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Characterization and stability of short-chain fatty acids modified starch Pickering emulsions

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ABSTRACT

Acetylated, propionylated and butyrylated rice and quinoa starches at different levels of modification and starch concentrations, were used to stabilize oil-in-water starch Pickering emulsions at 10% oil fraction. Short-chain fatty acid modified starch Pickering emulsions (SPEs) were characterized after emulsification and after 50 days of storage. The particle size distribution, microstructure, emulsion index, and stability were evaluated. An increase in starch concentration led to a decrease of emulsion droplet sizes. Quinoa starch has shown the capability of stabilizing Pickering emulsions in both the native and modified forms. The emulsifying capacity of SPEs was improved by increasing the chain length of SCFA. Modified quinoa starch with higher chain lengths (i.e. propionylated and butyrylated), at higher levels of modification, showed higher emulsion index (> 71%) and stability over the entire 50 days storage. At optimized formulation, SCFA-starch particles have the potential in stabilizing emulsions for functional foods, pharmaceutical formulations, or industrial food applications.

1. Introduction

Emulsions are defined as multi-phase systems consisting of at least two immiscible phases where one phase is dispersed into the other phase as small droplets. As emulsions are thermodynamically unstable systems, destabilization may occur which consequently leads to coalescence followed by gravitational separation. Coalescence of emulsion droplets happens when emulsion droplets merge as a result of the reorganization of oil-water interfaces, to minimize the free energy. Emulsion droplet stabilization is achieved through the reduction of interfacial tension between the phases, through increasing steric hindrances, and/or electrostatic repulsion between the droplets (Rayner et al., 2012). Emulsion droplet stabilization is often achieved by the addition of protein, surfactants or polysaccharides (Dickinson, 2010). The examples of food-grade emulsifiers for each of the emulsifier categories mentioned above are: sodium caseinate for protein-based surfactants, polysorbate 80 for low molecular weight surfactant and modified molecular starch for polysaccharide-based emulsifiers (Raikos et al., 2017). Polysorbate 80 and carboxymethylcellulose were shown to alter gut microbiota in a rat study, lowered the level of butyrate in fecal samples, and increased the tendency of low-inflammation of the intestinal tract (Chassaing et al., 2015). For these reasons, alternative food-based emulsifiers are of continued interest.

Emulsions stabilized by solid particles, commonly known as Pickering emulsions, have received attention from researchers during the past decades in terms of finding a solution to produce food-grade emulsifiers without the risk of causing health-related effects (Aveyard et al., 2003; Binks, 2002). Several solid particles such as flavonoids (Luo et al., 2012), modified cellulose (Duffus et al., 2016) and starch particles (Marku et al., 2012; Matos et al., 2013; Rayner et al., 2014; Timgren et al., 2011) have been widely studied.

The principle of Pickering emulsions stabilization is through the prevention of coalescence by the adsorption of solid particles at the interface of oil droplets. This provides a mechanical barrier by the formation of a layer of solid particles with subsequent stabilization against coalescence through volume exclusion and steric hindrances (Rayner et al., 2014). The development of this barrier depends on the contact angle as well as the shape and size of the particles (Li et al., 2018; Marefati et al., 2018). This is because particles should have partial dual wettability for both phases, making them being tightly held at the oil-water interface (Rayner et al., 2014). However, excessive droplet aggregation is not beneficial for the stability of Pickering emulsions as it can cause the emulsion droplets to cream or sediment.

Starch is an abundant biopolymer that has the potential for

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application as a material to generate Pickering emulsions. Starch is a polysaccharide consisted of amylose and amylopectin in the form of granules as the energy storage unit of plants. Amylose is a linear chain dominated by α-1,4-glycosidic linkages while amylopectin is branched and has a higher prevalence of α-1,6-glycosidic linkages (Bertoft, 2017). Starch particles in the native forms are naturally hydrophilic, due to the presence of hydroxyl groups (OH), making them readily dispersible in water (Colussi et al., 2015). Therefore, starch modification is required to improve its dual wetting properties for adsorption at the oil-water interface of droplets. One common approach for chemical modification of starch is using octenyl succinic anhydride (OSA). OSA increases the surface hydrophobicity of starch granules via its hydrophobic chain, hence promoting adsorption at the oil-water interface of emulsion droplets (Marefati et al., 2017b). OSA modified starch Pickering emulsions have been extensively studied showing improved and long-term stability against coalescence and barrier properties suitable for industrial applications (Marefati et al., 2017a; Marefati et al., 2015; Timgren et al., 2013; Yusoff & Murray, 2011). A recent study by Marefati and Rayner (2020) has reported that Pickering emulsions stabilized by OSA modified quinoa starch granules remained stable for 8 years. In addition, the hydrophobicity of starch towards the oil surface is depending on the level of modification, botanical origin and size of the starch granules (Marefati et al., 2018; Zainal Abiddin et al., 2018). However, the functionality provided by OSA could also be achieved by many general classes of fatty acids that could also provide health benefits. To have more suitable particles for use as functional ingredients, starch granules can be chemically modified by short-chain fatty acids (SCFA), such as acetate, propionate, and butyrate which may have potential as emulsion stabilizers.

According to the European Food Safety Authority (EFSA), the maximum level of acetylated starch, known as E1420 is 2.5% of its acetyl group, which corresponds to a maximum of 0.1 degrees of substitution (DS) (Alicja et al., 2017). Several studies have focused on acetylated starches (Colussi et al., 2015; Golachowski et al., 2015) and medium and long-chain fatty acid starches (Vanmarcke et al., 2017) by investigating physicochemical, textural, and morphological properties. However, limited study has been conducted for propionate and butyrate modified starches and their application as Pickering emulsifiers (Hong et al., 2018).

SCFAs are produced by fermentation of dietary fibre in the human intestine by gut microbiota at different ratios, where approximately 60% of these metabolites are acetate, 10% butyrate and the remaining is propionate (Ganapathy et al., 2013). In our study, SCFAs are selected instead of medium and long-chain fatty acids since SCFAs are essential in providing benefits to intestinal health in humans. The health benefits of SCFAs are achieved via becoming an intermediate in prevention from colon cancer by inducing apoptosis and also for their treatment since they act as chemotherapeutic substances (Matthews et al., 2012). The concept being for short-chain fatty acid starch Pickering emulsions (SPEs) to be applied in functional food applications.

This work aims to study the feasibility of the utilization of SCFA-starches to stabilize Pickering emulsions. For this purpose, SCFAs with 3 different chain lengths, i.e. acetate (C2:0), propionate (C3:0) and butyrate (C4:0), at 4 different modification levels were used to esterify native rice and quinoa starch granules. The microstructure and physical stability of the SPEs were evaluated after homogenization and after 50 days of storage. In addition, the effect of starch concentration on the microstructure and stability of the emulsions were investigated.

