibration sieve) steps. Finally, the starch suspension was neutralized and spray dried using a spray dryer (type Minor Production, Niro A/S, Denmark) at an

inlet and outlet temperature of 180 °C and 80 °C, respectively. At this point it should be noted that spray drying is widely used in the food industry as a gentle drying process which is even used for heat sensitive enzymes (You, Zhang et al. 2017). In this work, despite the high inlet and outlet temperatures of the air in the spray dyer, moist droplets never reach the air temperature and experience a much lower temperature than the air during drying that is the wet bulb temperature due to evaporative cooling during the constant rate period, Singh and Heldman (2001) and thus gelatinization is avoided. Only at the end of the drying time (3 to 5 seconds) does the temperature begin to rise in the now almost dry particles. The temperature within the water droplets and thus the starch particles during the drying process is always below the outlet temperature of 80°C and after separation in the cyclone the temperature decreases rapidly to about 50°C (below the peak temperature of gelatinization measured in excess water). Furthermore the peak temperature of gelatinization depends on the mass fraction of water in relation to starch (BeMiller and Whistler 2009). For example, the dried starch with a mass fraction of water of 0.12 gelatinizes at temperatures above 150°C. Since the mass fraction of water during spray drying is quickly reduced from 0.75 to 0.12 no gelatinization occurs. The absence of gelatinization was verified by the SEM photographs of the 3 starches and DSC thermographs described in section 2.4.2 and 2.4.3 below.

2.1.2 Quinoa / Amaranth

Quinoa and amaranth starch were separated according to the semitechnical process of Wilhelm, Themeier et al. (1998). Raw materials were *Amaranthus hypochondriacus* from Mexico and *Chenopodium quinoa* from Bolivia. All raw materials were procured as import products. In brief, the grains were dry-milled and the flour was suspended in water and mixed. Thereafter, for improvement of the protein separation from the starch suspension, the slurry went through enzymatic hydrolysis using a commercially available enzyme (Alcalase 2.4 L FG, Novozymes A/S, Bagsvaerd, Denmark) and then mixed with a screw loop mixer (type 50, DMT, Germany) and a high-pressure homogenizer (type 317HD4-3TBS, APV Gaulin, Germany). The starch and fiber were then separated by sieving. The proteins were separated from the starch in two steps, first using a decanter and then the remaining protein residues were manually removed by centrifugation. Finally, the starch was dried using a spray dryer (type Minor Production, Niro A/S, Denmark) at an input and output temperature of 180 °C to 80 °C respectively.

2.2 OSA Modification of starch granules

OSA modification reaction: 50.0 g of the starch was suspended in 200.0 g distilled water. The pH was adjusted to 8.2-8.4 by titration with a 0.5 N NaOH solution, and maintained constant during the reaction. Then a solution of OSA in acetone (100 mg OSA/mL solution) was added within 5-40 min and the temperature was kept constant (32.0± 0.5 °C). The total amount of added OSA was varied (0.6, 1.2, 1.8, 2.4 and 3.0% OSA) in relation to the dry matter of the starches. The reaction finished after 90-120 min. When the pH-value was constant at 8.3, no further addition of NaOH solution was necessary.

Isolation of the product: To the reaction slurry 190 g distilled water was added and the slurry was centrifuged (7 min, 5000 rpm). The sediment (89-92 g) was suspended again in 350 g distilled water and centrifuged. The second sediment was then suspended in 300 mL acetone stirred for 5 min and again centrifuged. The third sediment was first dried at room temperature over the night and then in a laboratory convection dryer, WTB binder (Type MB6, Binder GmbH, Germany) at 30 °C for 4 h. At this conditions the acetone was quantitatively evaporated and the starch was dried below its equilibrium water content. Finally, in order for the starches to reach their equilibrium moisture content, samples conditioned at room temperature for 2 days. All yields varied between 50.1 and 51.1 g.

2.3 Determination of the degree of modification

2000±0.5 mg of the modified starches were weighed in a 100 mL Erlenmeyer flask. Then 60 mL of distilled water was added. The suspension was stirred with a magnetic bar and the pH-value (about 8.4) was adjusted exactly to pH= 7.0±0.1 by addition of 0.1 N H_2SO_4 (Quinoa: 0.20-0.45 mL; Amaranth: 0.06-0.36 mL; Rice: 0.27-0.46 mL) until the pH-value was constant at the end of addition for at least 3 min. Then 20.00±0.03 mL of a 0.1 N NaOH solution were added and the Erlenmeyer flask was quickly closed with a stopper to minimize the adsorption of carbon dioxide from the air. The suspension was then stirred in a water bath at 35.0±0.5°C for 24 h. The minimum time of 24 h required for a quantitative hydrolysis of the ester was determined by kinetic studies. After 24 h, the suspension was cooled to room temperature and the excess of 0.1 N NaOH solution was back titrated to pH=7.0±0.1 with an 0.1 N H_2SO_4 solution and a pH-meter. All samples were investigated in triplicate. The blank volume of 0.1 N H_2SO_4 was determined by a linear or quadratic extrapolation of the mean values of the titration function. The blank values were 19.786 mL, 19.712 mL and 19.836 mL for rice, quinoa and amaranth starch, respectively.

2.4 Characterization of starch granules

2.4.1 Proximate analysis (protein, amylose/amylopectin content, dry matter)

The protein level of quinoa starch granules was determined using a nitrogen/protein analyzer (Flash EA 1112 Series, Thermo Scientific, USA). The amylose level (%, w/w) determined using a lectin Concanavalin A assay (Megazyme International, Ireland) which is a modified version of the method developed by (Yun and Matheson 1990). The dry matter of the isolated and OSA-modified starches was determined according to a modified version of previous method (Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFBG Dez 2008). Approximately 1 g of each sample was weighed with an accuracy of ±0.2 mg in a dry matter glass and dried at 130±1 °C for 90 min in a non-convection oven. The dry matter glass was then closed and cooled for 45 min in a desiccator to room temperature and weighed.

2.4.2 Scanning electron microscopy

Starch granules were characterized by scanning electron microscopy (SEM). The dried samples were coated with gold and examined under SEM (field emission SEM, JSM-6700F, JEOL, Japan) operated at 5 kV with a working distance of 8 mm. Lower detection imaging mode (LEI) was used to give clear three-dimensional images of the sample surface. The LEI detector combines both signals secondary and back scattered electrons during operation.

2.4.3 Characterization of gelatinization properties of starch

The gelatinization properties of starch granules were analyzed using a differential scanning calorimeter (DSC, Seiko 6200, Seiko instruments Inc., Japan), calibrated with indium (M_p = 156.6 °C). Starch dispersions were prepared and weighed into coated aluminum pans (TA Instruments, USA) at a ratio of 1:10 and gelatinization transition enthalpy (ΔH, J/g dry matter), gelatinization onset temperature (°C), gelatinization peak temperature (°C) and gelatinization conclusion temperature (°C) were determined. The scanning rate was 10°C/min from 10 to 120 °C.

2.4.4 Particle size of starch granules

The particle size distribution of starch granules was determined using a laser diffraction particle size analyzer (Mastersizer 2000 Ver. 5.60, Malvern, Worcestershire UK). 70 mg of starch was dispersed in a 7 mL of phosphate buffer (95%, 5 mM, pH 7, 0.2 M NaCl) using a rotor-stator high shear homogenizer (Ystral D-79828, Ballrechten-Dottingen, Germany) with 6 mm dispersing tool, at 22 000 rpm for 30 s. The sample was added to the flow system containing MilliQ-water and was pumped through the optical chamber at a pump velocity of 2000 rpm. The refractive index (RI) of the starch was set to 1.54 (Bromley and Hopkinson 2002) and the RI of the continuous phase was set to 1.33 (water) and the obscuration was between 10 and 20%. This is referred as starch buffer mix (SBM) throughout the text.

2.5 Formulations and Emulsification

2.5.1 Formulation with varying levels of OSA

5% v/v oil-in-water starch granules stabilized emulsions were prepared using Miglyol 812 (Caesar & Loretz GmbH, Germany) as dispersed phase, phosphate buffer (95%, 5 mM, pH 7, 0.2 M NaCl) as continuous phase and starch granules from 3 different botanical origins (i.e. rice, quinoa and amaranth) in native and different modification levels from 0.6-3.0% OSA to stabilize the emulsions.

7 mL emulsions were prepared in a glass test tube using 5% v/v of the dispersed phase and 95% of aqueous phase. 200 mg of starch/mL oil was used to stabilize the emulsions. The emulsions were homogenized using a rotor-stator high shear homogenizer (Ystral D-79828, Ballrechten-Dottingen, Germany) with 6 mm dispersing tool, at 22 000 rpm for 30 s. The samples were prepared in duplicates. The appearance of these emulsions are presented in Fig. 3. Thereafter the emulsions were characterized as described below in section 2.6.