2. Experimental

2.1. Materials

Native rice starch, *Oryza sativa* and native quinoa starch, *Chenopodium quinoa* were isolated according to a method described previously by Marefati et al. (2017b). Starches were esterified with acetic anhydride (CAS No: 108-24-7), propionic anhydride (CAS No: 123-62-6) and butyric anhydride (CAS No: 106-31-0) obtained from Carl Roth GmbH (Germany). The method of SCFA-starch modification and the quantification of the degree of substitution was presented in our previous work (Abdul Hadi, Wiege, Stabenau, Marefati, & Rayner, 2020). The detailed information on type and levels of modification of the starches used in this work are shown in Table 1. The continuous phase of emulsions was a phosphate buffer (5 mM, 0.2 M NaCl, pH 7) prepared with disodium hydrogen phosphate (Na_2HPO_4, CAS No: 10028-24-7, VWR, USA) and sodium dihydrogen phosphate (NaH_2PO_4, CAS No: 13472-35-0, VWR, USA). The dispersed phase used was medium chain triacylglyceride (MCT oil, Miglyol 812; CAS No: 52622-27-2) obtained from Caeser & Loretz GmbH (Germany).

Table 1
Types and levels of modification of rice and quinoa starch used in the preparation of Pickering emulsions.

Starch sample	Type of modification	Percent acyl (%)	DS
Rice	None (NR)	0	0
	Acetylated (RA)	0.95	0.0360
		1.85	0.0708
		2.70	0.1044
		3.49	0.1361
	Propionylated (RP)	1.06	0.0305
		2.02	0.0586
		3.29	0.0965
		4.28	0.1270
	Butyrylated (RB)	1.38	0.0319
		2.76	0.0648
		4.16	0.0989
		5.14	0.1235
Quinoa	None (NQ)	0	0
	Acetylated (QA)	0.87	0.0331
		1.72	0.0657
		2.54	0.0981
		3.42	0.1333
	Propionylated (QP)	1.04	0.0298
		2.19	0.0637
		3.25	0.0953
		4.12	0.1219
	Butyrylated (QB)	1.30	0.0300
		2.53	0.0591
		3.73	0.0883
		4.84	0.1160

2.2. Preparation of SCFA starch Pickering emulsions

2.2.1. SCFA-starch Pickering emulsions with different starch concentration

Oil-in-water emulsions (O/W) in 7 mL in total volume and 10% v/v oil fraction were prepared using the different types of SCFA-starch at different starch concentrations (50, 100, 200, 400 and 800 mg starch/mL oil) only for the highest level of modification. SCFA-starch was dispersed in phosphate buffer by mixing with vortex for 10 s in a glass test tube with a diameter of 2 cm. The corresponding amount of oil was added into the starch dispersion and the mixture was homogenized using a rotor-stator high shear mixer (Ystral, Germany) with a 6 mm dispersing tool at 22000 rpm for 60 s. Freshly prepared emulsions were let to stand for 30 min to allow the air bubbles to collapsed before further analyses were performed.

2.2.2. SCFA-starch Pickering emulsions at different levels of modification

The emulsifying properties of the three different SCFA-starches in the native form and four different levels of modification were investigated. The volume fraction of oil was 10% and the concentration of starch used was fixed at 200 mg starch per mL of oil. A similar emulsification procedure was used as described in the previous section to prepare the emulsions. To show the visual appearance of the emulsions, 4 mL of emulsions in a glass vial were let to stand and images were

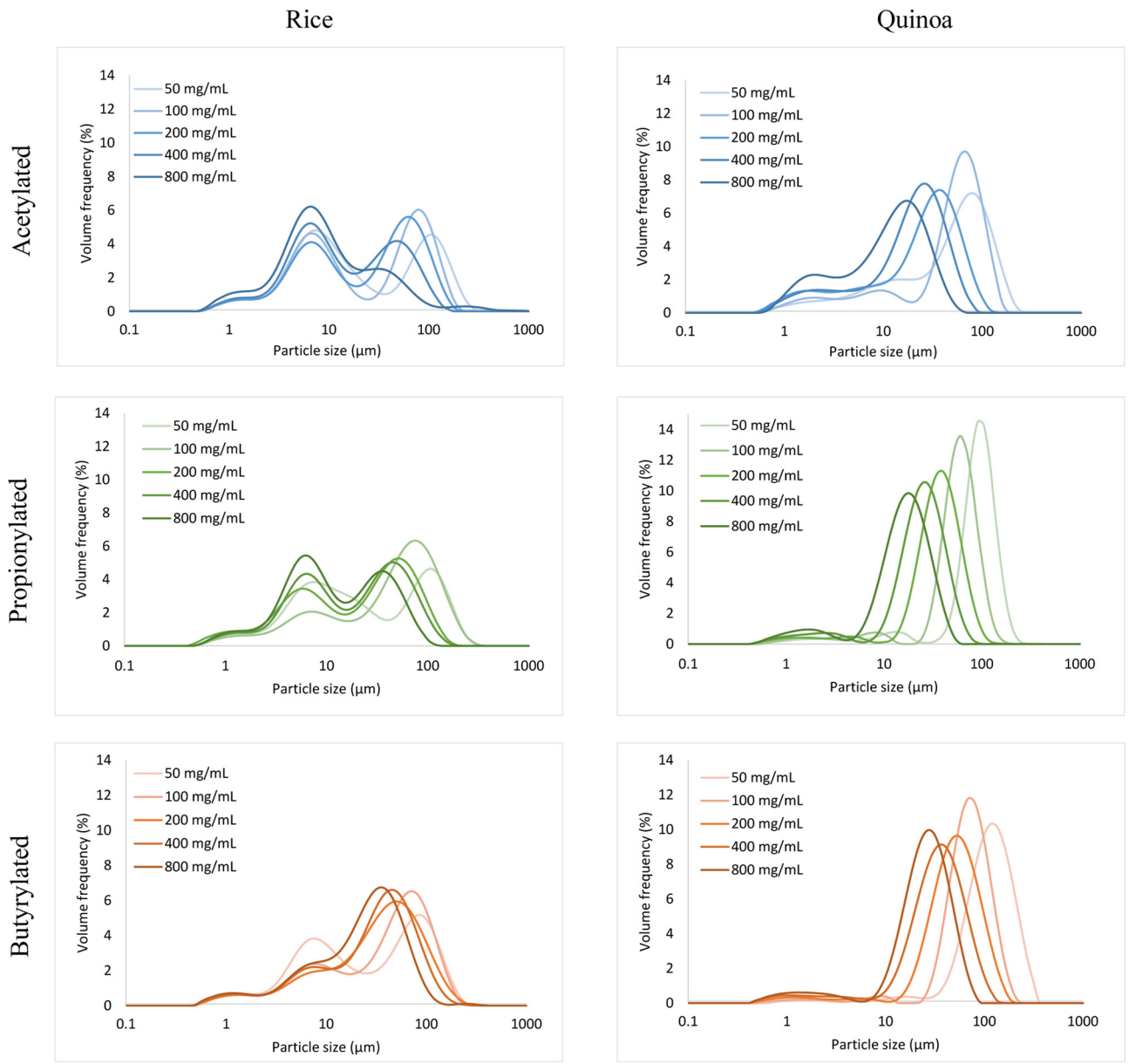

Fig. 1. Volume frequency particle size distribution of acetylated, propionylated and butyrylated rice and quinoa starch emulsions at the highest level of modification with 50, 100, 200, 400 and 800 mg starch/mL of oil.

captured on day 1 and day 50 using a digital camera.