2.5.2 Formulation with varying levels of starch

5% v/v oil-in-water starch granules stabilized emulsions were prepared using Miglyol 812 (Caesar & Loretz GmbH, Germany) as dispersed phase, phosphate buffer (95%, 5 mM, pH 7, 0.2 M NaCl) and starch granules from the 3 different botanical origin (i.e. rice, quinoa and amaranth) with 3.0% OSA modification to stabilize the emulsions (this is described in section 2.4.4).

7 mL of emulsions were prepared in a glass test tube using 5% v/v of the dispersed phase and 95% of aqueous phase. Different amounts of starch namely 50, 100, 200, 400, 800 mg/mL oil were added to stabilize the emulsions. The emulsions and the starch dispersions were homogenized using a rotor-stator high shear homogenizer (Ystral D-79828, Ballrechten-Dottingen, Germany) with 6 mm dispersing tool, at 22 000 rpm for 30 s. Thereafter, the emulsions were characterized as described below in section 2.6.

2.6 Emulsion characterization

2.6.1 Particle size distributions of Starch Pickering Emulsions

The particle size distributions of the starch granule stabilized emulsions were characterized with a laser diffraction particle size analyzer, Mastersizer 2000 (Malvern Instruments, UK). Each emulsion

was added to the flow system (Hydro SM small volume wet dispersion unit) containing MilliQ-water and was then pumped through the optical chamber where it was measured. The refractive index of starch particles was set to 1.54 (Bromley and Hopkinson 2002) and the refractive index of the continuous phase was set to 1.33 which is the refractive index of the water and the obscuration was between 10 and 20%. For each emulsion sample added to the flow system three measurements were performed, and all emulsions were prepared in duplicates and analyzed 3 times.

2.6.2 Microscopy

The emulsions were characterized by light microscopy using a camera (DFK 41AF02, The Imaging Source, Germany) that was attached to a light microscope (Olympus BX50, Japan) and both were connected to a computer. The emulsions were diluted 5 times with MilliQ water and then one drop was placed on a glass microscopic slide. In order to prevent deformation of droplets no cover glass was used. The microscopic images were taken using objective magnifications of 20× and 50×.

3. Results and discussion

3.1 Granules

3.1.1 Proximate analysis and degree of modification

The protein level of starches was highest for quinoa 0.538-0.687 followed by rice 0.271-0.328% and the lowest was amaranth with 0.032-0.112% (Table 1). Moreover, the protein content of native samples showed higher values which may be due to solubilization of proteins during modification as a result of exposure to alkali solution or being washed away by acetone at the end of modification process which could also result in further removal of proteins.

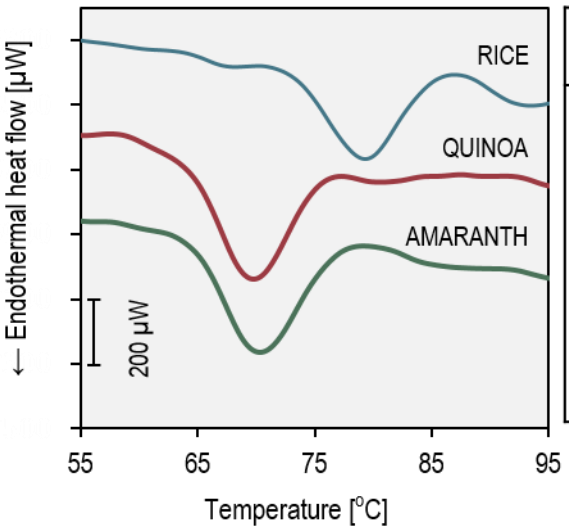
The amylose content of starches was lower for rice 4.43%±0.78 and somewhat similar for quinoa and amaranth with 20.95%±0.45 and 20.90%±0.98 which fall within the range of previous results in the literature (Juliano 1992, Inouchi, Nishi et al. 1999, Qian and Kuhn 1999, Tang, Watanabe et al. 2002, Lindeboom, Chang et al. 2005, Singh, Kaur et al. 2006, Kong, Bao et al. 2009).

3.1.2 Gelatinization properties of starch

The gelatinization temperature range (T_o-T_c) of different starches was the highest for rice 72.2-85.0 °C which was similar to the previously reported values in the literature and lower for quinoa and amaranth with 64.1-75.5 °C and 63.8-76.8 °C respectively which again was similar previously

reported values (Atwell, Patrick et al. 1983, Inouchi, Nishi et al. 1999, Qian and Kuhn 1999, Singh, Kaur et al. 2006, Kong, Bao et al. 2009, Li, Wang et al. 2016) (Fig. 1).

The higher gelatinization range for rice can be attributed to the higher amylopectin content since according to Fredriksson, Silverio et al. (1998) starch crystallinity increases with amylopectin content, and hence, starches with higher amylopectin content (i.e. lower amylose content) would expect to have higher onset, peak and conclusion temperature. In the same way both similarity and lower gelatinization range of quinoa and amaranth compared to rice can be described by similar and lower amylopectin content of those corresponding starches.



	RICE	QUINOA	AMARANTH
Onset temp [°C]	72.2 ± 0.2	64.1 ± 0.2	63.8 ± 0.7
Peak temp [°C]	79.4 ± 0.3	69.8 ± 0.2	70.3 ± 0.2
Conclusion temp [°C]	85.0 ± 0.2	75.5 ± 0.3	76.8 ± 0.9
Enthalpy [mJ/mg]	13.9 ± 3.1	15.4 ± 0.8	19.0 ± 2.9

Figure 1. DSC thermogram and thermal properties data for gelatinization of starches in buffer.

3.1.3 OSA modification

Different amount of OSA was bond to the starches at the same level of added OSA (i.e. 0.6, 1.2, 1.8, 2.4, 3.0) for different starches with different botanical origin (Table 1). Table 1 shows that the OSA reaction efficiency (RE) was the highest for quinoa, followed by amaranth and it was lowest for rice modified starches. The RE values for rice, quinoa and amaranth varied between 0.783-0.840, 0.903-0.987 and 0.862-0.918 and among all added OSA points respectively.

Table 1. Proximate Analysis and OSA levels

RICE

Sample	Dry matter (%)	OSA (%)	Degree of substitution (DS)	Reaction efficiency	Protein content (%)
R-Native	89.3±0.00	0	0	-	0.328±0.000
R-OSA-0.6	87.5±0.12	0.46 ± 0.01	0.0036	0.783	0.278±0.000

R-OSA-1.2	87.4±0.00	0.97 ± 0.03	0.0077	0.840	0.274±0.005
R-OSA-1.8	87.7±0.04	1.40 ± 0.05	0.0108	0.808	0.272±0.005
R-OSA-2.4	87.7±0.11	1.90 ± 0.05	0.0149	0.828	0.274±0.001
R-OSA-3.0	87.5±0.01	2.36 ± 0.02	0.0186	0.827	0.271±0.001

QUINOA

Sample	Dry matter (%)	OSA (%)	Degree of substitution (DS)	Reaction efficiency	Protein content (%)
Q- Native	88.6±0.04	0	0	-	0.687±0.001
Q-OSA-0.6	87.7±0.20	0.58 ± 0.01	0.0045	0.987	0.570±0.006
Q-OSA-1.2	87.6±0.25	1.14 ± 0.04	0.0091	0.979	0.548±0.003
Q-OSA-1.8	87.3±0.01	1.67 ± 0.02	0.0130	0.958	0.538±0.004
Q-OSA-2.4	87.7±0.06	2.13 ± 0.04	0.0168	0.923	0.547±0.002
Q-OSA-3.0	87.7±0.59	2.59 ± 0.02	0.0205	0.903	0.539±0.025

AMARANTH

Sample	Dry matter (%)	OSA (%)	Degree of substitution (DS)	Reaction efficiency	Protein content (%)
A-native	88.8±0.08	0	0	-	0.112±0.030
A-OSA-0.6	87.6±0.14	0.53±0.02	0.0041	0.908	0.036±0.001
A-OSA-1.2	87.8±0.21	1.07±0.01	0.0084	0.918	0.033±0.001
A-OSA-1.8	87.9±0.13	1.50±0.02	0.0117	0.862	0.032±0.001
A-OSA-2.4	88.2±0.18	2.06±0.03	0.0161	0.893	0.032±0.004
A-OSA-3.0	88.3±0.14	2.62±0.02	0.0208	0.915	0.032±0.004

3.1.4 Size and morphology of starch granules

The size distribution and granule morphology of native and 3.0% OSA modified rice, quinoa, and amaranth can be observed in Fig. 2. The details of particle size values for native starches can be found in Table 2. The volume mean diameter (D4,3) was consistent with what was expected with rice being the largest (6.92 µm) followed by quinoa (2.44 µm) and amaranth (1.48 µm) and comply with former results in the literature. The particle size distribution graph for the native rice granules showed a bimodal size distribution with small peak around 1 µm and a large peak around 7.6 µm. Comparing these results with previous results in the literature, unimodal and bimodal size distribution could be found (Zuo, Knoerzer et al. 2009, Wani, Singh et al. 2012). The amount of the small granules could depend on botanical source, as well as the isolation process as in some industrial processes the fine granules are lost in the separation step. In addition, depending on the measuring technique, the small peak may not be resolved and instead a wider peak is seen.