2.3. Characterization of SCFA-starch Pickering emulsions

2.3.1. Microstructure

The morphology of Pickering emulsions was observed by light microscopy (Olympus BX50, Japan). The emulsions were diluted in phosphate buffer with a ratio of 1:5 and two drops of emulsion were placed on the microscopic glass slide without a coverslip. The microscopic images were taken using an objective magnification of 20× and were analyzed using ImageJ (Image processing and analysis in Java software).

2.3.2. Stability kinetics towards gravitational separation

The stability of SPEs was analyzed by Static Multiple Light Scattering (S-MLS), Turbiscan Lab Expert (Formulaction Co., France) according to a method by Matos et al. (2018) with slight changes. The emulsions (4 mL) were placed into flat-bottom measuring cells. Measurement temperature was set at 24 °C, and scans were performed every 5 min for 24 h. The storage stability of SPEs was determined by re-scanning the samples after 7 and 50 days. The stability of SPEs by S-MLS was determined based on the Turbiscan Stability Index (TSI) which is obtained from the intensity of backscattering (BS) and transmission (TS) which are correlated to the physical stability of the emulsion system. In this experiment, global TSI (TSI_T) was taken into account to investigate the emulsion droplets migration phenomena, caused by sedimentation and/or creaming, as well as possible changes in size caused by coalescence and/or aggregation. TSI_T value is the measurement of the overall TSI from the bottom to the top of an emulsion phase.

The TSI values were calculated using the equation below:

$$TSI = \sum i \frac{\sum_i |scan_i - scan_{i-1}|}{H} \quad (1)$$

which sums up the evolution of TS or BS light at all measured position, based on a scan-to-scan difference, over the total sample height (H):

2.3.3. Emulsion Index (EI)

The emulsion index (EI) was identified by measuring the length of

Table 2
The size distribution of native rice starch suspension (NRS), native quinoa starch suspension (NQS), acetylated rice emulsions (RAE), propionylated rice emulsions (RPE), butyrylated rice emulsions (RBE), acetylated quinoa emulsions (QAE), propionylated quinoa emulsions (QPE), butyrylated quinoa emulsions (QBE) at the highest level of modification of SCFA.

Sample	Starch concentration/ oil volume (mg/mL)	Average ± SD		
		$D_{[3,2]}$ [μm]	$D_{[4,3]}$ [μm]	Mode $D_{[4,3]}$ [μm]
3.49-RAE	50	7.0 ± 0.2	44.2 ± 4.9	100.8 ± 14.4
	100	7.3 ± 0.3	40.6 ± 3.0	74.4 ± 4.0
	200	7.4 ± 0.3	35.5 ± 1.6	58.9 ± 1.0
	400	6.2 ± 0.2	24.0 ± 0.9	44.9 ± 1.0
	800	4.7 ± 0.1	20.4 ± 4.0	29.3 ± 0.2
4.28-RPE	50	7.5 ± 0.3	46.3 ± 6.4	102.0 ± 16.5
	100	10.0 ± 0.5	56.2 ± 10.7	73.4 ± 15.5
	200	6.9 ± 0.2	35.6 ± 3.1	55.4 ± 3.5
	400	6.7 ± 0.2	27.2 ± 1.6	42.5 ± 0.9
	800	5.7 ± 0.1	18.7 ± 0.7	33.3 ± 0.1
4.16-RBE	50	8.0 ± 0.4	44.3 ± 3.5	80.3 ± 5.5
	100	9.4 ± 0.4	48.5 ± 3.0	66.7 ± 2.5
	200	9.6 ± 1.3	42.3 ± 6.2	46.5 ± 8.8
	400	8.8 ± 0.6	36.7 ± 3.1	42.5 ± 1.0
	800	7.8 ± 0.5	28.5 ± 2.2	33.2 ± 1.5
3.42-QAE	50	11.1 ± 0.8	54.0 ± 4.0	74.1 ± 3.2
	100	11.3 ± 0.5	52.6 ± 2.1	62.3 ± 2.2
	200	7.7 ± 0.6	32.0 ± 2.0	39.7 ± 1.9
	400	6.9 ± 0.4	23.0 ± 2.8	24.3 ± 1.2
	800	4.9 ± 0.3	14.0 ± 1.3	16.2 ± 0.6
4.12-QPE	50	24.8 ± 3.7	86.1 ± 6.1	90.1 ± 5.3
	100	18.0 ± 1.7	55.3 ± 3.1	56.7 ± 2.0
	200	13.3 ± 1.2	36.0 ± 1.5	35.5 ± 1.2
	400	6.%2 ± 0.9	24.3 ± 1.2	24.5 ± 1.1
	800	1.2 ± 0.5	16.4 ± 0.9	16.7 ± 0.9
4.84-QBE	50	37.6 ± 5.1	104.5 ± 10.0	102.9 ± 9.7
	100	25.1 ± 2.1	65.7 ± 5.0	63.4 ± 3.8
	200	17.6 ± 1.5	49.2 ± 3.9	47.0 ± 2.8
	400	12.1 ± 2.1	33.3 ± 2.9	32.7 ± 2.4
	800	9.1 ± 0.2	23.1 ± 1.8	23.5 ± 1.6

the emulsion layer of emulsions by S-MLS. The equation used for the EI determination is stated below:

$$\text{EI}\ (\%) = \frac{\text{height of sedimentation or creaming layer}}{\text{height of total emulsion}} \times 100 \quad (2)$$

2.3.4. Particle size distribution of drops and particles

The particle size distribution of SPEs was measured by using a laser diffraction particle size analyzer, Mastersizer Hydro 2000 (Malvern Instrument, UK) at pump speed 2000 rpm with obscuration range 10-20%. The refractive indexes used for water and starch were 1.33 and 1.54, respectively. The average droplet sizes were calculated based on $D_{[4,3]}$ (volume-weighted mean diameter), $D_{[3,2]}$ (surface weighted mean diameter or Sauter mean diameter) and the mode of $D_{[4,3]}$.

3. Results and discussion

3.1. Particle size distribution of SCFA starch Pickering emulsions formulated with varying amounts of starch

Formulation of SPEs at various starch concentrations was carried out to determine the appropriate starch concentration for further characterization of SCFA starches at different modification levels in the next step. The emulsion droplet size of SPEs for both rice and quinoa starches decreased as the starch concentration increased (Fig. 1 and Table 2). Similar observations were reported for lauroylated amaranth and OSA modified starches (Leal-Castañeda et al., 2018; Marefati et al., 2017b). This is due to the total amount of stabilized surfaces increased by the presence of a sufficient amount of starch particles (Marefati et al., 2017b; Matos et al., 2016). At the highest amount of starch concentration, quinoa-SPEs showed smaller droplets compared to rice-SPEs. This was interpreted as quinoa starch granules that have smaller particle sizes compared to rice starch granules which provide more surfaces to covers a larger interfacial area at the same mass/oil volume (Timgren et al., 2013).