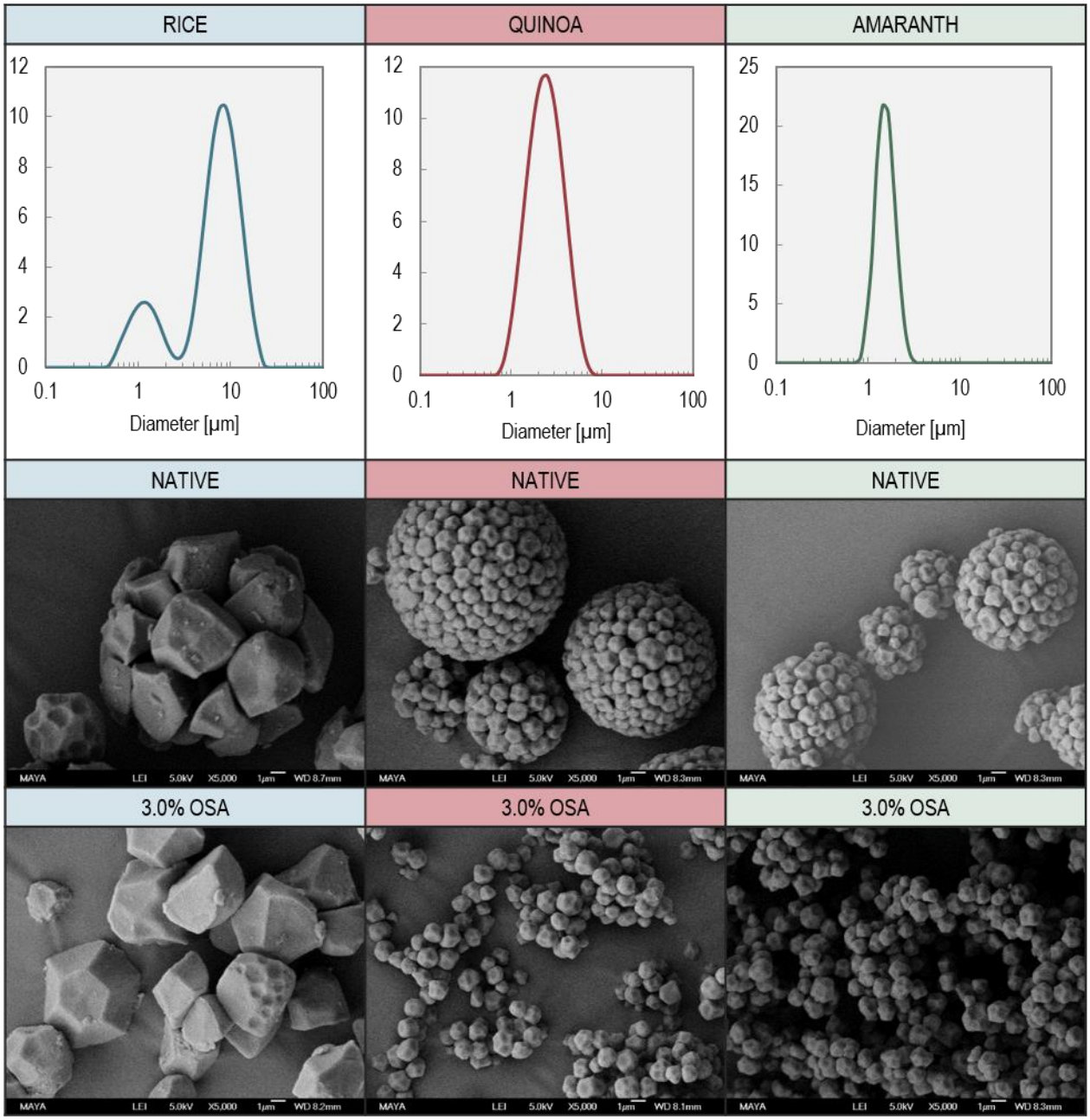


Figure 2. Size and morphology of granules. Top row: Particle size distribution of starch in buffer dispersions for Rice, Quinoa, and Amaranth. Middle row: SEM images of the various native starch granules. Bottom row: SEM images of the various starch granules after 3.0% OSA modification.

3.2 Effect of the degree of OSA modification on the emulsifying capacity

The appearance of the emulsions at all OSA modification levels and the morphology of the emulsions' droplets produced with 200 mg/mL oil of different starches at 3.0% is presented in Fig. 3. The starch particles can be seen on the surface of the emulsions' droplet which is the characteristic trait of Pickering emulsion. As can be seen in Fig. 3, these emulsions were not space filling and the droplets formed a sediment in the bottom of the test tubes due low oil fraction and high density of starch compared to the continuous phase respectively which agrees with the

previous results for quinoa (Rayner, Timgren et al. 2012). The cumulative and volume frequency particle size distribution of emulsions produced from native and modified starches at all OSA modification levels (i.e. 0.6, 1.2, 1.8, 2.4, 3.0%) at the same oil/starch ratios (200 mg/mL oil) are presented in Fig. 4 and Table 2. There seems to be a negative correlation between the level of modification and droplet size and the amount of free starch at the same oil/starch ratios meaning that the greater the degree of OSA the smaller the resulting emulsions droplet were at the same starch to oil ratio and starch type as can be seen in the right column of Fig. 4. Moreover, the degree of modification appears to be more influential on emulsifying capacity of amaranth. In addition, the thickness of the emulsions layer increased as the modification level increased. According to (Schröder, Sprakel et al. 2017), inter-particle interactions that are key factor to control the stability of the Pickering emulsions results in formation of three-dimensional network of aggregated droplets in continuous phase as can be confirmed by the micrographs in Fig. 3. The emulsifying capacity was observed to be higher for quinoa and rice and lower for amaranth at lower OSA levels and this concentration of starch (200 mg/mL). In addition, among different native starches, native quinoa showed to have better emulsifying capacity (Fig. 4, Table 2). This higher emulsifying capacity in native quinoa (as well as modified quinoa starches) could be attributed to higher protein level that may provide additional hydrophobic groups and results in higher hydrophobicity and better interfacial affinity which in turn can also result in lower amount of free starch as well. The amount of protein present as a minor component in starch granules is dependent on the botanical origin and purification method. According to Baldwin (2001), a substantial part of starch granule associated proteins are located at the surface of the starch granules. Due to the considerable surface area/g of the starches used in this study, which is 1.22, 1.98 and 2.86 m^2/g for native rice, quinoa and amaranth respectively, the presence of these proteins may significantly influence the overall surface properties of starch granules. From the data presented in Table 1, the amount of protein was found to be higher in quinoa than rice (by a factor of 2) and amaranth (by a factor of approximately 6 and 17 for native and modified starches respectively). If we assume that all the proteins are at the surface of the starch granules, the amount of protein/unit area for native rice, quinoa and amaranth will be 2.7×10^{-3}, 3.5×10^{-3}, 0.4×10^{-3} g/m^2 and for the modified rice, quinoa and amaranth we will have 2.2×10^{-3}, 2.7×10^{-3}, 0.1×10^{-3} g/m^2. Considering the intermediate size of quinoa starch granules with more similarity in size and shape to amaranth, the better hydrophobicity and the higher emulsification capacity of quinoa starch could be explained by this protein level difference. This is further manifested in the case of the native starches where there was no chemical hydrophobization and the trace amount of protein present in amaranth did not create a stable emulsion. It is also due to this higher hydrophobicity that lower amounts of free (non-adsorbed) starches can be seen in particle size measurements for quinoa compared to other starches (see Fig. 4). Vertical dashed lines

in Fig. 4 are the d(90) of the size distribution of starch granules dispersed in buffer. This gives an indication of the degree of free starch in the system. For amaranth in particular, there is a very high amount of free starch observed in the particle size distribution seen as a peak in the 1-2 µm range. By looking at the cumulative plot we can note that for native amaranth, there was no effective droplet stabilization as the majority of the oil was phase separated and 80% of the starch remained free in the continuous phase. Furthermore, between 30% and 50% of the cumulative volume of particles measured in the samples for 0.6% OSA, and 1.2% OSA amaranth starch stabilized emulsions were present as free starch and not adsorbed at the oil-water interface. For similar degrees of modification in quinoa the volume of free starch is observed to be in the range of 8 to 12% (see left column of Fig. 4). Therefore, the precise influence of the surface proteins of starch granules with respect to the optimization of emulsification capacity of starch granules could be topic of further investigations.

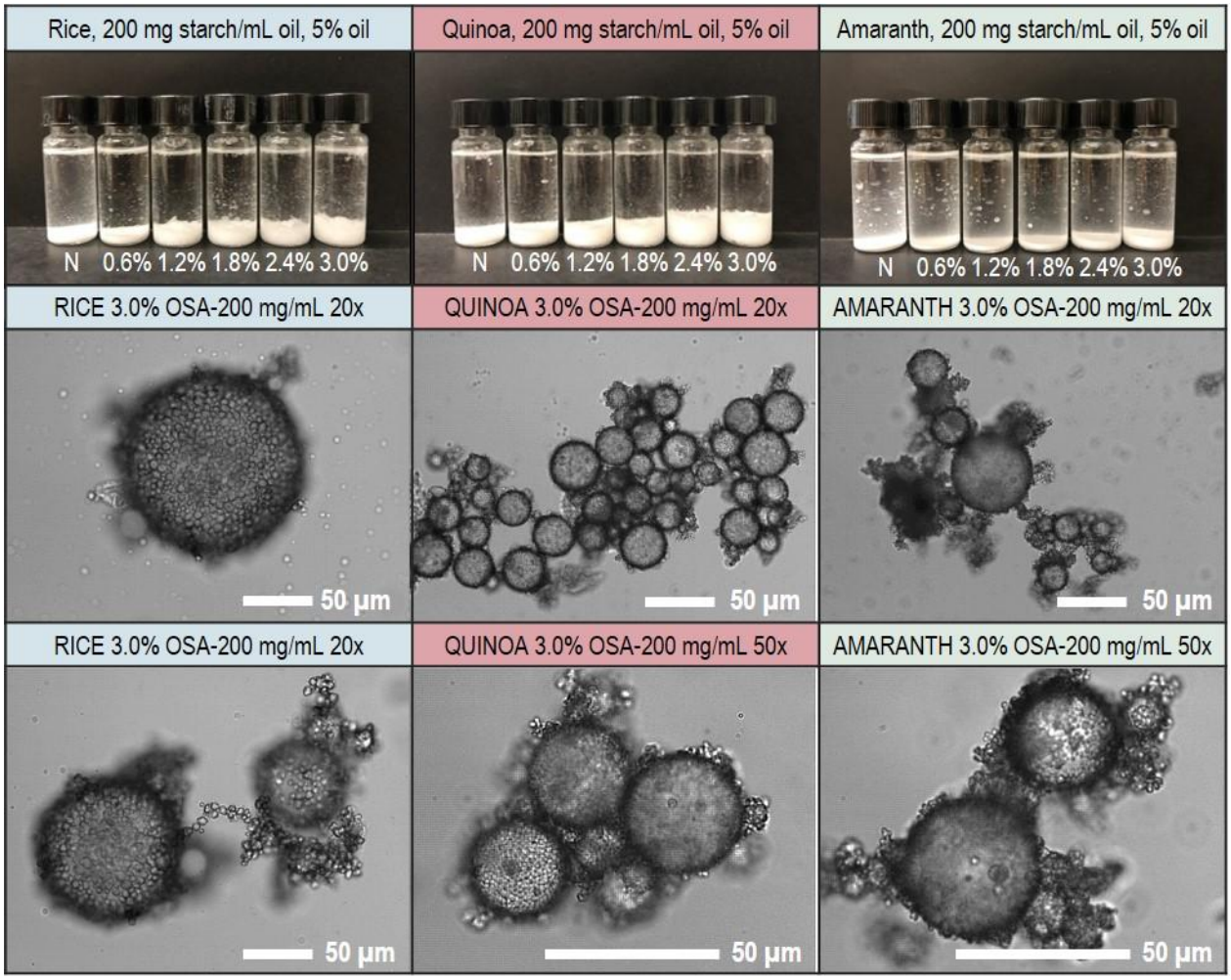
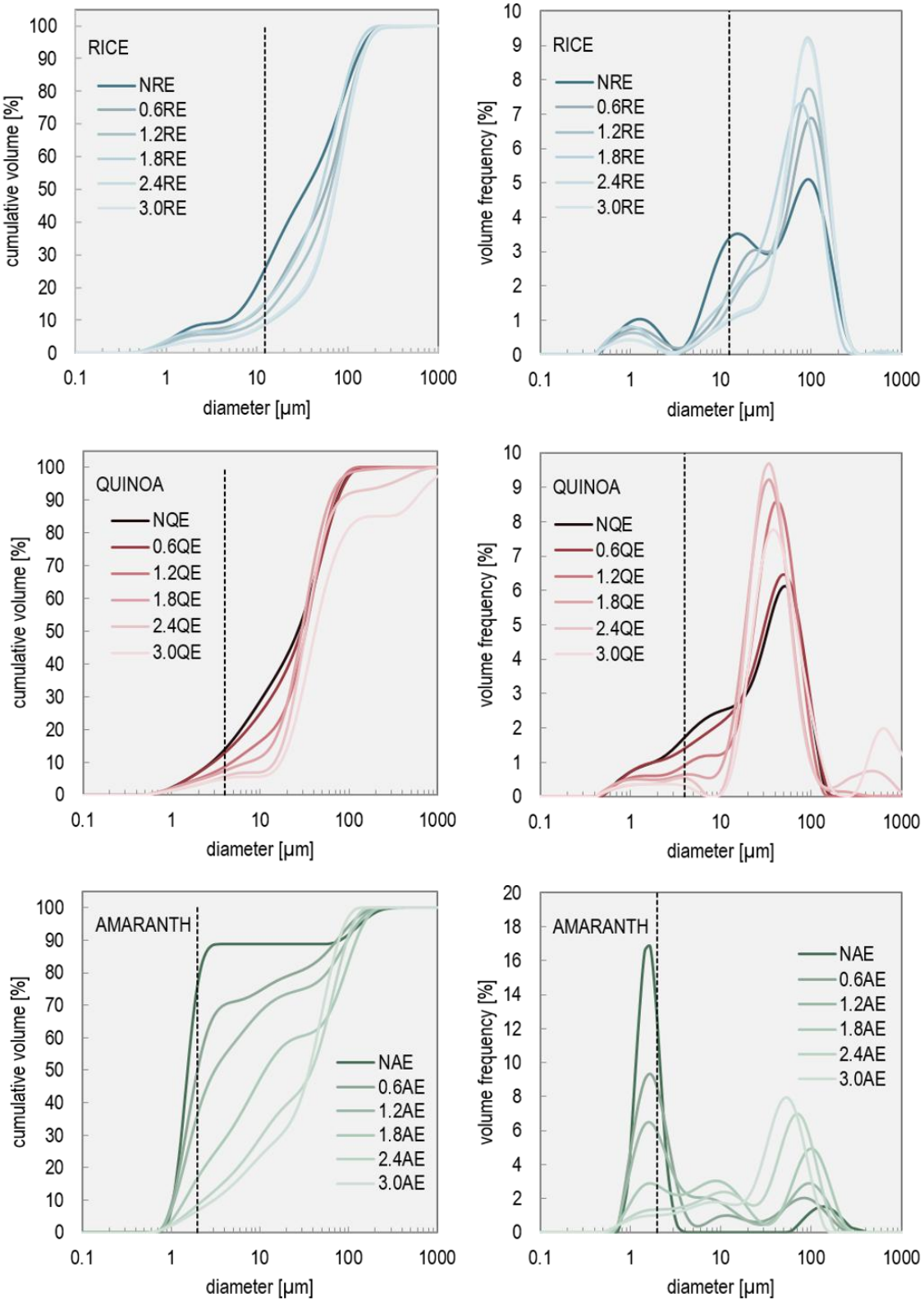


Figure 3. Images of starch granule stabilized emulsions (top row), Optical micrographs of 3.0% OSA modified starch granule stabilized emulsions (200 mg starch / mL oil) (two bottom rows)



402

403 **Figure 4.** Particle size distributions (left cumulative and right frequency) of starch granule stabilized emulsions (200 mg
404 starch/mL oil) for various levels of OSA modification: native emulsion, 0.6% OSA, 1.2%, 1.8%, 2.4%, 3.0%. Vertical
405 dashed lines are d_{90} of the granules size Rice: 12.3 µm, Quinoa 3.93 µm, and Amaranth 1.97 µm respectively.

406 **Table 2.** Different size measurements for: native starch granules dispersed in buffer, native emulsion, 0.6% OSA
407 emulsion, 1.2% OSA emulsion, 1.8% OSA emulsion, 2.4% OSA emulsion, 3.0% OSA emulsion for rice, quinoa and
408 amaranth respectively.