3.2. Particle size distribution of SCFA starch Pickering emulsions formulated with varying levels of modification

The size distribution of native rice granules showed a bimodal distribution with the mode $D_{[4,3]}$ of the first and the second peak were 1.2 ± 0.0 μm and 5.6 ± 0.0 μm, respectively. Meanwhile, native quinoa starch granules showed a unimodal distribution with the mode $D_{[4,3]}$ was 1.6 ± 0.0 μm. The emulsifying capacity of native quinoa starch emulsion (NQE) was better than native rice starch emulsion (NRE) as shown by the smaller emulsion droplet sizes represented by the mode $D_{[4,3]}$ of 51.8 ± 0.7 μm and 80.4 ± 7.2 μm, respectively. This agrees with the previous findings of (Marefati et al., 2017b), where they attributed the higher emulsifying capacity of quinoa starch particles, in addition to smaller granular sizes, to the higher protein level of quinoa which could play an important role in the optimization of the wettability of the starch particles and resulting in more stable emulsions. Although native rice starch has a lower emulsifying capacity, our findings revealed that modification of native rice starch granules by SCFA was able to improve its emulsifying capacity. This was indicated by the reduction in the droplet sizes and the growth in the volume frequency of emulsion as the levels of modification increased. Also, the amount of non-adsorbed starch decreased as the level of modification and chain length increased, except for acetylated starches with modification levels below 1.85% and propionylated starches with modification levels below 1.06% (Fig. 2). The hydrophobicity of starch increased as a result of the replacement of the hydroxyl groups of glucose

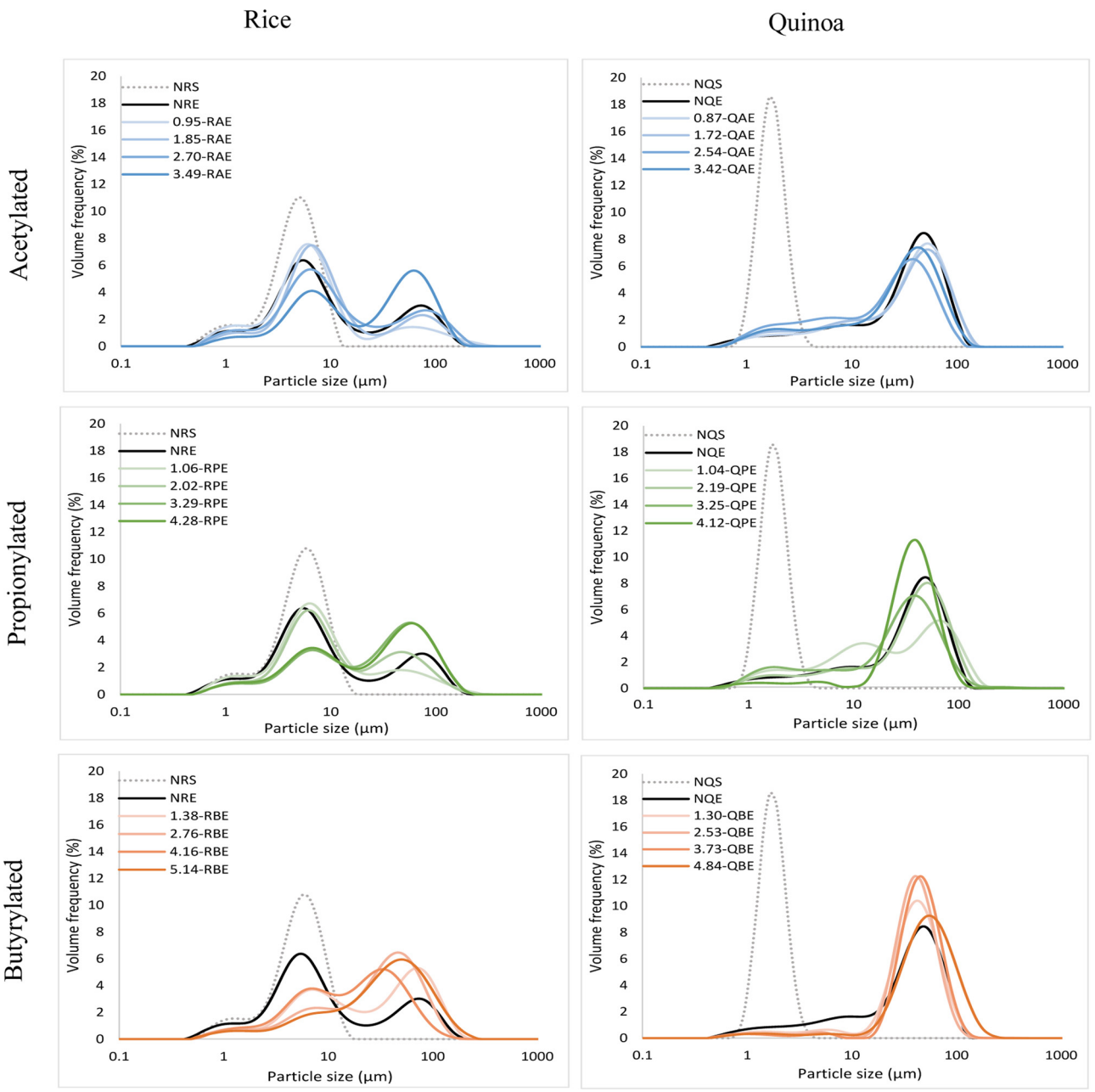

Fig. 2. Volume frequency particle size distribution of rice starch granules; NRS, quinoa starch granules; NQS, 200 mg starch/ mL oil of native rice and quinoa starch emulsions, rice-SPEs and quinoa-SPEs.

units in starch by the acyl group during the acylation which enhanced the emulsifying capacity (Hong, 2015). Low levels of acetyl (Fig. 2) led to the presence of more free starch in the continuous phase and low volume frequency of emulsion droplets compared to its corresponding native starch emulsion. This may have been caused by the removal of protein (which can act as natural hydrophobic domains) as a result of additional exposure to NaOH and washing during modification (Marefati et al., 2017b).

The mean droplet size of acetylated and propionylated quinoa stabilized emulsions decreased as the level of SCFA modification increased (Table 3). However, this trend was not observed in butyrylated quinoa emulsions where the droplet sizes increased as the level of modification increased. This may be due to that the measured value for the droplet size of emulsions from butyrylated quinoa included aggregated droplets, instead of single emulsion droplets (Fig. 4). Butyrylated quinoa emulsion at the lowest level of modification showed similar emulsifying capacity than the highest level of acetylated and propionylated quinoa emulsions. Thus, to achieve better emulsifying capacity, with a low degree of modification, increasing the carbon chain length of short fatty acid could be one solution which in turn, improves starch adsorption at the oil droplet surfaces. Comparing the volume frequency (Fig. 2) and cumulative particle size distributions (supplementary data 2), rice-SPEs had more free starch compared to quinoa-SPEs, which was supported by the micrographs (Fig. 4). However, as the acyl chain length and level of modification of SCFA increased, the amount of free starch was reduced. Assuming starch granules are tightly organized on the surface of oil droplet with packing density (φ) of 0.9, maximum surface coverage and estimation of emulsions droplet size were calculated. Due to larger sizes and lower surface available, the maximum surface coverage at the same oil content is higher for rice. Therefore, at the same starch concentration, the droplets are bigger for rice than quinoa (Table 3 and supplementary data 1). After 50 days of storage, emulsion droplet size

Table 3
Mean emulsion droplet size distributions of native starch emulsions and SPE (200 mg starch per mL oil) at different levels of modification.

Samples	DAY 1			DAY 50		
	$D_{[3,2]}$ [μm]	$D_{[4,3]}$ [μm]	Mode of $D_{[4,3]}$ [μm]	$D_{[3,2]}$ [μm]	$D_{[4,3]}$ [μm]	Mode of $D_{[4,3]}$ [μm]
NRS	3.4 ± 0.0	5.2 ± 0.0	5.6 ± 0.0	-	-	-
NRE	4.9 ± 0.2	25.9 ± 2.5	80.4 ± 7.2	3.5 ± 0.4	22.9 ± 3.5	107.1 ± 21.9
0.95-RAE	3.9 ± 2.5	18.2 ± 2.5	59.3 ± 8.1	8.3 ± 0.6	62.3 ± 15.7	91.4 ± 21.7
1.85-RAE	5.0 ± 0.2	21.0 ± 1.8	71.2 ± 4.7	6.1 ± 0.1	54.6 ± 11.0	101.0 ± 21.7
2.70-RAE	4.9 ± 0.2	26.6 ± 2.7	77.3 ± 5.8	7.0 ± 0.3	48.3 ± 8.6	87.8 ± 17.5
3.49-RAE	7.4 ± 0.3	35.5 ± 1.6	58.9 ± 1.0	7.9 ± 0.2	55.8 ± 6.8	81.4 ± 10.1
1.06-RPE	4.2 ± 0.1	16.4 ± 4.2	45.3 ± 6.6	4.0 ± 0.1	16.0 ± 3.4	43.6 ± 7.9
2.02-RPE	4.9 ± 0.4	23.8 ± 3.4	43.7 ± 7.3	6.2 ± 0.3	110.7 ± 40.7	93.4 ± 17.8
3.29-RPE	7.2 ± 0.3	36.1 ± 2.4	55.5 ± 2.8	3.7 ± 0.0	14.6 ± 0.5	52.4 ± 1.7
4.28-RPE	6.9 ± 0.2	35.6 ± 3.1	55.4 ± 3.5	10.0 ± 0.5	63.4 ± 13.3	92.9 ± 18.2
1.38-RBE	7.7 ± 0.6	45.6 ± 9.8	67.2 ± 2.5	6.6 ± 0.2	45.3 ± 12.3	72.4 ± 24.4
2.76-RBE	9.2 ± 0.4	34.2 ± 2.2	43.7 ± 2.3	6.4 ± 0.3	32.0 ± 2.2	53.9 ± 1.9
4.16-RBE	6.7 ± 0.3	24.0 ± 4.3	34.7 ± 7.5	6.8 ± 0.5	35.1 ± 7.7	42.6 ± 3.1
5.14-RBE	9.6 ± 1.3	42.3 ± 6.2	46.5 ± 8.8	9.6 ± 1.6	43.5 ± 10.8	48.1 ± 11.8
NQS	1.5 ± 0.0	1.7 ± 0.1	1.6 ± 0.0	-	-	-
NQE	9.4 ± 0.4	40.9 ± 1.3	51.8 ± 0.7	7.8 ± 0.2	36.4 ± 0.8	49.1 ± 0.6
0.87-QAE	8.5 ± 0.6	36.4 ± 1.7	48.4 ± 0.8	8.2 ± 0.7	48.2 ± 5.0	65.8 ± 3.4
1.72-QAE	8.2 ± 0.5	37.3 ± 2.1	49.3 ± 1.3	7.6 ± 0.4	38.0 ± 1.7	54.7 ± 0.8
2.54-QAE	6.2 ± 2.5	26.8 ± 1.1	35.2 ± 0.7	5.7 ± 1.3	31.8 ± 1.9	56.5 ± 0.5
3.42-QAE	7.7 ± 0.6	32.0 ± 2.0	39.7 ± 1.9	6.8 ± 0.2	35.4 ± 1.3	48.5 ± 0.8
1.04-QPE	9.5 ± 0.5	40.9 ± 1.4	50.3 ± 1.5	7.6 ± 0.4	38.2 ± 1.3	57.9 ± 0.1
2.19-QPE	9.4 ± 1.2	40.0 ± 4.0	45.8 ± 3.0	8.8 ± 0.5	37.6 ± 1.5	47.0 ± 0.9
3.25-QPE	7.0 ± 0.6	32.1 ± 2.8	36.3 ± 1.6	6.2 ± 0.4	30.0 ± 3.9	36.3 ± 2.5
4.12-QPE	13.3 ± 1.2	36.0 ± 1.5	35.5 ± 1.2	12.3 ± 1.5	34.9 ± 1.1	35.0 ± 0.8
1.30-QBE	14.5 ± 0.4	38.2 ± 1.0	38.9 ± 0.9	13.7 ± 1.3	41.6 ± 3.7	40.3 ± 2.7
2.53-QBE	15.1 ± 0.6	39.1 ± 1.3	38.3 ± 1.1	14.4 ± 1.4	39.9 ± 3.0	38.7 ± 2.3
3.73-QBE	17.8 ± 2.0	47.3 ± 3.4	45.7 ± 2.8	16.9 ± 1.8	45.5 ± 3.6	45.1 ± 2.7
4.84-QBE	17.6 ± 1.5	49.2 ± 3.9	47.0 ± 2.8	19.3 ± 2.4	58.1 ± 9.9	48.6 ± 4.1

(-) represent not determined

Fig. 3. Images of 200 mg starch per mL oil of native starch suspensions, native rice and quinoa starch emulsions, and SPEs on day 1 and day 50. The percentage in the images represents the percent of the acyl groups.