RICE					
Sample	**Mode [µm]**	**D [4, 3] [µm]**	**Span**	**D [3, 2] [µm]**	**d (0.5) [µm]**
NRSBM	7.69±0.12	6.92±0.17	1.66±0.01	3.29±0.15	6.77±0.14
NRE	90.2±12.2	52.9±4.95	3.58±0.53	7.88±0.49	34.7±2.39
0.6RE	94.4±11.5	66.3±12.3	2.50±0.71	10.5±2.66	56.3±17.7
1.2RE	36.0±8.68	23.9±6.50	1.50±0.19	11.7±1.75	65.1±5.10
1.8RE	42.2±5.00	27.8±2.35	1.50±0.28	9.27±0.67	50.3±3.45
2.4RE	87.8±12.9	80.8±12.8	1.73±0.06	15.6±1.70	74.9±10.9
3.0RE	86.8±6.84	76.6±5.99	1.77±0.10	15.2±1.79	72.2±4.56
QUINOA					
Sample	**Mode[µm]**	**D [4, 3] [µm]**	**Span**	**D [3, 2] [µm]**	**d (0.5) [µm]**
NQSBM	2.22±0.15	2.44±0.10	1.21±0.06	2.02±0.09	2.22±0.11
NQE	47.8±5.33	32.6±3.39	2.61±0.13	7.36±0.41	26.9±3.79
0.6QE	46.1±7.82	34.3±4.07	2.40±0.11	7.86±0.64	29.6±4.43
1.2QE	39.2±2.13	35.2±2.26	1.91±0.05	9.86±0.63	32.6±2.39
1.8QE	31.8±0.66	34.5±3.59	1.83±0.07	10.3±0.39	29.3±0.90
2.4QE	31.8±1.32	62.1±4.75	2.67±1.47	13.4±0.85	32.5±2.11
3.0QE	36.4±4.18	48.2±8.19	1.88±0.08	13.6±2.04	36.7±4.17
AMARANTH					
Sample	**Mode[µm]**	**D [4, 3] [µm]**	**Span**	**D [3, 2] [µm]**	**d (0.5) [µm]**
NASBM	1.42±0.03	1.48±0.03	0.63±0.01	1.40±0.03	1.43±0.03
NAE	No emulsion	-	-	-	-
0.6AE	1.52±2.08	18.9±0.12	36.5±4.92	2.05±0.10	1.99±0.09
1.2AE	1.50±0.10	26.3±6.72	29.0±1.22	2.57±0.22	3.30±0.73
1.8AE	95.0±9.48	42.6±3.09	10.6±2.53	4.47±0.35	11.7±2.31
2.4AE	66.1±1.46	43.0±0.92	2.44±0.06	7.15±0.18	38.3±0.32
3.0AE	49.8±0.28	37.9±1.02	2.09±0.06	8.19±0.40	35.7±1.18

409

410 ***3.3 Effect of starch concentration on emulsion droplet size***

411 The particle size distribution values and particle size distribution as a function of starch
412 concentration for 3.0% OSA level can be found in Table 3 and Fig. 5. Except in very low
413 concentration for rice and amaranth modified starches, there was a negative correlation between the
414 amount of starch and the particle size. It was shown that modified quinoa starch had a good overall

emulsifying capacity and it was the best emulsifier in the lower starch concentrations among the starch varieties tested. Furthermore, it was also shown that, in higher concentrations of starch and adequate level of OSA modification, (>400 mg/mL oil) amaranth had the highest emulsification capacity among the different starches.

Table 3. Different size measurements for: 3.0% OSA modified starch emulsions, with 50, 100, 200, 400, 800 mg starch/ mL of oil for rice, quinoa, and amaranth respectively.

RICE					
Sample	**Mode [μm]**	**D [4, 3] [μm]**	**Span**	**D [3, 2] [μm]**	**d (0.5) [μm]**
50 mg/mL	123.2±17.5	101.6±11.79	1.86±0.09	19.8±2.83	97.4±10.44
100 mg/mL	131.0±15.0	107.8±9.67	1.79±0.06	24.1±0.88	103.9±8.76
200 mg/mL	95.6±7.59	80.6±6.45	1.89±0.15	18.8±1.60	74.6±5.25
400 mg/mL	51.9±2.31	49.9±4.04	1.90±0.08	13.7±0.74	44.2±2.45
800 mg/mL	28.6±4.29	34.0±2.78	2.06±0.20	9.34±1.01	26.0±3.74
QUINOA					
Sample	**Mode [μm]**	**D [4, 3] [μm]**	**Span**	**D [3, 2] [μm]**	**d (0.5) [μm]**
50 mg/mL	104.5±9.29	104.80±7.66	1.16±0.18	35.0±4.28	99.9±6.47
100 mg/mL	67.6±1.29	69.6±2.91	1.12±0.10	24.9±0.57	66.0±1.78
200 mg/mL	32.7±1.09	48.8±3.13	2.49±0.29	14.9±0.57	34.9±1.17
400 mg/mL	25.6±3.13	35.8±2.58	2.34±0.78	10.7±1.29	25.7±2.49
800 mg/mL	18.7±0.71	20.7±2.68	1.55±0.04	8.34±0.42	17.6±0.89
AMARANTH					
Sample	**Mode [μm]**	**D [4, 3] [μm]**	**Span [μm]**	**D [3, 2] [μm]**	**d (0.5) [μm]**
50 mg/mL	128.9±14.0	100.3±9.77	1.89±0.03	13.4±0.48	100.6±9.61
100 mg/mL	74.5±4.57	46.7±7.10	3.11±0.83	7.21±0.81	36.9±12.81
200 mg/mL	45.4±9.54	38.2±2.24	3.34±0.76	6.89±0.27	27.1±3.56
400 mg/mL	15.6±8.15	23.5±1.70	5.05±2.22	4.94±0.69	12.2±3.31
800 mg/mL	11.6±2.85	12.0±0.29	3.28±0.36	3.78±0.09	8.15±0.96

By taking a mass balance over the amount of particles available for stabilizing the emulsions droplets assuming no free starch in the limited coalescence regime the theoretical droplet diameter of emulsion droplets can be estimated by:

$$\frac{1}{D} = \frac{m_p}{\varphi\ 4\ d_p\ \rho_p\ V_{disp}} \qquad \text{Eq. 2.}$$

Where the emulsions droplet size is D, the mass m_p and density ρ_p of particles, and the volume of dispersed phase V_d and φ is the packing density assumed to be $\varphi \approx 0.907$, i.e. hexagonal close

packing of spheres in a plane (Arditty, Schmitt et al. 2004). If we compare the theoretical droplet size for a formulation with a certain starch granule size and amount of starch we find that in the case of quinoa and amaranth the experimental droplet sizes to be 1.7 to 5 times and 3.4 to 4.9 times larger than the predicted theoretical dimeter respectively. However, in the case of rice, the measured droplet sizes we closed to be predicted values being 0.6 to 2.2 times larger. This suggests that the rice granules are performing better than the quinoa and amaranth if we adjust for the effect of their size. This could be attributed to rice's bimodal particle size distribution (Fig. 2) with the smaller fraction contributing more to the apparent emulsifying capacity.

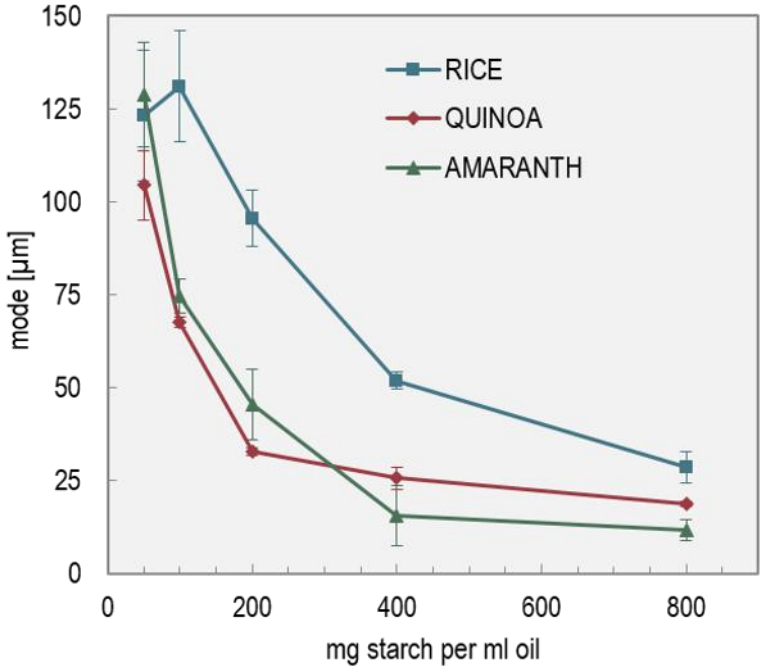


Figure 5. Particle size (mode of D43) for: 3.0% OSA modified starch emulsions.