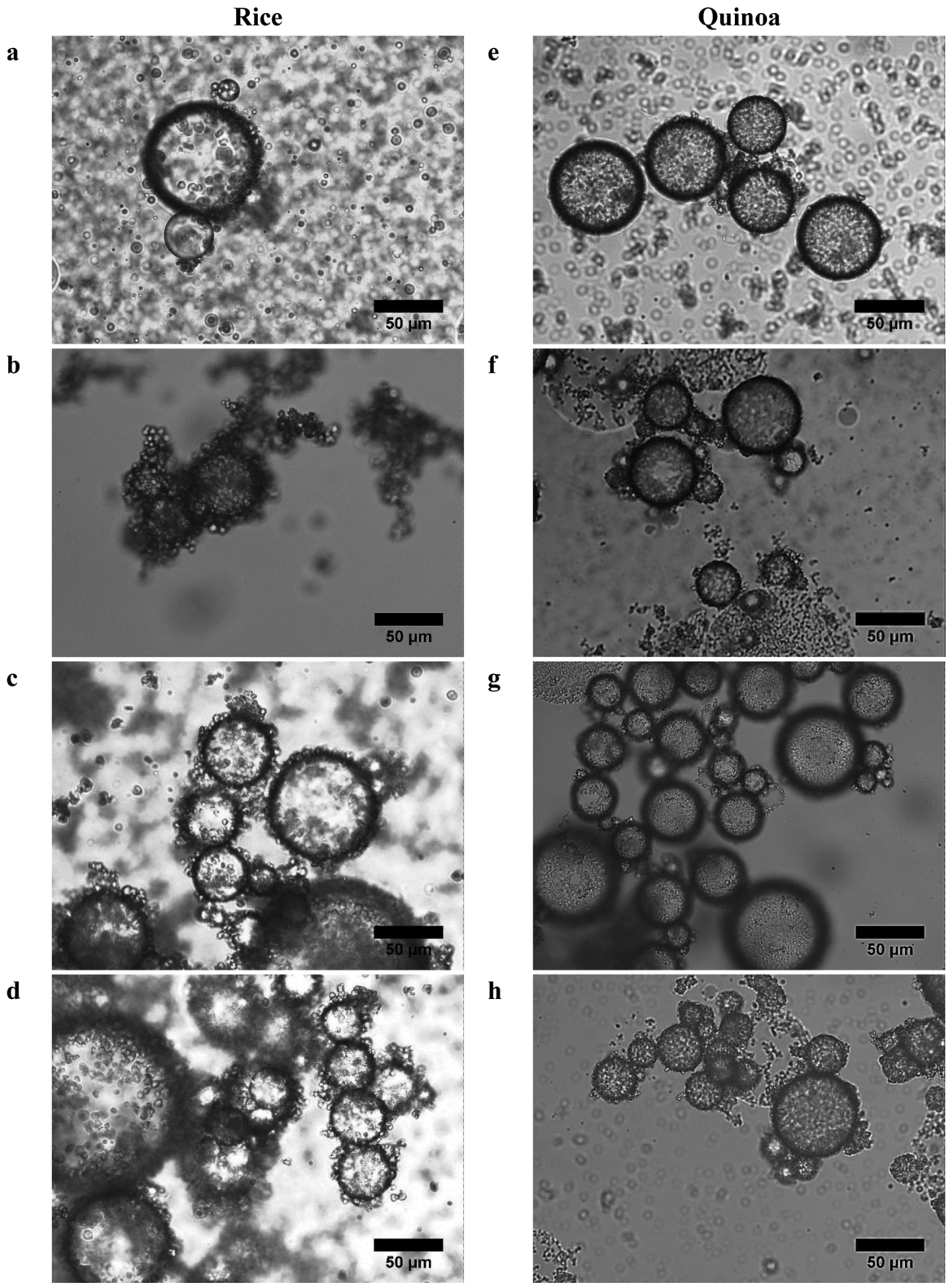

Fig. 4. Micrograph images of 200 mg starch per mL oil by using native starches and highest modification levels of SCFA-starches a. NRE, b. RAE, c. RPE, d. RBE, e. NQE, f. QAE, g. QPE and h. QBE.

distributions were re-measured. The rice-SPEs showed changes in droplet size being larger than 50 µm with the exceptions at 1.06% propionyl, 4.16% butyryl and 5.14% butyryl (Table 3). In contrast, most of the quinoa-SPEs showed stable emulsion in which the size of the droplets was below 50 µm that may be resulting in being less susceptible to aggregation or coalescence (Table 3).

3.3. Micro- and macro-structure of SCFA starch Pickering emulsions formulated in varying levels of modification

In freshly prepared emulsions, the S-MLS showed that rice-SPEs at all levels of modification and chain lengths led to free starch and/or emulsion sediment at the bottom of the measuring cell or a thin layer of

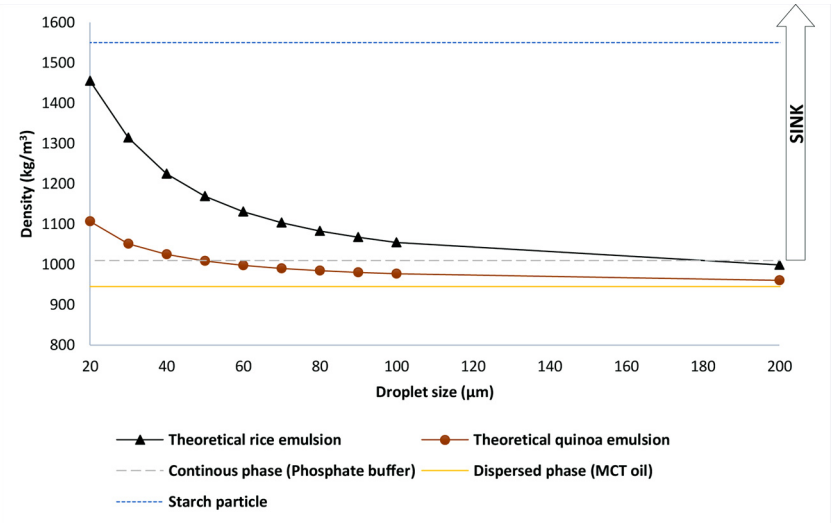

Fig. 5. Theoretical emulsion droplet density (see supplementary data 2), if the density is above the CTS plane, the particle or droplet will sink.

emulsion phase. On the other hand, in the case of quinoa-SPEs, more emulsion phase was formed in all cases with subsequent sedimentation or creaming depending on the resulting emulsion droplet buoyancy (i.e. the relative volume of oil to the amount of starch covering it). Theoretical calculation of emulsion droplet density was performed by the inclusion of the density of starch particles (Fig. 5). It shows that rice starch emulsions tend to sedimentation due to higher droplet density compared to quinoa starch emulsions at all levels of modification. This estimation was supported by Fig. 3 where a thin layer of sediment was observed in native and rice-SPEs. Since quinoa-SPEs had lower emulsion drop density, it was expected that it would have formed a cream layer on top. However, the cream layer was only present at above 2.54% acetyl and 3.25% propionyl. The occurrence of a sediment layer for the quinoa-SPEs was due to small emulsion droplet and close packing of starch adsorb on the oil droplets. This increased the effective density of particle-laden drops of the dispersed relative to the continuous phase, hence droplets tended to sink. This situation was confirmed by microscopy images (Fig. 4), which showed that quinoa-SPEs had smaller emulsion droplet size compared to rice-SPEs and its native form.

3.4. Emulsion index (EI) of SCFA starch Pickering emulsions

The quinoa-SPEs had higher EI (58.7% ± 0.9 - 83.0% ± 0.7) compared to rice-SPEs (12.0% ± 0.8 - 36.5% ± 0.9). Meanwhile, native rice emulsion had a lower EI (9.5% ± 0.1) compared to native quinoa emulsion (44.4% ± 0.5) and the lowest EI among all formulations. There was no overall correlation between the degree of modification and the EI. Gravitational separation occurred in rice-SPEs immediately after they were analyzed, as it can be observed in TS and BS profiles (Fig. 6). TS intensity was high and fairly flat in the middle section of the measuring cell while two BS peaks are observed at the bottom and top sections of the measuring cell which indicates that both sedimentation and creaming have rapidly occurred. Meanwhile, for quinoa-SPEs, it took a long time for emulsion droplets to migrate, due to lower density differences between dispersed and continuous phases (Fig. 6). This is because the droplet size of quinoa-SPEs was smaller than rice-SPEs hence smaller density difference between the continuous phases in the emulsion system (Rayner et al., 2014). Another notable result was low EI was observed at 4.16-RBE (31.8% ± 0.4) compared to 2.76-RBE (36.5% ± 0.7). However, the BS intensity of 4.16-RBE (44%) was higher compared to 2.76-RBE (36%) which means high BS intensity showing the emulsion is in a compact and dense position state.

3.5. Stability kinetics of SCFA starch Pickering emulsions

The TSI integrates all the variations detected in the samples in terms of size and/or concentration. Therefore, an increase in the TSI value during the storage is interpreted as emulsion droplets migration leading to local variations of the concentration in the bottom and top of the sample (sedimentation or creaming processes), or an increase in mean droplet sizes (caused by coalescence) that can lead to global variations in the middle of the sample. So, it is necessary to analyze TSI_T (Table 4), as well as emulsion macrostructure and microstructure by visual inspection and microscopy.

The BS intensity of freshly prepared SPEs showed a flat line with measurable BS intensity throughout the height of the measuring cell as the chain length increased. Except for acetylated rice emulsions where the fast separation was observed. This situation showed that the emulsion droplets were well distributed in the system after emulsification (Fig. 6).

It appears from Table 4 that the TSI_T of acetylated-rice-SPEs decreased at all levels of modification and measurement interval (day 1 and day 50). This finding showed that acetylated rice starch at a high level of modification led to more stable emulsions compared to native rice starch. The same trend was found with propionylated and butyrylated rice starches except for propionylated rice starch emulsions analyzed at after 50 days, where high instability was observed with high TSI_T values. This could be explained by the differences in droplet size as reported in Table 3.

In contrast, SCFA-quinoa-SPEs showed better stability observed with low TSI_T values. The low value of TSI_T was obtained from SCFA-SPEs, compared to its native starch emulsions is an indication of better stability. As a general trend, it was observed that, as the level of modification increases, the TSI_T measurement decreased which means the emulsion system is more stable. There was an exception at 2.53% and 3.73% butyryl that can be explained by the movement of the initial cream layer (day 1) (Fig. 3), which rapidly settled to the bottom resulting in increased TSI_T. However, this is not an indication of instability since the droplet sizes maintained after 50 days of storage. Even though the TSI_T of all samples increased after 50 days of storage, as the size of the droplets remained constant during storage, the emulsions were considered stable.

As expected, native rice and quinoa starch emulsions showed higher TSI_T compared to SCFA-SPEs indicating the emulsions were unstable with low emulsion index. Phase separation of emulsions was seen to have occurred within 3 hours. Further investigation was carried out by identifying the destabilization of emulsion systems by measuring the BS

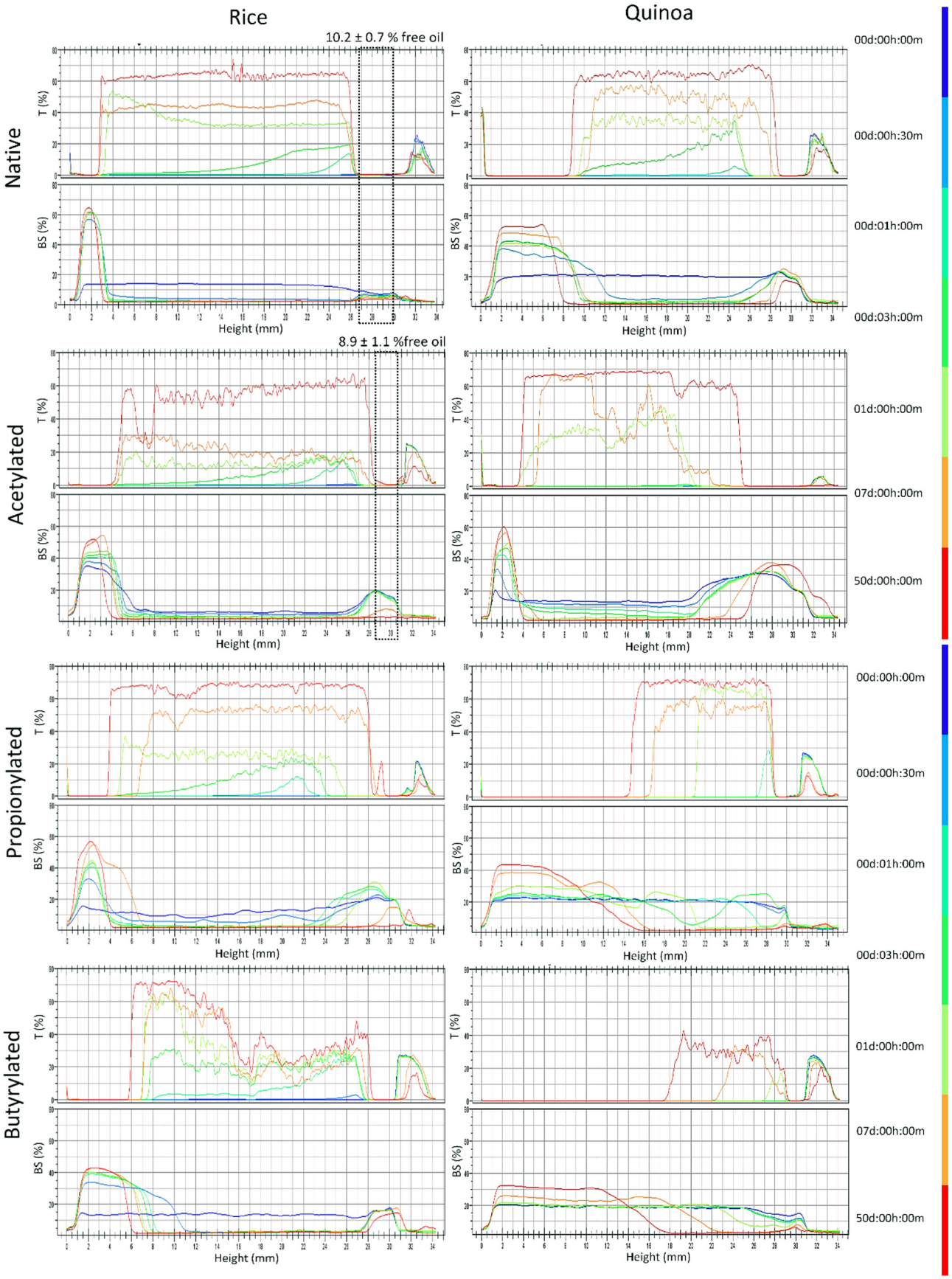

Fig. 6. The transmission (T) and backscattering (BS) profiles of emulsions with 10% oil and 200 mg starch/mL oil by using native starches and highest modification levels of SCFA-starches; a. NRE, b. RAE, c. RPE, d. RBE, e. NQE, f. QAE, g. QPE and h. QBE. Black dotted rectangular represents free oil.