4. Conclusions

This study showed that starch granules from small granule botanical sources have the capacity to stabilize emulsions. In addition, starch granules from quinoa have good emulsifying capacity in both native and OSA modified form and especially better emulsifying capacity in the lower starch concentrations compared to the rice and amaranth. Native rice was also able to stabilize emulsion droplets. This may be due to higher protein contents of quinoa and rice starch granules in the native form that can optimize the hydrophobicity, which could be the topic of further investigations.

In the case of modified starches and when enough starch is available for stabilization (starch concentrations >400 mg/mL oil), smaller size of amaranth granules seems to be optimum.

Lastly, the bimodal nature of rice starches could be subjected to future studies for exploring the effect of size on the emulsifying capacity of starch granules from the same plant.

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617

Table(s)

RICE					
Sample	**Dry matter (%)**	**OSA (%)**	**Degree of substitution (DS)**	**Reaction efficiency**	**Protein content (%)**
R-Native	89.3±0.00	0	0	-	0.328±0.000
R-OSA-0.6	87.5±0.12	0.46 ± 0.01	0.0036	0.783	0.278±0.000
R-OSA-1.2	87.4±0.00	0.97 ± 0.03	0.0077	0.840	0.274±0.005
R-OSA-1.8	87.7±0.04	1.40 ± 0.05	0.0108	0.808	0.272±0.005
R-OSA-2.4	87.7±0.11	1.90 ± 0.05	0.0149	0.828	0.274±0.001
R-OSA-3.0	87.5±0.01	2.36 ± 0.02	0.0186	0.827	0.271±0.001
QUINOA					
Sample	**Dry matter (%)**	**OSA (%)**	**Degree of substitution (DS)**	**Reaction efficiency**	**Protein content (%)**
Q- Native	88.6±0.04	0	0	-	0.687±0.001
Q-OSA-0.6	87.7±0.20	0.58 ± 0.01	0.0045	0.987	0.570±0.006
Q-OSA-1.2	87.6±0.25	1.14 ± 0.04	0.0091	0.979	0.548±0.003
Q-OSA-1.8	87.3±0.01	1.67 ± 0.02	0.0130	0.958	0.538±0.004
Q-OSA-2.4	87.7±0.06	2.13 ± 0.04	0.0168	0.923	0.547±0.002
Q-OSA-3.0	87.7±0.59	2.59 ± 0.02	0.0205	0.903	0.539±0.025
AMARANTH					
Sample	**Dry matter (%)**	**OSA (%)**	**Degree of substitution (DS)**	**Reaction efficiency**	**Protein content (%)**
A-native	88.8±0.08	0	0	-	0.112±0.030
A-OSA-0.6	87.6±0.14	0.53±0.02	0.0041	0.908	0.036±0.001
A-OSA-1.2	87.8±0.21	1.07±0.01	0.0084	0.918	0.033±0.001
A-OSA-1.8	87.9±0.13	1.50±0.02	0.0117	0.862	0.032±0.001
A-OSA-2.4	88.2±0.18	2.06±0.03	0.0161	0.893	0.032±0.004
A-OSA-3.0	88.3±0.14	2.62±0.02	0.0208	0.915	0.032±0.004

Table(s)

RICE					
Sample	**Mode [µm]**	**D [4, 3] [µm]**	**Span**	**D [3, 2] [µm]**	**d (0.5) [µm]**
NRSBM	7.69±0.12	6.92±0.17	1.66±0.01	3.29±0.15	6.77±0.14
NRE	90.2±12.2	52.9±4.95	3.58±0.53	7.88±0.49	34.7±2.39
0.6RE	94.4±11.5	66.3±12.3	2.50±0.71	10.5±2.66	56.3±17.7
1.2RE	36.0±8.68	23.9±6.50	1.50±0.19	11.7±1.75	65.1±5.10
1.8RE	42.2±5.00	27.8±2.35	1.50±0.28	9.27±0.67	50.3±3.45
2.4RE	87.8±12.9	80.8±12.8	1.73±0.06	15.6±1.70	74.9±10.9
3.0RE	86.8±6.84	76.6±5.99	1.77±0.10	15.2±1.79	72.2±4.56
QUINOA					
Sample	**Mode[µm]**	**D [4, 3] [µm]**	**Span**	**D [3, 2] [µm]**	**d (0.5) [µm]**
NQSBM	2.22±0.15	2.44±0.10	1.21±0.06	2.02±0.09	2.22±0.11
NQE	47.8±5.33	32.6±3.39	2.61±0.13	7.36±0.41	26.9±3.79
0.6QE	46.1±7.82	34.3±4.07	2.40±0.11	7.86±0.64	29.6±4.43
1.2QE	39.2±2.13	35.2±2.26	1.91±0.05	9.86±0.63	32.6±2.39
1.8QE	31.8±0.66	34.5±3.59	1.83±0.07	10.3±0.39	29.3±0.90
2.4QE	31.8±1.32	62.1±4.75	2.67±1.47	13.4±0.85	32.5±2.11
3.0QE	36.4±4.18	48.2±8.19	1.88±0.08	13.6±2.04	36.7±4.17
AMARANTH					
Sample	**Mode[µm]**	**D [4, 3] [µm]**	**Span**	**D [3, 2] [µm]**	**d (0.5) [µm]**
NASBM	1.42±0.03	1.48±0.03	0.63±0.01	1.40±0.03	1.43±0.03
NAE	No emulsion	-	-	-	-
0.6AE	1.52±2.08	18.9±0.12	36.5±4.92	2.05±0.10	1.99±0.09
1.2AE	1.50±0.10	26.3±6.72	29.0±1.22	2.57±0.22	3.30±0.73
1.8AE	95.0±9.48	42.6±3.09	10.6±2.53	4.47±0.35	11.7±2.31
2.4AE	66.1±1.46	43.0±0.92	2.44±0.06	7.15±0.18	38.3±0.32
3.0AE	49.8±0.28	37.9±1.02	2.09±0.06	8.19±0.40	35.7±1.18

Table(s)

RICE					
Sample	**Mode [µm]**	**D [4, 3] [µm]**	**Span**	**D [3, 2] [µm]**	**d (0.5) [µm]**
50 mg/mL	123.2±17.5	101.6±11.79	1.86±0.09	19.8±2.83	97.4±10.44
100 mg/mL	131.0±15.0	107.8±9.67	1.79±0.06	24.1±0.88	103.9±8.76
200 mg/mL	95.6±7.59	80.6±6.45	1.89±0.15	18.8±1.60	74.6±5.25
400 mg/mL	51.9±2.31	49.9±4.04	1.90±0.08	13.7±0.74	44.2±2.45
800 mg/mL	28.6±4.29	34.0±2.78	2.06±0.20	9.34±1.01	26.0±3.74
QUINOA					
Sample	**Mode [µm]**	**D [4, 3] [µm]**	**Span**	**D [3, 2] [µm]**	**d (0.5) [µm]**
50 mg/mL	104.5±9.29	104.80±7.66	1.16±0.18	35.0±4.28	99.9±6.47
100 mg/mL	67.6±1.29	69.6±2.91	1.12±0.10	24.9±0.57	66.0±1.78
200 mg/mL	32.7±1.09	48.8±3.13	2.49±0.29	14.9±0.57	34.9±1.17
400 mg/mL	25.6±3.13	35.8±2.58	2.34±0.78	10.7±1.29	25.7±2.49
800 mg/mL	18.7±0.71	20.7±2.68	1.55±0.04	8.34±0.42	17.6±0.89
AMARANTH					
Sample	**Mode [µm]**	**D [4, 3] [µm]**	**Span [µm]**	**D [3, 2] [µm]**	**d (0.5) [µm]**
50 mg/mL	128.9±14.0	100.3±9.77	1.89±0.03	13.4±0.48	100.6±9.61
100 mg/mL	74.5±4.57	46.7±7.10	3.11±0.83	7.21±0.81	36.9±12.81
200 mg/mL	45.4±9.54	38.2±2.24	3.34±0.76	6.89±0.27	27.1±3.56
400 mg/mL	15.6±8.15	23.5±1.70	5.05±2.22	4.94±0.69	12.2±3.31
800 mg/mL	11.6±2.85	12.0±0.29	3.28±0.36	3.78±0.09	8.15±0.96

Figure(s)

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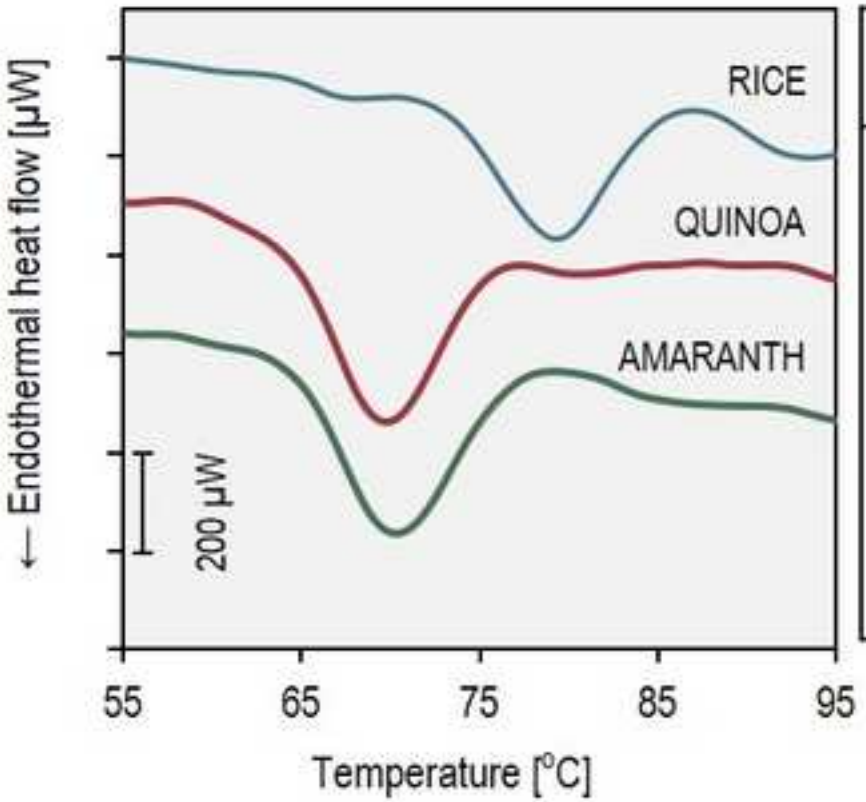

	RICE	QUINOA	AMARANTH
Onset temp [°C]	72.2 ± 0.2	64.1 ± 0.2	63.8 ± 0.7
Peak temp [°C]	79.4 ± 0.3	69.8 ± 0.2	70.3 ± 0.2
Conclusion temp [°C]	85.0 ± 0.2	75.5 ± 0.3	76.8 ± 0.9
Enthalpy [mJ/mg]	13.9 ± 3.1	15.4 ± 0.8	19.0 ± 2.9

Figure(s)

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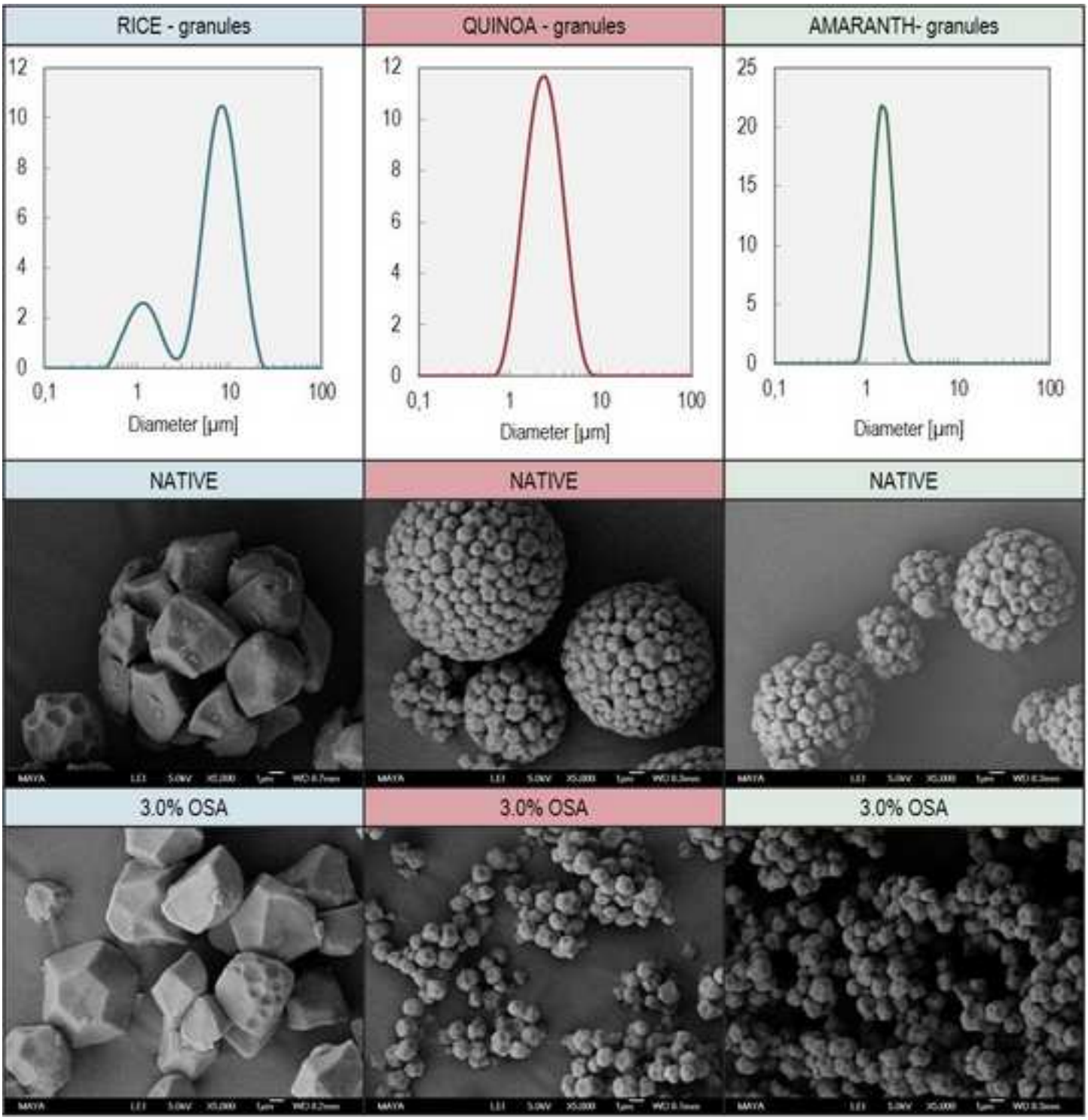

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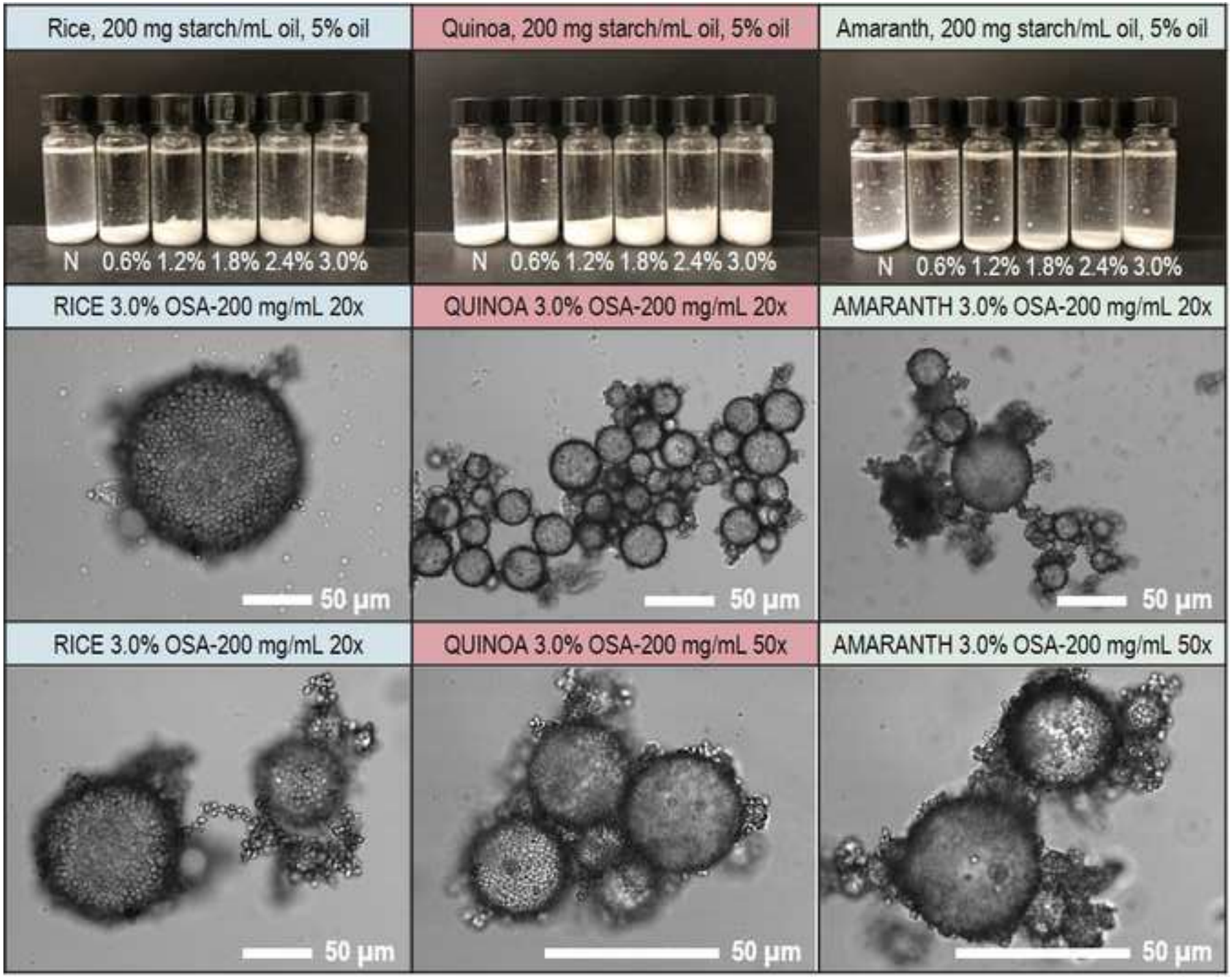