Table 4
The emulsion index (EI) and global TSI of native starch and SCFA-starch emulsions with formulation 10% oil and 200 mg starch/ mL oil at different levels of modification.

Sample	Day 1		Day 50
	EI (%)	TSI_T	TSI_T
NRE	9.5 ± 0.1	35.3 ± 1.0	48.2 ± 0.1
0.95-RAE	12.0 ± 0.8	35.8 ± 0.6	59.7 ± 0.1
1.85-RAE	22.2 ± 0.4	39.2 ± 0.6	58.4 ± 0.0
2.36-RAE	19.0 ± 0.5	28.5 ± 0.8	50.8 ± 0.1
3.49-RAE	28.6 ± 0.6	11.4 ± 0.2	42.4 ± 0.1
1.06-RPE	30.1 ± 0.9	31.7 ± 0.1	101.0 ± 0.1
2.02-RPE	24.0 ± 0.5	20.0 ± 0.5	60.5 ± 0.1
3.29-RPE	36.4 ± 0.5	32.9 ± 3.6	99.0 ± 0.2
4.28-RPE	38.1 ± 0.1	21.5 ± 1.6	97.5 ± 0.1
1.38-RBE	26.9 ± 2.2	23.9 ± 1.2	54.1 ± 0.1
2.76-RBE	36.5 ± 0.7	16.5 ± 0.5	56.3 ± 0.2
4.16-RBE	31.8 ± 0.4	19.8 ± 0.2	53.1 ± 0.0
5.14-RBE	36.5 ± 0.9	24.4 ± 0.9	56.3 ± 0.2
NQE	44.4 ± 0.5	35.9 ± 2.2	52.9 ± 0.7
0.87-QAE	58.7 ± 0.9	30.6 ± 5.0	64.4 ± 0.2
1.72-QAE	49.0 ± 3.1	24.6 ± 0.7	67.9 ± 0.1
2.54-QAE	36.0 ± 1.9	32.7 ± 0.7	54.6 ± 0.1
3.42-QAE	44.0 ± 0.6	18.8 ± 1.1	120.9 ± 0.6
1.04-QPE	50.0 ± 0.5	32.4 ± 0.5	52.4 ± 0.1
2.19-QPE	48.0 ± 0.5	30.9 ± 0.4	42.0 ± 0.1
3.25-QPE	48.0 ± 0.9	24.9 ± 0.8	54.0 ± 0.2
4.12-QPE	71.0 ± 1.2	25.8 ± 0.7	50.2 ± 0.1
1.30-QBE	66.0 ± 0.3	20.8 ± 0.3	37.9 ± 0.1
2.53-QBE	70.0 ± 1.2	57.6 ± 0.6	74.7 ± 0.1
3.73-QBE	75.0 ± 0.2	22.4 ± 3.0	66.3 ± 0.0
4.84-QBE	83.0 ± 0.7	2.9 ± 0.34	21.4 ± 0.1

or TS variations. In this work, the BS is more preferable to detect the emulsion destabilization as the emulsions appear turbid due to the attribution of droplets. These emulsions have high obscuration which somehow reflected the light as backscattering, thus the backscattered light is more sensitive in detecting emulsion droplets. Meanwhile, the TS intensity is suitable to evaluate diluted starch particle dispersions as the transmitted light that passes through the dispersion can be detected by the S-MLS. Comparing the percent of BS (Fig. 6), the layer formed at the bottom of the measuring cell in the case of quinoa-SPEs increased as the SCFA-chain increased. However, after 50 days, it was observed that the layer became compact/packed resulting in increased BS. This was supported by Peng et al. (2016) which stated that the aggregation and sedimentation of large emulsion droplets increase BS intensity. Meanwhile, a decreasing BS at the top over the time means that a clarification process is taking place (with simultaneous sedimentation at the bottom) or it can be also caused by coalescence of emulsion droplets.

As the acyl chain increased, low BS intensity was observed at the bottom part of rice-SPEs and quinoa-SPEs. The height of the bottom layer present became wider as shown in Fig. 6d, g and h. This resulted in the layer of emulsion droplets being less compact, with other droplets and free starch granules. High BS intensity monitored at the bottom part particularly in native rice emulsion was mainly due to the presence of free starch as starch granules that are solid and can organize themselves more compactly as they sediment which caused a higher intensity of the light to reflect. After 50 days of storage, the free oil layer was observed in native, acetylated and propionylated below 3.29% rice starch emulsions (Fig. 3). The amount of free oil was also measured (Fig. 6a and b), by the height of the oil layer at the top and cell dimensions. It was shown that native and 3.49% acetylated rice starch emulsions had 10.2% ± 0.7 and 8.9% ± 1.1 of free oil at the top of the Pickering emulsions system, respectively.

4. Conclusions

The results of this investigation showed that starch acylation by SCFA has the potential for improving the emulsifying capacity for both rice and quinoa starches. The substitution of hydrophilic OH group found on the starch molecules with hydrophobic acyl group of SCFA by esterification increased the emulsification capacity of rice and quinoa starch granules. The increase of starch concentration, level of modification and SCFA acyl chain length, reduced the emulsion droplet sizes. Butyrylated and propionylated quinoa starch granules at the highest level of modification produced the smallest emulsion droplet size, higher EI, less free oil and free starch and were stable up to 50 days. Thus, SCFA starch granules can be used as emulsifying particles in Pickering emulsion with an appropriate formulation and modification level. Formulation of Pickering emulsions stabilized by SCFA modified starch granules could be a route for supplemention of short-chain fatty acid in functional foods application.

CRediT authorship contribution statement

N. Abdul Hadi: Conceptualization, Methodology, Investigation, Visualization, Writing- Original draft. **A. Marefati:** Validation, Visualization, Supervision, Writing - Review & Editing. **M. Matos:** Validation, Supervision, Writing - Review & Editing. **B. Wiege:** Resources, Writing - Review & Editing. **M. Rayner:** Validation, Supervision, Writing - Review & Editing.

Declaration of Competing Interest

None

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.carbpol.2020.116264.

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