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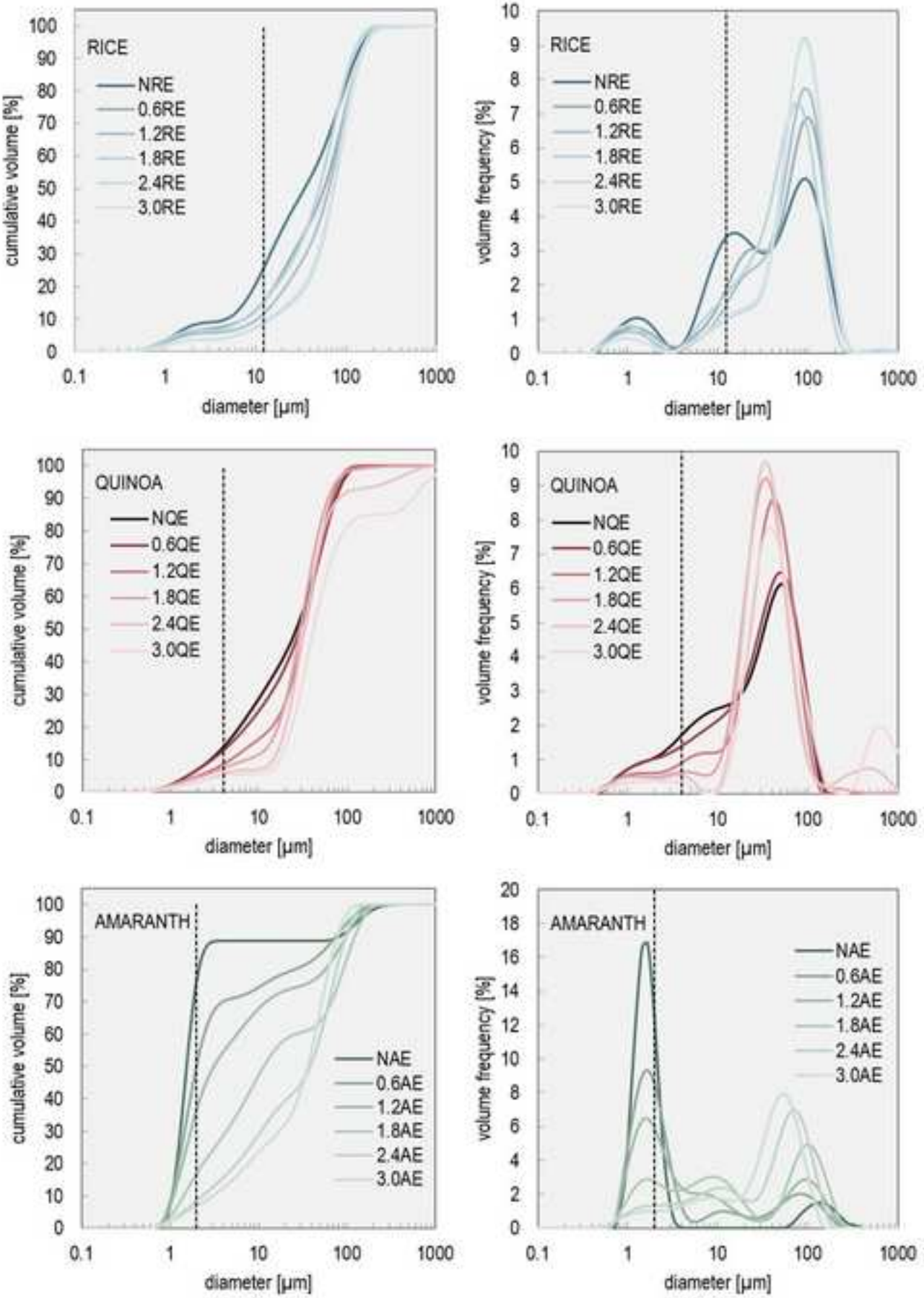

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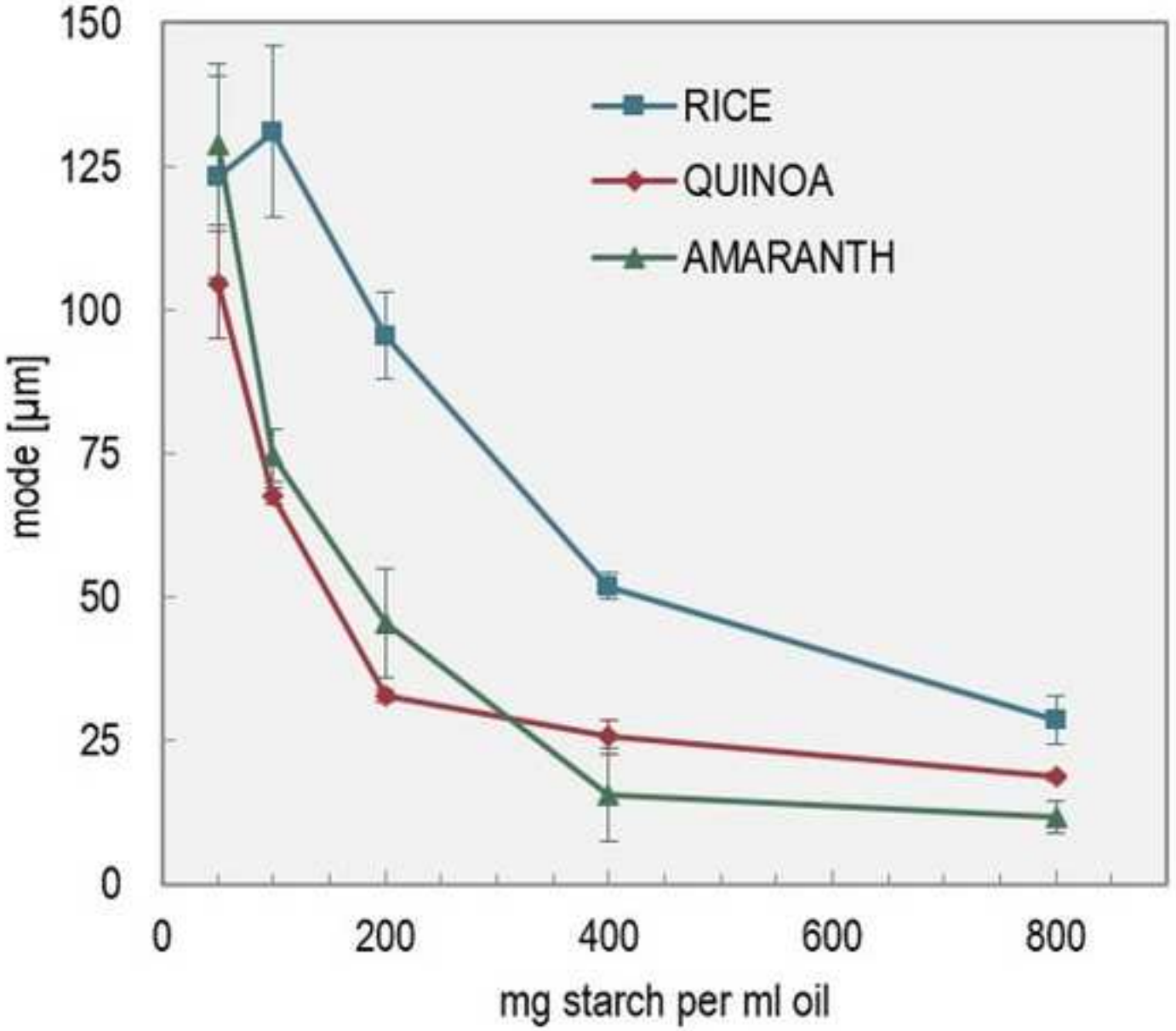
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